

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

Katja ŠUKLJE<sup>1</sup>, Helena BAŠA ČESNIK<sup>1</sup>, Lucija JANEŠ<sup>1</sup>, Veronika KMECL<sup>1</sup>,  
Andreja VANZO<sup>1</sup>, Alain DELOIRE<sup>2,3</sup>, Paolo SIVILOTTI<sup>4</sup> and Klemen LISJAK<sup>1\*</sup>

1: Agricultural Institute of Slovenia, Central Laboratories, Hacquetova ulica 17, 1000 Ljubljana, Slovenia

2: Stellenbosch University, Department of Viticulture and Oenology, Private Bag X1, Matieland 7602, South Africa

3: NWGIC, Charles Sturt University, Boorooma Street, Wagga Wagga, NSW, Australia, 2650

4: University of Nova Gorica, Wine Research Center, Vipavska 13, 5000 Nova Gorica, Slovenia

## 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 methoxy-pyrazines 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  $\beta$ -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, methoxy-pyrazines, 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 méthoxy-pyrazines 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  $\beta$ -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, méthoxy-pyrazines, 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<sup>2</sup>/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-sulfanylhhexyl 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<sub>13</sub>-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

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<sup>2</sup>) 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<sub>2</sub> 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<sub>4</sub><sup>+</sup> 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 (β-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<sub>2</sub> 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<sub>2</sub> 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), [<sup>2</sup>H<sub>2</sub>]-3-sulfanylhexyl acetate (d3SHA) and [<sup>2</sup>H<sub>2</sub>]-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 ≤ 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<sup>2</sup>/kg, as seen in

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 m<sup>2</sup> 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	m <sup>2</sup> leaf area/kg fruit	CELAP (m <sup>2</sup> /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	16 Aug 11	23 Aug 11	30 Aug 11
	<i>véraison</i>			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).

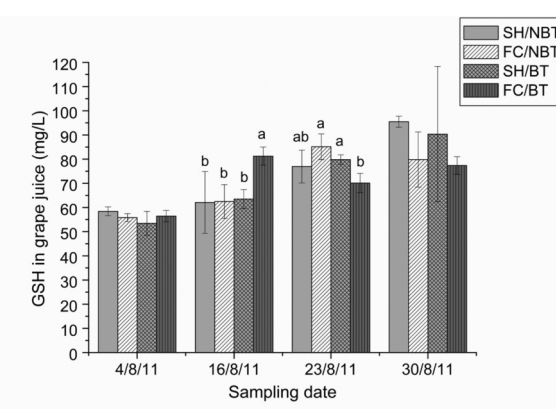
first and second sampling date in the SH/NBT treatment, but there was no significant difference among the treatments at harvest. In addition, shoot hedging and bunch thinning had no significant effect on pH value during the grape maturation.

