Clonal differences and impact of defoliation on Sauvignon blanc (*Vitis vinifera L.*) wines: a chemical and sensory investigation

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ABSTRACT

BACKGROUND: The aim of this study, performed on Sauvignon blanc clones SB11 and SB316, grafted on the same rootstock 101-14 Mgt (*V. riparia* x *V. ruperstris*) and grown at two adjacent vineyards, was twofold; i) to study wine chemical and sensory composition of both clones within an unaltered canopy and ii) to determine the effect of defoliation (e.g. bunch microclimate) on wine chemical and sensory composition.

RESULTS: Orthogonal Projection to Latent Structures Discriminate Analysis (OPLS-DA) was applied to the concentration profiles of volatile compounds derived from GC-MS data. The loadings directions inferred that 3-isobutyl-2-methoxypyrazine (IBMP) discriminated control treatments (shaded fruit zone) of both clones from defoliation treatments (exposed fruit zone), whereas 3-sulfanyl-hexan-1-ol (3SH), 3-sulfanylhexyl acetate (3SHA), hexanol, hexyl hexanoate and some other esters discriminated defoliated treatments from the controls. The OPLS-DA indicated the importance of IBMP, higher alcohol acetates and phenylethyl esters, for discrimination of clone SB11 from clone SB316 irrespective of the treatment. Defoliation in the fruit zone significantly decreased perceived greenness in clone SB11 and elevated fruitier aromas, whereas in clone SB316 the effect of defoliation on wine sensory perception was less noticeable regardless the decrease in IBMP concentrations.

CONCLUSION: These findings highlight the importance of clone selection and bunch microclimate to diversify produced wine styles.

Key words: Methoxypyrazines, esters, thiols, viticulture, sensory

INTRODUCTION

Through history the grapevine has been an important global agricultural crop. With the domestication of *Vitis vinifera* L., selected germpalsms were asexually propagated, yielding clones genetically identical to parental plants as long as spontaneous mutation did not arise.[1] During the last two centuries, clonal selections were performed to improve vineyard health and production traits by vegetative propagation. These traits included improved flavour, colour, yield, berry size, precocity and disease resistance to mention a few. Knowledge has been gathered on the genetic diversity between clones of a single variety[1, 2] but very little evidence on the chemical and sensory properties of wines originating from different clones has been reported. Concentrations of 3-isobutyl-2-methoxypyrazine (IBMP) vary significantly between different Merlot and Carmenere clones[3,4] and variability in thiols precursors and free varietal thiols concentrations within selected clones of Sauvignon blanc were noted.[5] Recently, the effect of 131 Malbec clones on the anthocyanins profile variability and the putative genes involved in these differentiation was also reported.[6]

The Sauvignon blanc wine aromatic profile can be generally attributed to three distinct and very potent groups of compounds; thiols, esters and methoxypyrazines.[7] The 3-sulfanyl-hexan-1-ol (3SH) and 3-sulfanylhexyl acetate (3SHA) in Sauvignon blanc wines are often associated with tropical fruit aromas such as passion fruit, mango, guava, gooseberry, grapefruit and have detection threshold as low as 60 and 4 ng L^{-1}, respectively in model wine solution.[8,9] The sensory detection threshold of 4-methyl-4-sulfanylpentan-1-on (4MSP) is even lower, 0.8 ng L^{-1} and it is often described as having box-tree and passion fruit like odours.[10] The varietal thiols 3SH and 4MSP are present in grapes as non-volatile forms bound to cysteine and glutathione.[11-1313] The release of 3SH and 4MSP from their precursors during alcoholic fermentation is proposed to be regulated by nitrogen catabolic repression,[14] thus juice nitrogen status and yeast physiology are indisputably linked to thiol release.

Furthermore Pinu[15] showed the role of nitrogenic and sulphuric compounds on thiols releases and also emphasised the role of carboxylic and linoleic acids on 3SH, 3SHA and 4MSP release during fermentation.

Esters are also known to contribute to the fruity aromas of wines. The two main groups of esters, contributing significantly towards young white wine fruity aromas, are ethyl esters of fatty acids (EEFAs) and higher alcohol acetates (HAAs).[16,17] The EEFAs are responsible for the tree fruit notes whereas HAAs contribute towards tropical fruit odours. Other minor group of esters can act synergistically with other volatiles in wines by enhancing or masking specific sensory attributes.[18] Nevertheless, even though esters are undisputedly yeast-derived aromatic compounds and varietal thiols are released during fermentation from their non-odorous precursors, their concentrations and profiles can be altered in the vineyard by modifying the grape nitrogen status, the amino acids profile and the lipid composition.[8, 15, 19, 20]

In contrast to the above described thiols and esters, methoxypyrazines, namely IBMP and 3-isopropyl-2-methoxypyrazine (IPMP), are responsible for the green, vegetative, green pepper and asparagus aromas of Sauvignon blanc wines.[21] The detection threshold of IBMP in water and white wine is as low as 2 ng L^{-1} [21] and its concentrations in wines are strongly correlated with those observed in the corresponding grapes.[22] Increased light penetration in the bunch zone early in grape development (before veraison) is of utmost importance for decreasing the IBMP and IPMP concentrations in grapes at harvest and the effect of temperature on methoxypyrazines concentrations cannot be overlooked.[23-25]A recent study on Sauvignon blanc suggested modifications in wine chemical and sensory composition when grapes were exposed to different light environment achieved either by defoliation or reduction of UV-light.[20]

To better understand the importance of Sauvignon blanc clonal diversity on wine composition and sensory perception, two commercially used clones in South Africa (SB316 and SB11) were studied.

Clone SB11 is anecdotally considered among grape growers and wine makers as a greener clone, with strongly perceived asparagus and grass aromas, whereas SB316 is often described as fruiter clones with strong fig aroma and some green pepper/asparagus notes (KWV (http://www.agriworldsa.com/article-archive/viticulture/clones_2007.pdf)). No scientific evidence however exists to support these empirical observations. Therefore, the aim of this work was twofold: i) to study wine chemical composition and sensory characteristic of both clones (SB316 and SB11) within an unaltered canopy (a shaded fruiting zone) and ii) to determine if defoliation at the fruiting zone would impact the aromatic composition of wines from both clones in a similar manner. For the study a specific site was chosen in the Western Cape region (South Africa), characterised by a temperate to cool climate during the fruit ripening period, due to the sea breeze influence which cools down the vines during the hottest hour of the day.[20] The choice of the site allowed to strongly open the canopy at the fruit zone by removing all leaves and laterals, without damaging the fruit (e.g. sunburn and/or berry shrivelling).

MATERIALS AND METHODS

Vineyard and layout. The experiment was performed on two adjacent commercial *Vitis vinifera* L. Sauvignon blanc vineyards in the Elgin valley, located in the Southern coastal region of South Africa (34°16′50″S, 19°05′09″E), with an approximate distance of 7.5 km from the Atlantic ocean and an elevation of 412 m. This wine growing region is considered to be among the coolest in South Africa with Huglin index[26] for 2012-2013 vintage determined as 1886 units, inferring that the experimental site is classified as having a temperate to cool climate due to the sea breeze influence during the ripening period. The experimental plot

scions were Sauvignon blanc clones SB11 and SB316 grafted on 101-14 Mgt rootstock (*V. riparia* x *V. ruperstris*) and both plots were established in 2003. Clone SB11 is South African selected clone, with average production and vigour. Wines are often described as balanced and of good quality (KWV (http://www.agriworldsa.com/article-archive/viticulture/clones_2007.pdf)). Contrary, clone SB316 is originating from France and is described as having average yield and vigour. Wines are typical of cultivar and aromatic (KWV (http://www.agriworldsa.com/article-archive/viticulture/clones_2007.pdf)). Row orientation for both experimental plots was Northwest-Southeast, with spacing between rows 2.5 m and between vines 1.8 m. The vines in both plots were trained on a double cordon with vertical shoot positioning and similar vineyard management was applied to both of the plots throughout the season. To examine the differences between clones SB316 and SB11 within an unaltered canopy (referred from here on as SB316 control and SB11 control) and the effect of defoliation on both clones (referred to as SB316 and SB11 defoliation) a checkerboard experimental layout was designed across nine rows with three treatment repetitions in each experimental vineyard. Each treatment repetition consisted of eight consecutive vines replicated across three rows, therefore 24 vines per repetition. The SB316 and SB11 control treatment represented the canopy as managed by vineyard manager, subjected to no leaf removal during the season, implicating that bunches were in a permanently shaded situation throughout the growing and ripening period. Defoliation treatments were performed two weeks before veraison on the 22 January 2013 by completely removing leaves and lateral shoots from the fruiting zone on the morning side of the canopy (north-eastern side) up to 40 cm above the cordon. The afternoon side of the canopy (south-western side) remained unaltered (Figure 1) as described previously.[20] Mesoclimatic temperature data were collected from the automatic weather station situated within a vineyard and located 260 m from the experimental plots. The microclimatic bunch temperature was

monitored between 25 January 2013 until harvest on 25 March 2013 at 15-minute intervals using TinyTag® TGP-4520 dual channel external loggers (Gemini Data Loggers Ltd., Chichester, United Kingdom) with the two flying lead thermistor probes positioned inside the representative bunch on each side of the canopy. The canopy external leaf area perimeter (CELAP) was calculated to estimate the percentage of leaves removed as well as to indicate possible differences in vigour between the two clones.[27] The exposed leaf area measurements were calculated from 6 measurements per treatment per clone.

General analyses of maturity Prior to fermentation, a set of physiochemical parameters relating to maturity was analysed in the must. The total soluble solids (TSS) were measured using a PAL-1 digital handheld refractometer (Atago, Tokyo, Japan) with automatic temperature correction. The pH and titratable acidity (TA) were determined by sodium hydroxide titration to an end point of pH 8.2 with a Metrohm titrator and sample changer (785 DMP Titrino with a LL-Unitrode Pt1000 electrode - Metrohm AG, Herisau, Switzerland).

Small-scale vinifications. Grapes from both of the clones and treatments were harvested on 26 March 2013 when TSS of grape juice reached values between 20 and 22 °Brix. Similarly, as described previously[20], only totally exposed bunches from the north-eastern (morning) side of the canopy were harvested for the defoliated treatments and shaded bunches from the afternoon facing side of the canopy were not considered (Figure 1). For the control treatments all the bunches within the canopy were harvested as these treatments were considered as homogeneous in terms of light and temperature exposure. Grapes were harvested manually with care to ensure consistency in the method of harvesting, especially for the defoliated treatments. Approximately 20 kg of grapes was randomly harvested per treatment replicate, therefore approximately 60 kg of grapes in total per treatment per clone and transported to the Stellenbosch University experimental cellar for vinification. The grapes from the three

treatment replicates were pooled and stored overnight at + 4 °C. All grape crushing and juice handling occurred in the presence of solid carbon dioxide and nitrogen gas (N_2) to avoid oxidation. Grapes were crushed with 40 mg L^{-1} of sulphur dioxide (SO_2) which was added during the crushing. After cold maceration at + 4 °C for 24 hours, the grape slurry was pressed. The pressed juice was placed in buckets in which solid carbon dioxide was placed and thereafter an enzyme was added at 2 g hL^{-1} (Rapidase Vino Super, DSM Food Specialists B.V., Netherlands) to facilitate sedimentation. The bucket headspace was purged with N_2 before the buckets were sealed and placed overnight at + 4 °C for sedimentation. Clear juice was racked off sediment using tube and divided by placing four liters of juice into each 4.5 L glass fermenter which was filled beforehand with N_2 and a pellet of solid carbon dioxide. The divided grape juice was inoculated with 30 g hL^{-1} of DV10 yeast (Lallemand, South Africa) prepared in accordance to the manufacturer's instructions and fermentations in triplicate were conducted in a temperature controlled room at + 15 °C. The progress of fermentation was monitored daily by mass loss due to CO_2 evaporation and fermentations were considered dry when the weight of a fermenter did not change for two consecutive days. This was confirmed by enzymatic sugar analyses. The wines were cold stabilised at – 4 °C for 14 days, after which the free SO_2 levels were adjusted to 35 mg L^{-1} and the wines bottled. Bottled wines were stored at + 4 °C until wine chemical analyses and wine sensory evaluation was performed approximately 3 months after the end of fermentation.

Amino acids. Three bunches per treatment replicate, were sampled randomly from the harvest crates and stored at – 20 °C. Juice samples were collected by pressing the thawed bunches by hand in a plastic bag. The pressed juice was filtered through 0.45 µm Sartorius Minisart RC 25 filters (Fischer Scientific, Johannesburg, South Africa) into vials which were capped and stored at – 20 °C prior to analyses. Amino acids quantification was performed by high performance liquid chromatography (HPLC), Agilent 1100 (Agilent Technologies,

Waldbronn, Germany) by pre-column derivatization and fluorescence detection based upon the method previously described[28] with some modifications to the derivatization, injection and gradient flow. The eluents were, 10 mM sodium tetraborate, 10 mM sodium phosphate and 5 mM sodium azide pH 8.2 (solvent A) and methanol:acetonitrile:water 45:45:10 (v:v) (solvent B). The following binary gradient system at flow rate 1.5 mL min^{-1} was employed: 0-0.5 min (2% B); 0.5-10.7 min (27.5% B); 10.7-12.7 min (27.5% B); 12.7-17.1 min (38.5% B); 17.1-20.8 min (57% B); 20.8-20.9 min (100% B); 20.9- 24.0 min (100% B); 24.0-24.1 min (2% B). A Zorbax Eclipse plus C18 Rapid Resolution column (4.6 x 150 mm, 3.5 µm particle size; Agilent Technologies) operated at 40 °C was used following derivatization of the amino acids. The injection programme was changed to include the reaction with iodoacetic acid before all other derivatization steps (Table S1). Derivatization was performed using three different reagents; iodoacetic acid (Sigma Aldrich, Aston Manor, South Africa) for cysteine[29], o-phthaldialdehyde (OPA, Sigma Aldrich) for primary amino acids and fluorenylmethyloxycarbonyl chloride (Sigma Aldrich) for secondary amino acids.[28] Internal standards, norvaline (Sigma Aldrich) and sarcosine (Sigma Aldrich) at 20 mg L^{-1} concentration respectively were spiked to each sample prior to derivatization.

Esters. Esters were quantified according to the method developed[16] with some modifications as described.[20] Briefly, 10 mL of wine was spiked with a 20 µL mix of an internal standards consisting of [$^2H_3$]-ethyl butyrate at 40 mg L^{-1}, [$^2H_{11}$]-ethyl hexanoate at 20 mg L^{-1}, [$^2H_{15}$]-ethyl octanoate at 20 mg L^{-1}, [$^2H_{23}$]-ethyl dodecanoate at 4 mg L^{-1}, and [$^2H_5$]-ethyl cinnamate at 12 mg L^{-1}. A mix of isotopically labelled esters was prepared from commercial deuterated esters provided by C/D/N isotope (Pointe-Claire, Canada). A 5 mL aliquot of this wine was placed into a 20 mL head space-solid phase micro extraction (HS-SPME) vial previously filled with 1.5 g of sodium chloride (NaCl). Samples were analysed by gas chromatograpy coupled to a mass spectrometry detector (GC-MS) in selected ion monitoring mode (SIM)[16]

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). Higher alcohols were measured in a semi-quantitative way (peak area ratio, compounds/internal standard) by the same method, but with a MS-Scan mode performed simultaneously to the MS-SIM for esters.[20]