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).**

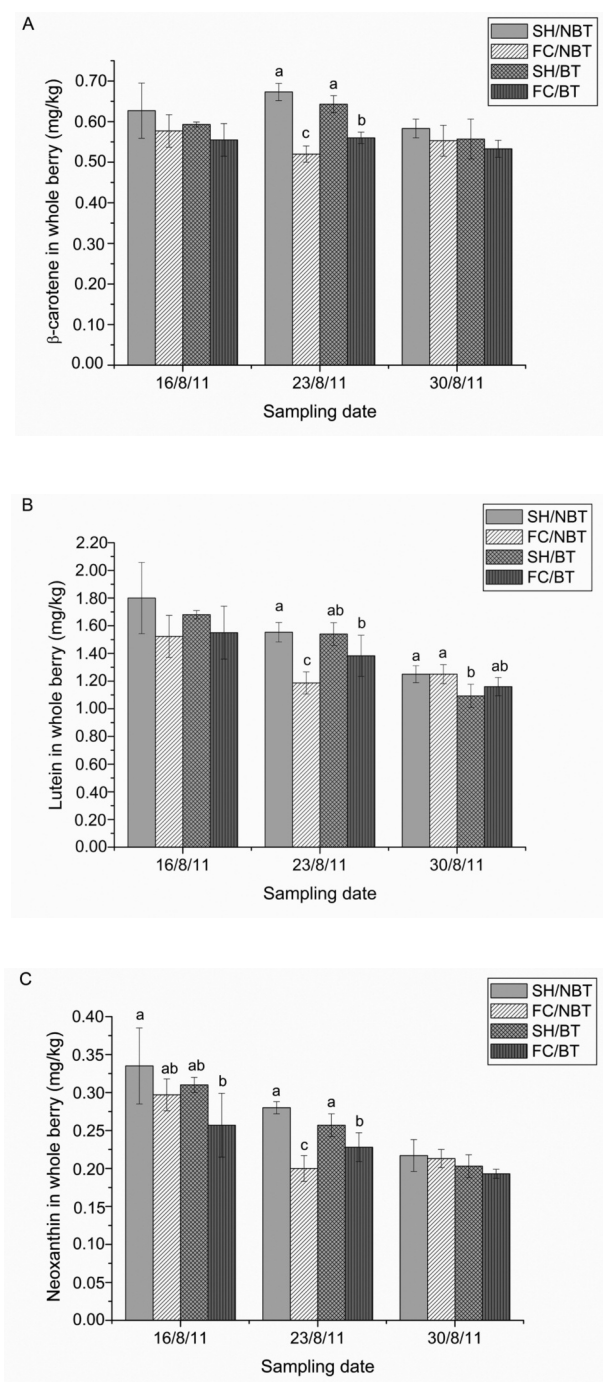
methoxy pyrazine (IPMP) concentration was already below the LD at the first sampling date. The IBMP concentration depends largely on temperature and bunch exposure to light (BELANCIC and AGOSIN, 2007; SCHEINER *et al.*, 2010). The fast decrease in IBMP concentration in our study was probably related 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 <i>véraison</i>	16 Aug 11	23 Aug 11	30 Aug 11 harvest
<i>trans</i> -caftaric acid (mg/L expressed as <i>trans</i> -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
<i>cis</i> -caftaric acid (mg/L expressed as <i>trans</i> -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
<i>trans</i> -coutaric (mg/L expressed as <i>trans</i> -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
<i>cis</i> -coutaric (mg/L expressed as <i>trans</i> -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
<i>SUM</i> (mg/L expressed as <i>trans</i> -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*-ferric 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  $\beta$ -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  $171 \pm 43$  mg N/L and  $164 \pm 36$  mg N/L for SH/NBT and FC/NBT, respectively, and between  $122 \pm 28$  mg N/L and  $114 \pm 15$  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  $\beta$ -carotene and neoxanthin at harvest (Figure 2). The concentration of lutein at harvest was significantly lower in the treatments with bunch thinning.  $\beta$ -carotene and lutein were the predominant carotenoids in grape berry. With the exception of  $\beta$ -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  $\beta$ -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.