Thiols. The determinations of 3SH and 3SHA were conducted using the method with an extraction and concentration step followed by derivatization.[30] Briefly, isotopically labelled 3SH ([$^2H_2$]-3SH) and 3SHA ([$^2H_2$]-3SHA), synthetised at Auckland University, New Zealand, were added to 180 mL of wine containing 3 g L^{-1} SO_2. Polyvinylpolypyrolidone (Sigma Aldrich), at a concentration of 5 g/L, was added and the mixture was stirred for 10 min. After centrifugation at 6200 *x g* for 5 min, the supernatant was recovered and 50 g/L of sodium chloride (Merck, Modderfontein, South Africa) added and the pH was adjusted to 5 using solid calcium carbonate (Merck). Sodium borohydride (Sigma Aldrich), at a concentration of 3.84 g L^{-1}, was slowly added to the mixture followed by 110 mL of dichloromethane (Merck) after which extraction took place for 20 min with vigorous stirring. After phase separation, the dichloromethane layer was recovered, washed with 100 mL water and dried over 3 g anhydrous sodium sulphate (Sigma Aldrich). The extract was evaporated to 3 to 5 mL under vacuum and afterwards evaporation continued at 30 °C under a gentle stream of N_2 to the final volume of approximately 200 µL. During this last step, 300 µL of methanol (Merck) was added in order to replace the dichloromethane as solvent. The extracted sample was derivatized manually prior to instrumental analysis by the addition of 5 g L^{-1} OPA solution in methanol and 10 g L^{-1} of ethanolamine in 80 mM borate buffer at pH 7.3 were added to 50 µL of wine extract. The mixture was vortexed and allowed to sit for 5 min before injection. Separation was performed with a Waters Acquity ultra high pressure

liquid chromatography (UHPLC) (Waters, Milford, MA, USA) connected to a Waters Xevo triple quadrupole mass spectrometer (Waters) using a Waters Acquity UPLC BEH C18 2.1 x 100 mm, 1.7 µm particle column, fitted with a guard cartridge (Waters VanGuard C18 2.1 x 5 mm, 1.7 µm particle) and the detection was performed in multiple reaction mode (MRM).[30]

3-isobutyl-2-methoxypyrazine. The method to quantify IBMP by HS/SPME-GC/MS was used as previously described.[25, 31] An internal standard of final concentration 25 ng L^{-1} deuterated IBMP (CDN Isotopes, Pointe-Claire, QC, Canada) was added to the wine. Then 1.6 mL of wine was transferred into a 20-mL headspace vial containing 3 g of NaCl, and 6.4 mL of deionised water and 2 mL of 4M NaOH were added. The samples were homogenized with a vortex shaker and then extracted by HS-SPME. The extraction program consisted of shaking the vial at 500 rpm for 5 min at 50 °C, inserting a DVB-CAR-PDMS fibre (Supelco, Bellefonte, PA, USA) into the headspace for 30 min at 50 °C with shaking. The fibre was positioned in the injector for desorption at 250 °C for 12 min in the GC injector set in splitless mode (splitless: 1 min). The samples were thereafter analysed by GC-MS using a HP-5MS fused silica capillary column (30-m, 0.25-mm, 0.25-µm film thickness; Agilent Technologies, Little Falls, DE, USA) and a 6890 gas chromatograph coupled to a 5975C mass spectrometer (Agilent Technologies) equipped with Enhanced Chemstation version D.01.02.16 software (Agilent Technologies). The carrier gas was helium N55 with a column-head pressure of 6.7 psi. The oven temperature was programmed at 40 °C for 5 min, raised at 5 °C min^{-1} to 100 °C held 1 min, 3 °C min^{-1} to 145 °C held 3 min and 25°C min^{-1} to 250 °C. The mass spectrometer was operated in electron ionisation mode at 70 eV with SIM mode as described.[31] The method was validated in a Chardonnay wine assessing the linearity from 2 to 50 ng L^{-1} (5 points calibration curve, r^2= 0.9996), the repeatability at 20 ng L^{-1} (variation coefficient: 4.9%), the recovery at 10 ng L^{-1} (104%). LOD and LOQ were respectively calculated at 2 ng L^{-1} and 0.7 ng L^{-1}.

Wine sensory evaluation. Wine sensory analyses were performed three months after bottling. Twenty-seven wine professionals or students (44% women and 56% men) from the Stellenbosch area (South Africa) participated in the study, with the majority having professional experience in the wine industry (93%). Details of the gender, age and the profession of the participants are listed in Table S2. A sorting task associated with a description was used to characterize the differences between the wines. Samples (25 mL) per wine were presented simultaneously in a randomized order for each participant. Samples were served in covered black ISO glasses (to avoid dispersion of odorants) and coded with a random three-digit number.[32,33] Participants were first asked to smell and taste each wine, following the order of presentation, and then to sort the wines into groups on the basis of perceived similarities and differences. They were allowed to form an unlimited number of groups as needed and to sort as many wines as determined to fit into each group. After the initial smelling and tasting of the samples, the participants were allowed to smell and taste the samples as many times as needed and in any order. After they had performed the sorting task, the participants were asked to describe each group they had formed using a list of 19 aroma descriptors (Table 1) based on a list generated in a previous experiment on Sauvignon blanc.[20] Participants were asked to choose the most relevant terms from the list to describe the characteristics that define each group (a maximum of five descriptors per group). They were allowed to smell and taste the wines again but were not allowed to change the groupings.

Statistical analysis. One-way and two-way analyses of variance (ANOVA) for variables clone and defoliation were performed on the chemical data using Statistica, Version 12 (StatSoft, Tulsa, OK, USA) 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 three replicates of one treatment. Orthogonal Projection to Latent

Structures Discriminate Analysis (OPLS-DA) was conducted on the concentration profiles of volatile compounds derived from GC-MS data. Prior to OPLS-DA compound concentrations were preprocessed by Log10 scaling, mean centre and standardisation of variance using a Pareto equation. Logarithmic scaling was considered appropriate since concentrations vary by large orders of magnitude across the different compounds and scaling using the Pareto equation was used to ensure weighting of variables with large concentrations did not dominant compounds with small to medium concentrations.[34] Orthogonalisation was performed to improve model interpretation by removing variance not associated with the experimental factors by projecting the data matrix onto a vector of sample class (clone or defoliation) and removing a maximum of one latent variable prior to determining the final models. OPLS-DA was conducted using external parameter orthogonalization in the PLS toolbox version 7.8 (Eigenvector Research Inc, Wenatchee, WA, USA) within Matlab version 7.14.0.739 (The Mathworks, Natick, MA, USA).

For wine sensory evaluation, dissimilarities between samples were analysed using non-metric multidimensional scaling (MDS). MDS allows the representation of the perceptive proximities between wines on a map. The wines are represented by points which are positioned such that the distances between the pairs of points reflect as well the distances between the pair of wines. Therefore, two wines which have regularly been sorted together by the assessors are close on this representation and two wines which have rarely been sorted together are far apart. Where the MDS configuration perfectly reproduces the input data, the stress value is zero. Stress values below 0.1 are considered an excellent fit, values between 0.1 and 0.2 are an adequate fit and values above 0.2 are a poor fit.[35] Then, assuming that the terms assigned to a group of wines characterize all the wines of this group, the citation frequency of each term was computed for each wine. Only terms cited by a minimum of 6 participants per wine were considered for further analysis. Pearson correlations were

calculated between citation frequencies of each term and the coordinates of the wines per dimension of the MDS map.[36,37] These correlations constitute the coordinates of the terms in the MDS configuration and allow interpretation of the underlying dimensions that differentiate the products. In the same way, correlations were also calculated between concentrations of the chemical compounds and the coordinates of the wines on the MDS map. Finally, coordinates of samples in the MDS map were submitted to hierarchical cluster analysis to determine clusters of wines with similar characteristics and so to support the interpretation of the clusters of wines in the MDS map.[38]

RESULTS AND DISCUSSION

Vineyard characterisation and basic parameters of maturity The CELAP**,** a measure of the leaf surface exposed to the sun, for clone SB316 and SB11 control was 6.32 and 6.66 m^2 $vine^{-1}$, respectively. Defoliation resulted in decreased CELAP value of 7.6 and 7.5% for clone SB316 and SB11, respectively. High CELAP values measured indicated that the vines did not experience water constraint and nutrient deficiency and that the remaining exposed leaf area was enough to achieve the ripening of the fruit. This was further confirmed by vine visual inspection. Daily mesoclimatic maximum temperatures exceeded 30 °C only 8 times during the season. Microclimatic temperatures revealed interesting trends in the daily temperature evolution for both treatments. Temperature in the bunch zone of the control treatment was lower until 1 pm compared to temperatures in the bunch zone of the defoliated treatments. Conversely, in the afternoon, bunch zone temperatures of control treatment were higher when compared to those measured in the defoliated treatments (Figure 2). The drop in the bunch zone temperature of defoliated treatments in the afternoon is partly due to the movement of the sun on shaded back part of the canopy. However, the graduate decrease in the bunch zone temperature of defoliated treatments from 10 am onwards could be also due to the cold see breeze. The decrease in the temperature, as observed herein, resulting from the onset of the

sea breeze from the Atlantic Ocean in the beginning of the afternoon was already well reported.[20,25,39]

Defoliation did not influence the TSS values in clone SB316 at harvest, where the values were 20.5 and 20.2 °Brix for the control and defoliation treatment, respectively. However, a higher TSS concentration (22.1 °Brix) was measured in SB11 exposed compared to 19.8 °Brix in the SB11 control treatment. TA concentrations were relatively high and ranged from 8.6 and 9.9 g L^{-1} for the defoliated treatments of clones SB11 and SB316 respectively and 10.2 and 11.4 g L^{-1} for the control treatments of SB11 and SB316 respectively. Grapes grown in cooler climates generally results in higher TA concentrations, what can be also contributed to higher malic acid concentrations.[40-42]

Juice amino acids. Amino acids were analysed in the grape juice and divided into groups according to their importance for yeast metabolism during fermentation.[43] Branched amino acids where significantly influenced by clone and treatment (Table 2). Clone SB11 control was significantly higher in concentrations of all branched amino acids when compared to the defoliated treatment of the same clone and to the clone SB316. Similarly, Gregan *et al.*[44] also reported a decrease in amino acids in Sauvignon blanc grapes with defoliation. In contrast, defoliation in clone SB316 had no effect on branched amino acids in corresponding juice. Of the preferred amino acids, no significant differences between clones and treatments were observed, with the exception of glutamic acid and serine, which were higher in clone SB11 control compared to the defoliation treatment of the same clone; as well as when compared to clone SB316. Other amino acids, such as γ-aminobutric acid, proline (PRO), methionine (MET), threonine, cysteine, glycine, tyrosine (TYR), tryptophan and histidine were significantly altered by the choice of clone and the highest concentrations were measured in SB11 control. A significant variation in yeast assimilable nitrogen between nine different clones of Cabernet franc was recently reported.[45] Different managements and climatic

conditions could promote the selection of natural mutations what could result in different grape quality traits between two clones studied.

Wine chemical analyses. Wines were analyzed approximately three months after fermentation for chemical compounds partnering to the aromatic expression of Sauvignon blanc wines (Table 3). In order to clarify the overall impact of treatments on wine chemical composition, a number was assigned to each measured volatile chemical data in Table 3 and OPLS-DA was applied to the treatments and wine volatile chemical composition to observe possible trends in the data set. The first two principal components (PC) for OPLS-DA scores and loadings for variable "defoliation" are presented in Figure 3A, B, explaining 66.8% of variation in data set. A clear grouping of the treatments, irrespective of the clone can be observed according to the PC 1. The loadings directions infer that IBMP is negatively loaded on PC2 and discriminates control treatments of both clones from defoliation treatments, which is in agreement with previous measures of this compound from defoliated treatments.[20, 23] The 3SH, 3SHA, hexanol, hexyl hexanoate and some other esters are positively loaded on PC2 and are discriminating defoliated treatments from the control. The OPLS-DA scores and loadings for variable "clone" are presented in Figure 4A, B. The first two PCs represented with the model are accounting for 67.1% of variation in data set. A separation between the clones irrespective of the defoliation can be observed according the PC1, accounting for 51.9% of variation. The majority of compounds, including IBMP, HAAs, phenylethyl esters and others, were positively loaded on PC1 and discriminating clone SB11 from clone SB316.

The data highlighted by this chemometric approach are supported by the detailed results shown in Table 3. The thiols (3SH and 3SHA) concentrations were significantly influenced by clone, defoliation and the clone*defoliation interaction. Defoliation significantly increased the 3SH concentration in the corresponding wines, irrespective of the clone. Similarly,

defoliation also significantly elevated the 3SHA concentration in clone SB11, but had no effect in clone SB316. This is well supported by our previous work, where 3SH concentrations in Sauvignon blanc wines increased with defoliation treatments in the vineyard.[20] The concentrations of 3SH and its precursors were shown to be increased under the environmental stress such as ultraviolet radiation.[20, 46] For the thiols release during the fermentation also lipids, carboxylic acids, amino acids, nitrogen status of juice and glutathione concentrations play an important role.[15, 47,48] A significant effect of clone and defoliation was observed for IBMP concentrations in wines. Defoliation decreased the IBMP concentrations by 47% and 70% in clone SB11 and SB316, respectively, what is in agreement with previous works.[23-25] Higher concentrations of IBMP in wines from clone SB11 compared to SB316 could be due to the genetic variability between clones, if we consider that macro and mesoclimatic environments were similar at both instances. This findings confirms a three-fold variation in the IBMP concentrations between different Carmenere clones.[4]

Contrary to thiols and IBMP, defoliation had no effect on the total concentration of the three most odorously important groups of esters (EFFAs, HAAs and ethyl esters of branched acids), irrespective of the clone. However, some variations in individual EFFAs could be observed. Ethyl butyrate concentrations were significantly modified by the choice of clone, whereas ethyl decanoate and dodecanoate were found in higher concentrations in the SB316 defoliation treatment. A significant clone effect was observed for the HAAs, with higher concentrations found in clone SB11, particularly for phenylethyl acetate. A further important consideration is that the clone SB11 not only exhibited the highest concentrations of HAAs in the wines but also of some amino acids in the grapes. In parallel, also relative concentrations of higher alcohols in the wines were higher in SB11 clone compared to SB316 clone with the exception of phenylethanol. However, grape amino acids and wine higher alcohols are not

always positively correlated and the link is not always direct[49] as observed in the present study. On the other hand, recent works have suggested that higher levels of grape juice amino acids favor HAAs yeast biosynthesis probably by up-regulating acetyl-transferase activities.[16, 50] It is well-established that acetyl-transferase expression is the limiting factor of HAAs synthesis in yeast.[51] Therefore, the variations of HAAs concentrations between clones could be linked to the clonal differences in term of grape amino acids composition. In the present study, this up-regulation was probably more important in the production of phenylethyl acetate. The drop of the phenylethyl acetate concentration in parallel with the decrease of grape amino acids levels observed after leaf removal in clone SB11 supports our hypothesis. In contrast, defoliation of clone SB11 increased the level of hexyl acetate of which the concentrations are directly dependant on the pre-fermentative concentrations of its precursors, hexan-1-ol, hexenal, (E)-2-hexen-1-ol, and (E)-2-hexenal, which are in turn derived from lipids degradation.[19] Similar results were recently found in Sauvignon blanc wines from grapes subjected to defoliation.[20] This might indicate that increasing the light quantity at bunch level can up-regulate grape lipoxygenase acitivities. Ethyl esters of branched acids and cinnamates esters were not altered by either the selection of clone or defoliation or their interaction, with the exception of ethyl 2-methylbutyrate which was influenced by the clone effect. Besides clone, defoliation and their interaction had a significant effect on ethyl propionate concentrations in wines. Some other minor groups of esters were also monitored and exhibited variation in concentrations between clones and between treatments. Interestingly, defioliation stimulated the production of most of the long carbon chain esters (C8 to C12), regardless the alcohol group, in clone SB316. This trend might reflect a modification of yeast lipids metabolism due to grape composition variations. Such a link, between yeast fatty acids metabolism and grape composition, has been recently suggested in Sauvignon blanc wines.[20]