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

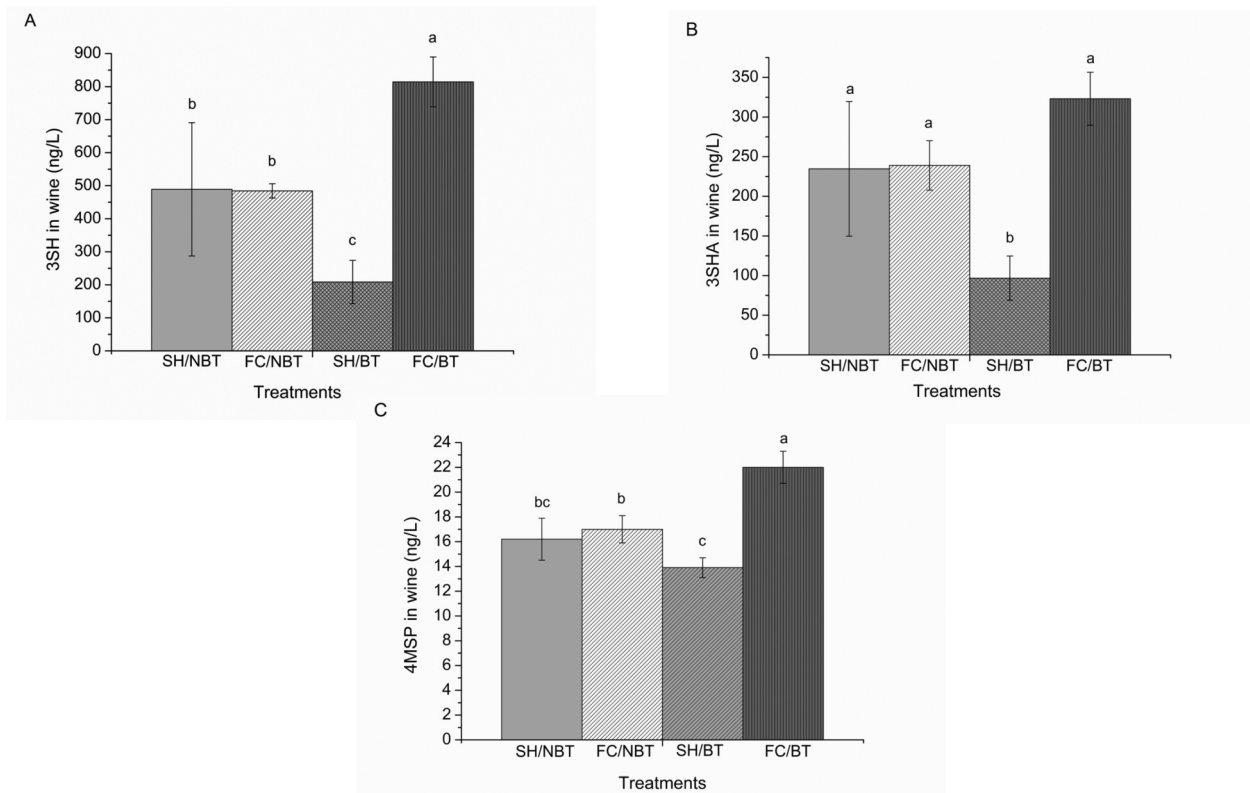
differ significantly between the other three treatments (Table 5) and were slightly higher than the values reported by DU TOIT *et al.* (2007), who found levels of up to 35 mg/L.

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.**

Treatments	Must (before yeast inoculation)					Wine (4 months after bottling)
	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.

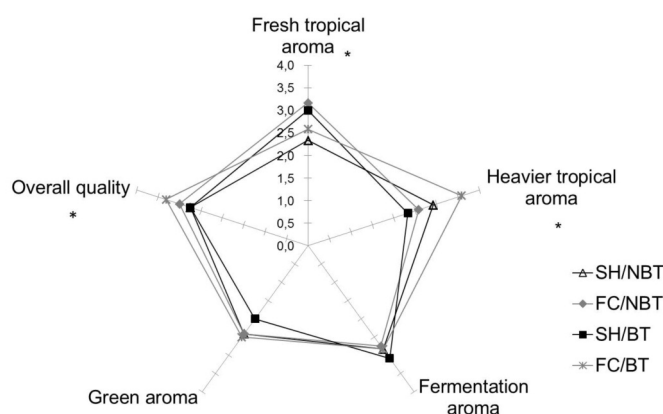
The tasting panel confirmed the heavier tropical aroma of Sauvignon blanc in the FC/BT wines, as revealed by the higher concentration of 4MSP and 3SH found in this treatment (Figure 4), whereas the perception of the heavier tropical aroma was the lowest in the wines from SH/BT treatment.

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).**

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<sup>2</sup>/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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