Wine sensory evaluation. Sensory data were subjected to hierarchical cluster analyses to indentify groups of samples as sorted by panellists (Figure 5). A clear grouping of treatments replicates existed and wines from all the clones and treatments were classified into separate groups as significantly different by the panellist. The only exception were wines from clone SB316 defoliated treatment, for which no clear grouping of replicates existed. Therefore, the first wine replicate from SB316 defoliation treatment was grouped with wines from clone SB11 defoliation treatment, whereas the second wine replicate of this treatment was grouped with SB316 control wines. Further on, sensory data were subjected to a two-dimensional MDS configuration which provided a stress value of 0.058, indicating an excellent representation of the original data[35] (Figure 6). All 19 terms presented to panellists listed in Table 1 were considered for the statistical analysis, but only terms showing a significant correlation within at least one of the dimensions of the MDS map were represented in Figure 6. Ten sensory descriptors and four out of six groups of chemical compounds were significantly correlated with one of the dimensions ($p \leq 0.10$, Table S3). Clone SB11 control wines were positively loaded on the Dimension 2 (Dim 2) together with green, asparagus, green pepper, cooked beans/peas and grassy descriptors and IBMP. In addition, tropical fruit, banana, floral and acid descriptors were negatively correlated to Dim 2 together with SB11 defoliated treatment and thiols. It is well established, that IBMP in white wines in concentrations above 2 ng L^{-1} contributes to green wine aromas.[21] High IBMP concentrations were long known to repress wine fruity nuances, whereas it was recently shown that 3SH also acts in a similar manner by repressing greenness originating from IBMP in Sauvignon blanc wines.[21, 52] Wines from SB316 control were described by the sensory panel as less green and fruitier than wines from the SB11 control and less fruity and greener than the SB11 wines from defoliation treatment, regardless of the similar IBMP concentrations. These wines were also correlated with ethyl esters of branched acids. The differentiation between the SB316

control and SB11 wines from defoliation treatment could be due to the higher concentrations of some esters and thiols in the SB11 defoliated treatment, as reported previously.[7]

This study documented the chemical and sensory variation of wines produced from two different Sauvignon blanc clones grafted on the same rootstock and grown in two neighbouring vineyards (similar mesoclimatic conditions). Furthermore, a modification in the wine chemical and sensory composition for each clone as a response to defoliation, performed before veraison, was elucidated. Significant interactions between clones and the treatments have been observed for the thiols, IBMP and some esters concentrations. Defoliation significantly decreased perceived greenness in clone SB11 and elevated fruitier aromas, whereas in clone SB316 the effect of defoliation on wine sensory perception was less noticeable regardless the decrease in IBMP concentrations. Due to the treatments which allowed to create significant microclimatic differences at the fruit zone level (e.g. fully shaded versus exposed fruit) and the choice of the site subjected to sea breeze influence, it is unlikely that replicates between vintages would have shown different results as already demonstrated.[20] Adapting Sauvignon blanc clone to the site (climate x soil) should allow, applying different strategies of canopy manipulation, producing more diverse wine styles.

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Supporting information available

Table S1. Derivatization programme for amino acid analysis.

Table S2. Frequency table of the gender, age and profession of the participants (N=27) in the wine sensory evaluation.

Table S3. Pearson's correlation coefficients (r) between descriptors and chemical compounds and dimensions of the MDS map, p-values indicate the significance of r (ns: not significant).

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FIGURES CAPTIONS

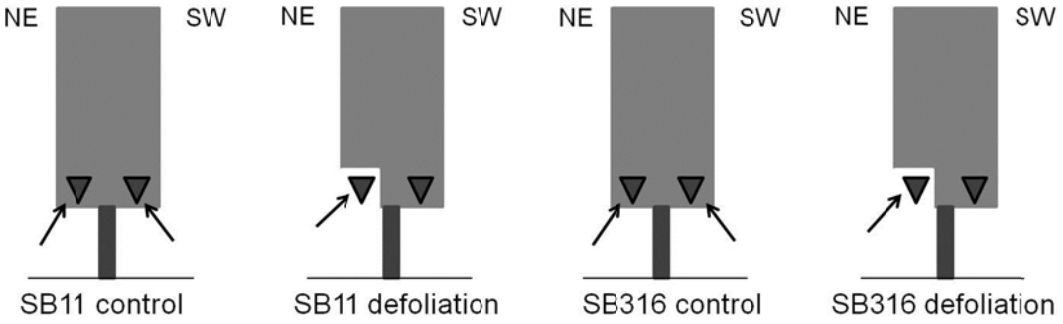

Figure 1 Schematic representation of the treatments applied in this experiment. The arrows indicate the side bunches were harvested from in each treatment, with NE indicating the Northeastern side and SW the Southwestern side of the canopy.

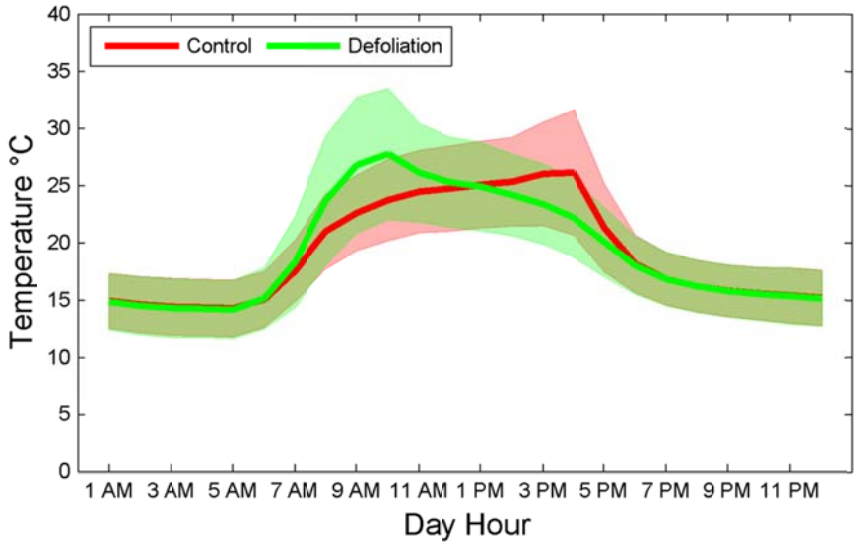

Figure 2 The mean hourly bunch temperatures from the 25 January 2013 to 25 March 2013. Shaded belt represents the standard deviation of temperatures across the monitoring period.

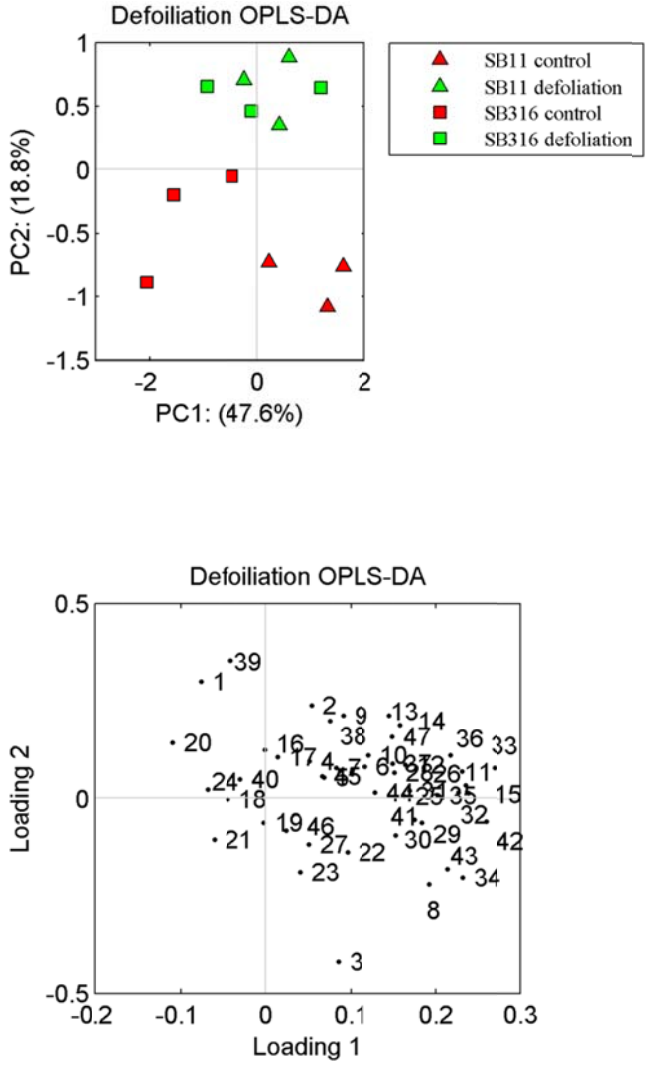

Figure 3 Orthogonal Projection to Latent Structures Discriminate Analysis (OPLS-DA) for the first two principal components for variable "defoliation" (A) scores of wine volatile chemical composition as analysed by GC-MS and (B) loadings of wine volatile chemical composition as analysed by GC-MS. Each number was assigned to a wine volatile measured and noted in Table 3.

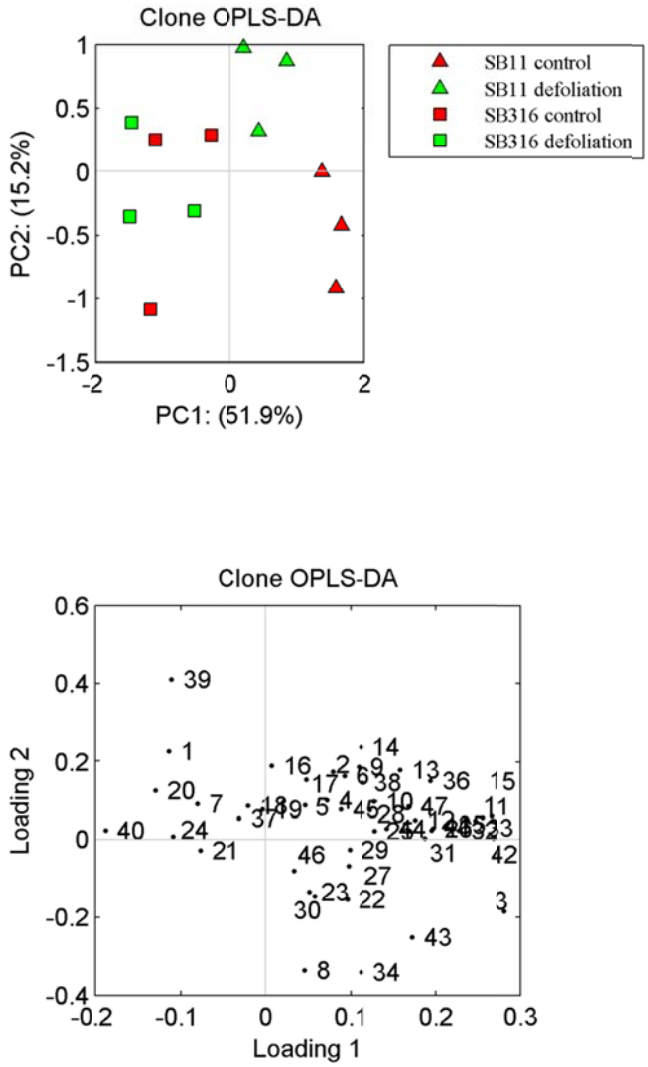

Figure 4 Orthogonal Projection to Latent Structures Discriminate Analysis (OPLS-DA) for the first two principal components variable "clone" (A) scores of wine volatile chemical composition as analysed by GC-MS and (B) loadings of wine volatile chemical composition as analysed by GC-MS. Each number was assigned to a wine volatile measured and noted in Table 3.

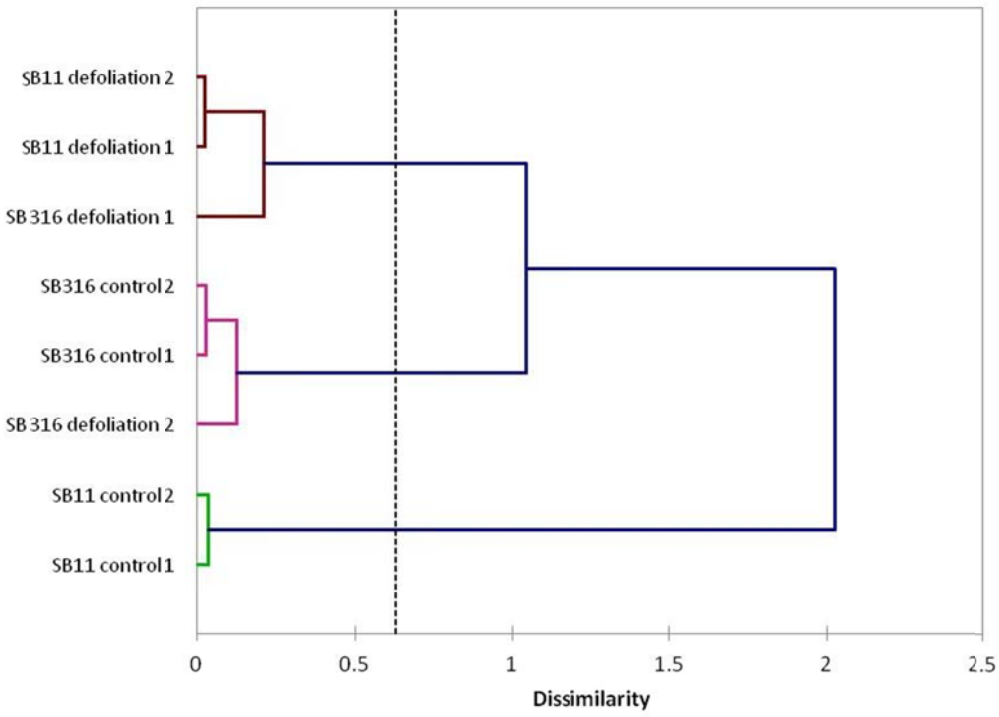

Figure 5 Hierarchical cluster analyses applied to sensory data.

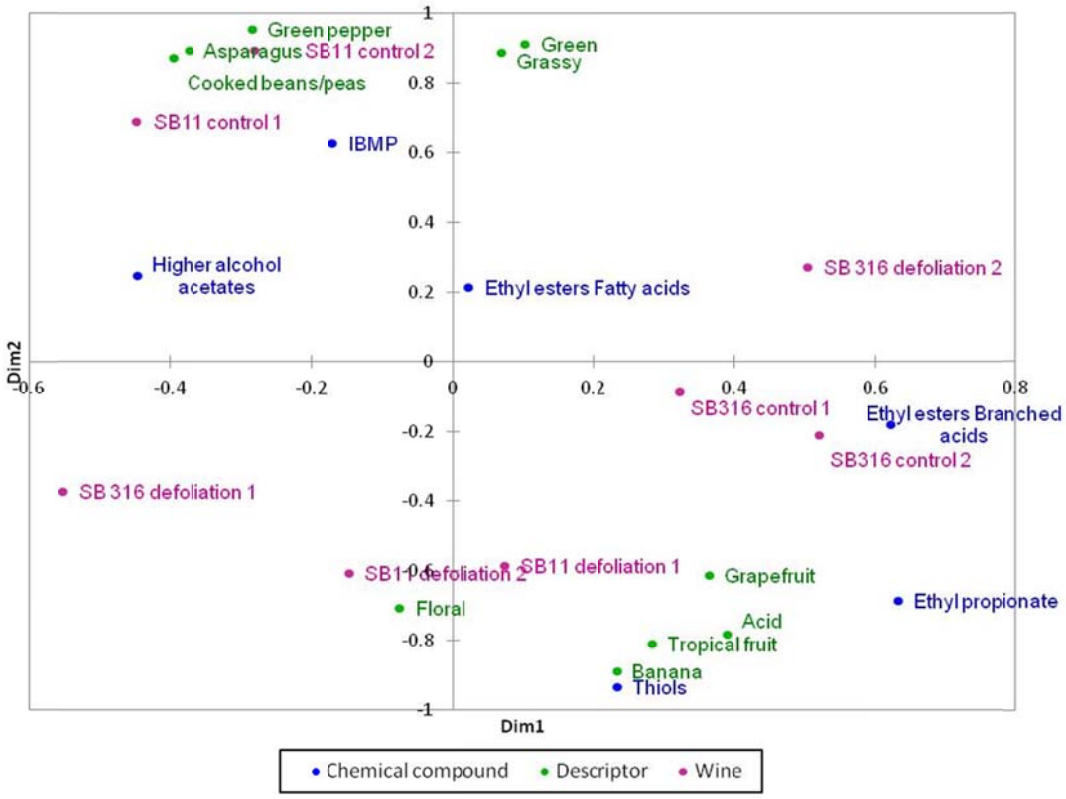

Figure 6 Two-dimensional multidimensional scaling (MDS) configuration of the eight sorted Sauvignon blanc wines (clones SB316 and SB11) and correlations of the sensory terms and chemical compounds with the dimensions.

TABLES

Table 1 List of the 19 terms used in the wine sensory evaluation verbalisation task

Green	Floral	Guava
Asparagus	Mineral	Passion fruit
Cooked beans/peas	Citrus	Pineapple
Grassy	Grapefruit	Acidic
Green pepper	Gooseberry	Bitter
Tropical fruit	Banana	Sweet
		Balanced

Table 2 Sauvignon blanc grape juice amino acids average concentrations (mg L^{-1}) at harvest as influenced by clone and treatment

	SB11 control	SB11 defoliation	SB316 control	SB316 defoliation	Clone	Defoliation	Clone*Defolitaion
	concentration[a]				p values[b]		
Yeast preferred amino acids							
Aspartic acid	17.9±6.2[a]	18.9±10.3[a]	27.2±5.8[a]	15.2±3.1[a]	0.500	0.205	0.138
Aspargine	10.3±1.6[a]	8.4±2.6[a]	6.7±2.9[a]	7.4±0.6[a]	0.097	0.643	0.33
Glutamic acid	50.5±5.0[a]	43.8±8.5[ab]	37.7±14.0[ab]	32.4±2.7[b]	**0.041**	0.269	0.892
Glutamine	252.9±23.5[a]	196.3±105.6[a]	135.9±92.7[a]	152.9±17.9[a]	0.089	0.645	0.4
Alanine	209.9±20.9[a]	163.4±40.2[a]	152.3±52.6[a]	164.8±20.1[a]	0.214	0.439	0.196
Arginine	778.5±59.1[a]	665.5±140.1[a]	578.8±189.3[a]	654.5±46.6[a]	0.178	0.801	0.223
Serine	98.0±1.6[a]	72.3±15.4[b]	60.5±19.3[b]	62.9±4.3[b]	**0.012**	0.147	0.089
Branched amino acids							
Valine	59.4±10.3[a]	35.7±10.6[b]	19.1±8.0[c]	16.3±3.5[c]	**<0.001**	**0.029**	0.068
Leucine	76.6±15.9[a]	45.3±14.6[b]	24.3±10.3[bc]	19.7±2.9[c]	**<0.001**	**0.033**	0.092
Isoleucine	46.9±10.6[a]	27.5±8.4[b]	13.6±6.2[bc]	11.2±0.9[c]	**<0.001**	**0.035**	0.082
Phenyl alanine	79.0±11.5[a]	52.6±18.5[b]	28.8±10.3[c]	16.0±4.3[c]	**<0.001**	**0.024**	0.366
Other amino acids							
Γ-aminobutric acid	403.2±38.9[a]	316.7±23.7[b]	273.5±50.5[b]	312.4±60.3[b]	**0.034**	0.391	**0.043**
Hydoxyproline	3.8±0.9[ab]	3.3±0.3[b]	4.8±0.5[a]	2.3±0.2[c]	0.84	**0.002**	**0.014**
Proline	350.2±73.7[a]	275.2±45.1[a]	241.7±93.1[ab]	145.8±15.6[b]	**0.012**	**0.049**	0.784
Methionine	20.0±3.0[a]	10.9±6.9[b]	5.9±2.8[b]	4.5±0.8[b]	**0.002**	**0.052**	0.133
Lysine	9.7±1.4[a]	8.7±1.2[a]	6.8±2.3[a]	8.7±1.0[a]	0.15	0.634	0.164
Threonine	105.0±4.4[a]	82.0±17.1[ab]	66.1±17.8[b]	75.0±8.2[b]	**0.017**	0.382	0.069
Cysteine	17.7±1.1[a]	12.9±1.5[b]	9.9±3.5[b]	11.2±1.7[b]	**0.004**	0.197	**0.037**
Glycine	8.1±0.6[a]	6.0±0.4[b]	4.6±1.5[b]	5.3±0.6[b]	**0.003**	0.216	**0.025**
Tyrosine	3.6±0.5[a]	2.6±0.0[b]	1.8±0.3[c]	1.6±0.1[c]	**<0.001**	**0.013**	**0.049**
Triptophan	30.3±7.8[a]	20.0±7.7[ab]	10.4±3.9[bc]	4.0±0.6[c]	**0.002**	0.052	0.132
Histidine	52.4±0.4[a]	39.8±14.0[ab]	24.7±12.7[b]	26.6±2.9[b]	**0.006**	0.361	0.225
Ornithine	5.8±0.6[a]	5.8±2.0[a]	4.9±2.2[a]	5.5±0.5[a]	0.483	0.773	0.728

[a] ANOVA was used to compare data. Means followed by different letters in a row are significant at $p \leq 0.05$ (Fischer's LSD). All quoted uncertainty is the standard deviation of three replicates per treatment

[b] Significance of two way ANOVA for treatment, clone and interaction treatment*clone. Bold numbers indicate significant differences

Table 3 Average concentrations of wine volatiles measured in finished wines in μg L^{-1}, unless stated otherwise, as influenced by clone and treatment with previously reported perception thresholds μg L^{-1}

Compound	Assigned number	Perception threshold	SB11 control	SB11 defoliation	SB316 control	SB316 defoliation	Clone	Defoliation	Clone*defoliation
			concentrations[a]				p values[b]		
3SH (ng/L)	1	0.060 [53]	466.55±16.47[c]	652.43±17.54[a]	581.05±10.77[b]	630.07±20.55[a]	**0.002**	**<0.001**	**<0.001**
3SHA (ng/L)	2	0.004 [54]	156.51±4.36[b]	204.43±3.60[a]	158.50±1.46[b]	143.45±17.88[b]	**<0.001**	**0.016**	**<0.001**
IBMP (ng/L)	3	0.002 [21]	19.51±1.66[a]	10.02±1.84[b]	12.45±0.81[b]	3.57±0.55[c]	**<0.001**	**<0.001**	0.703
Ethyl butyrate	4	20 [55]	648.36±22.96[ab]	658.10±26.10[a]	615.61±16.98[bc]	606.11±14.19[c]	**0.007**	0.992	0.442
Ethyl hexanoate	5	5 [55]	1713.59±26.28[a]	1710.29±27.98[a]	1669.56±106.17[a]	1750.64±47.87[a]	0.959	0.304	0.267
Ethyl octanoate	6	2 [55]	1366.93±62.25[a]	1312.04±75.68[a]	1227.72±238.69[a]	1367.28±35.51[a]	0.591	0.589	0.232
Ethyl decanoate	7	200 [56]	249.43±49.12[b]	270.81±13.98[b]	268.16±49.43[b]	389.83±59.40[a]	**0.032**	**0.028**	0.097
Ethyl dodecanoate	8	640 [57]	57.03±15.38[b]	46.29±8.78[b]	42.75±3.08[b]	91.19±29.55[a]	0.164	0.096	**0.018**
Ethyl esters of fatty acids			4035.35±140.41[a]	3997.53±71.47[a]	3823.80±407.02[a]	4205.05±99.88[a]	0.988	0.221	0.144
Propyl acetate	9		174.03±7.81[b]	190.12±5.37[a]	154.49±4.64[c]	160.16±5.78[c]	**<0.001**	**0.014**	0.172
Isobutyl acetate	10	2100 [58]	109.45±4.07[a]	107.87±2.42[a]	91.51±2.53[c]	100.06±3.08[b]	**<0.001**	0.087	**0.022**
Butyl acetate	11	1830 [58]	10.38±0.14[a]	9.90±0.59[a]	6.89±0.29[b]	6.34±1.03[b]	**<0.001**	0.186	0.927
Isoamyl acetate	12	860 [58]	7337.18±201.20[a]	7158.68±516.30[a]	5728.34±86.21[b]	6103.26±339.46[b]	**<0.001**	0.618	0.182
Hexyl acetate	13	670 [58]	436.86±16.52[b]	489.00±18.62[a]	369.65±23.52[c]	378.27±18.00[c]	**<0.001**	**0.026**	0.087
Octyl acetate	14	800 [58]	1.89±0.12[a]	2.07±0.26[a]	1.74±0.45[a]	2.15±0.31[a]	0.838	0.139	0.543
Phenylethyl acetate	15	250 [55]	740.83±45.64[a]	587.82±21.97[b]	425.69±47.54[c]	414.97±21.02[c]	**<0.001**	**0.004**	**0.009**
Higher alcohol acetates			8810.63±161.01[a]	8545.46±554.13[a]	6778.31±83.57[b]	7165.22±381.26[b]	**<0.001**	0.770	0.144

Ethyl isobutyrate	16	15 [55]	10.85±0.27[a]	11.60±0.33[a]	11.25±1.47[a]	10.60±0.45[a]	0.529	0.919	0.167
Ethyl 2-methylbutyrate	17	1 [55]	1.07±0.03[a]	1.11±0.03[a]	1.05±0.08[ab]	0.98±0.04[b]	**0.033**	0.468	0.076
Ethyl isovalerate	18	3 [56]	1.70±0.03[a]	1.68±0.08[a]	1.75±0.15[a]	1.61±0.09[a]	0.851	0.198	0.262
Ethyl Phenylacetate	19	73 [59]	0.34±0.03[a]	0.30±0.02[a]	0.34±0.07[a]	0.32±0.02[a]	0.706	0.317	0.711
Ethyl esters of branched			13.96±0.23[a]	14.69±0.46[a]	14.39±1.76[a]	13.50±0.56[a]	0.515	0.893	0.179
Ethyl propionate	20	2100 [58]	129.32±2.64[b]	150.13±7.14[a]	153.56±4.31[a]	153.47±3.22[a]	**<0.001**	**0.005**	**0.005**
Ethyl dihydrocinnamate	21	1.6 [56]	0.50±0.02[ab]	0.48±0.01[b]	0.55±0.04[a]	0.53±0.01[a]	**0.009**	0.202	0.589
Ethyl cinnamate	22	1.1 [56]	0.17±0.01[a]	0.16±0.05[a]	0.15±0.01[a]	0.15±0.02[a]	0.707	0.317	0.717
Cinnamates			0.67±0.03[a]	0.63±0.05[a]	0.70±0.04[a]	0.68±0.02[a]	0.132	0.262	0.541
Methyl butyrate	23		0.80±0.04[a]	0.64±0.10[b]	0.66±0.02[b]	0.70±0.06[b]	0.301	0.145	**0.024**
Methyl hexanoate	24		1.89±0.06[c]	1.97±0.09[c]	2.09±0.05[b]	2.25±0.04[a]	**<0.001**	**0.014**	0.331
Methyl octanoate	25		1.28±0.10[ab]	1.19±0.09[ab]	1.09±0.13[b]	1.33±0.15[a]	0.732	0.338	0.050
Methyl decanoate	26		0.29±0.03[a]	0.27±0.03[a]	0.22±0.04[a]	0.30±0.08[a]	0.548	0.298	0.101
Methyl esters			4.25±0.18[ab]	4.06±0.11[b]	4.06±0.23[b]	4.57±0.21[a]	0.179	0.174	0.012
Ethyl valerate	27		1.37±0.12[a]	1.26±0.09[ab]	1.24±0.04[ab]	1.08±0.09[b]	**0.016**	**0.032**	0.727
Ethyl heptanoate	28		0.21±0.01[a]	0.20±0.01[ab]	0.18±0.02[b]	0.22±0.01[a]	0.323	**0.078**	0.012
Ethyl nonanoate	29		0.19±0.03[a]	0.17±0.01[a]	0.17±0.04[a]	0.24±0.06[a]	0.426	0.295	0.100
Ethyl undecanoate	30		0.04±0.01[ab]	0.03±0.01[ab]	0.03±0.00[b]	0.04±0.01[a]	0.547	0.174	0.072
Ethyl esters of odd carbon number fatty acids			1.81±0.16[a]	1.66±0.10[ab]	1.61±0.06[b]	1.58±0.04[b]	**0.037**	0.168	0.344
Isobutyl butyrate	31		0.13±0.01[a]	0.11±0.01[a]	0.09±0.00[b]	0.10±0.01[b]	**<0.001**	0.499	0.121
Isobutyl hexanoate	32		0.15±0.02[a]	0.13±0.01[ab]	0.11±0.01[c]	0.11±0.01[bc]	**0.002**	0.563	0.115

Isobutyl octanoate	33		0.14±0.01[a]	0.13±0.01[ab]	0.09±0.01[b]	0.12±0.04[ab]	0.093	0.324	0.147
Isobutyl decanoate	34		0.10±0.03[ab]	0.08±0.01[b]	0.06±0.01[b]	0.14±0.05[a]	0.333	0.132	**0.020**
Isobutyl esters			0.51±0.07[a]	0.45±0.03[ab]	0.35±0.03[b]	0.48±0.09[a]	0.079	0.345	**0.027**
Isoamyl butyrate	35		0.80±0.03[a]	0.69±0.01[b]	0.57±0.06[c]	0.59±0.02[c]	**<0.001**	0.069	**0.010**
Isoamyl hexanoate	36		1.06±0.07[a]	1.02±0.09[a]	0.81±0.17[a]	1.00±0.16[a]	0.117	0.358	0.178
Isoamyl octanoate	37	125 [56]	1.88±0.41[b]	2.07±0.16[b]	1.86±0.27[b]	3.06±0.69[a]	0.086	**0.023**	0.073
Isoamyl esters			3.74±0.50[ab]	3.78±0.14[ab]	3.23±0.49[b]	4.65±0.85[a]	0.588	0.054	0.064
Hexyl butyrate	38		0.01±0.00[ab]	0.02±0.00[a]	0.01±0.00[ab]	0.01±0.00[b]	**0.042**	0.831	0.074
Hexyl hexanoate	39		0.00±0.00[ab]	0.01±0.00[b]	0.01±0.00[ab]	0.01±0.00[a]	0.309	**0.034**	0.693
Hexyl octanoate	40		0.02±0.00[b]	0.02±0.00[b]	0.02±0.00[b]	0.04±0.00[a]	0.003	**0.005**	0.033
Hexyl esters			0.036±0.004[b]	0.044±0.003[b]	0.041±0.005[b]	0.057±0.004[a]	**0.005**	**<0.001**	0.086
Phenylethyl butyrate	41		2.31±0.08[a]	1.93±0.04[b]	1.74±0.20[ab]	1.66±0.06[b]	**<0.001**	**0.008**	0.055
Phenylethyl hexanoate	42		0.88±0.05[a]	0.67±0.09[b]	0.55±0.14[b]	0.56±0.12[b]	**0.007**	0.132	0.111
Phenylethyl octanoate	43		0.20±0.02[a]	0.16±0.03[a]	0.15±0.02[a]	0.21±0.10[a]	0.971	0.706	0.159
Phenylethyl esters			3.39±0.06[a]	2.76±0.07[b]	2.44±0.34[b]	2.43±0.27[b]	**<0.001**	**0.035**	**0.042**
Isobutanol[c]	44	40000	11.77±0.58[a]	10.86±0.04[b]	9.52±0.16[c]	9.93±0.45[c]	**<0.001**	0.278	**0.016**
Isoamyl alcohol[c]	45		360.95±3.03[a]	352.24±8.46[ab]	331.72±10.71[bc]	328.25±19.55[c]	**0.005**	0.406	0.715
Phenylethanol[c]	46	14000 [56]	18.81±2.42[a]	17.83±1.04[a]	17.87±0.83[a]	17.62±1.25[a]	0.523	0.502	0.687
Hexanol[c]	47	8000 [55]	190.18±9.05[a]	208.97±15.37[a]	158.26±8.87[b]	158.51±14.84[b]	**<0.001**	0.221	0.232
Higher alcohols[c]			581.71±8.41[a]	589.89±21.56[a]	517.37±10.21[b]	514.30±12.18[b]	**<0.001**	0.760	0.507

[a] ANOVA was used to compare data. Means followed by different letters in a row are significant at $p \leq 0.05$ (Fischer's LSD). All quoted uncertainty is the standard deviation of three replicates of one treatment

[b] Significance of two way ANOVA for treatment, clone and interaction treatment*clone. Bold numbers indicate significant differences.

[c] indicates compounds where semi-quantitative data are shown, showing a peak area ratio.

[53, 54] model wine solution (12% ethanol and 5 g L^{-1} tartaric acid, pH 3.2); [21] white wine; [55] model wine solution (10:90, ethanol/water, w:w); [56] model wine solution (11:89 ethanol/water, v:v,7 g L^{-1} glycerine, 5 g L^{-1} tartaric acid, pH 3.4); [57] model wine (9.5:90.5 ethanol/water w:w); [58] dearomatised red wine; [59] red wine.