The reduction of plant sink/source does not systematically improve the metabolic composition of Vitis vinifera white fruit

Hubert Alem, Hernán Ojeda, Peggy Rigou, Rémi Schneider, Laurent
Torregrosa

HAL Id: hal-03254781
https://hal.inrae.fr/hal-03254781v1

Submitted on 3 Feb 2023

The reduction of plant sink/source does not systematically improve the metabolic composition of *Vitis vinifera* white fruit.

Hubert Alem[1,2], Hernán Ojeda[1], Peggy Rigou[3], Rémi Schneider[4], Laurent Torregrosa[1,5*]

[1]*UE INRAe de Pech Rouge, University of Montpellier, INRAe, 11430, Gruissan, France.*

[2]*Cátedra de Fruticultura, Facultad de Agronomía, Av. San Martín 4453, C1417DSE CABA, Argentina.*

[3]*SPO, University of Montpellier, CIRAD, INRAe, Institut Agro, 2, place P. Viala, 34060 Montpellier Cedex, France.*

[4]*Oenobrands SAS, 2196 bd Lironde, 34980 Montferrier Sur Lez, France.*

[5]*AGAP, University of Montpellier, CIRAD, INRAe, Institut Agro, 2, place P. Viala, 34060 Montpellier Cedex, France.*

Corresponding author: *laurent.torregrosa@supagro.fr*

Abstract

The quality of wine grapes depends on the balance between primary and secondary metabolites. Unlike many perennial crops that accumulate starch in the fruits before ripening, the non-climacteric grapes ripe with no previous carbon reserves. Based on the assumption that fruit carbon sink is limiting metabolite accumulation in grapes, bunch thinning is performed to limit plant Sink/Source (S/S). We studied the effects of severe bunch thinning on the accumulation of primary metabolites and on four families of glycosylated aroma precursors (GAPs) at the arrest of fruit phloem unloading of two white grape *Vitis vinifera* cvs. At plant level, crop reduction resulted in significant losses of metabolites to be accumulated in the fruits: i.e. up to 72% for sugars, 75% for organic acids and GAPs. Nevertheless, S/S manipulation could not modify the balance between GAPs and primary metabolites or increase the concentration in GAPs in the physiologically ripe grape.

Keywords: Fleshy fruit, grapevine, primary metabolites, glycosylated aroma precursors, bunch thinning.

1

1. Introduction

Vitis vinifera grape is a non-climacteric fleshy fruit. During the first phase of development, berries accumulate organic acids which represent the main contributors of the osmotic potential of the fruit. At the end of the first phase of growth, phloem unloading shifts from the symplasmic to the apoplasmic pathway, triggering a sudden acceleration of sugar import associated with a second phase of growth. When phloem unloading stops, grapevine berries display their maximum volume and quantity of solutes per fruit (Bigard, Romieu, Sire, Veyret, Ojeda & Torregrosa, 2019). During grape ripening, the concentrations of metabolites change as a function of the balance between biosynthesis and metabolization and growth-related dilution effects (Bigard et al., 2018). A range of metabolites and inorganic solutes determines the quality of the white wine grape, such as sugars, organic anions (i.e. tartaric and malic acids), cations (potassium) and a range of aroma compounds.

During *V. vinifera* fruit ripening, hexoses are accumulated up to 1 mol/l with glucose/fructose around 1 and only traces of sucrose. Malic acid is metabolized and diluted, while tartaric acid concentration only depends on fruit expansion (Bigard et al., 2019). Within the secondary metabolites, terpenes, C_{13}-norisoprenoids, phenols and non-terpenic alcohols are the most abundant grape aroma compounds. Non-volatile glycosylated aroma precursors (GAPs) represent 80-90% of the aroma potential in *V. vinifera* fruits (Razungles, Gunata, Pinatel, Baumes & Bayonove, 1993). Aglycones that are released during wine processing or their derivatives determine the final wine aroma profile. Depending on their molecular structure, aglycon hydrolysis may take a few months for terpenes to longer periods for C_{13}-norisoprenoids for instance (Parker, Capne, Francis & Herderich, 2018). In *V. vinifera,* only a few grape varieties are considered as aromatic, i.e. containing free aromas directly perceivable from the fresh fruit or juice. Sauvignon Blanc and Muscat varieties are the best examples for wine grape varieties. Indeed, Sauvignon Blanc accumulates high levels of methoxypyrazines that confer asparagus and bell pepper flavors (Allen & Lacey, 1998), whereas Muscat grapes contain high levels of free monoterpenols, mainly linalol, nerol and geraniol responsible of a pronounced aroma of rose (Gunata, Bayonove, Baumes &

2

Cordonnier, 1985). Most of white wine grapes are described as neutral since they only present small amounts of free aromas. Chardonnay, the most planted white wine grape variety in the world, exhibits most aromatic compounds in the form of glycosides of monoterpenols and C_{13}-norisoprenoids (Schneider, Charrier, Moutounet & Baumes, 2004).

A wide range of practices have been developed to regulate vine development or to manipulate grape microclimate, with the objective to increase the accumulation of metabolites of interest in the ripe fruit (Alem, Rigou, Schneider, Ojeda & Torregrosa, 2018). The level of carbon assimilated during the season is directly dependent on climatic characteristics, i.e. light radiation and temperature (Greer & Weedon, 2012) and vapor pressure deficit (VPD). As for other perennial species, the balance between carbon sinks and sources (S/S), i.e. crop load versus vegetative organ development, has to be regulated to ensure the sustainability of the crop production. However, the regulation of fruit load is complicated as it depends on environmental conditions of the previous year but also seasonal factors. During the season, plant sink/source can be regulated by shoot (Bernizzoni, Civardi, Van Zeller, Gatti & Poni, 2011) or bunch thinning (Gil et al., 2013), shoot tipping and leaf removal (Reynolds, 1989). The effects of these practices on fruit composition are variable depending on the year, the variety and the site of experimentation (Alem et al., 2018). In other perennial crops, fruit thinning is widely-used to regulate trophic competition with the main goal to get larger or better fruits (Link, 2000; Belhassine et al., 2019).

In grapevine, most previous studies about the effect of bunch thinning on fruit development were only based on the monitoring of the concentrations of metabolites. However, in the absence of consistent physiological landmarks, concentration and accumulation effects were often confused. Indeed, after phloem unloading stops at physiological ripening completion, fruit shriveling becomes the major driver of solute concentrations (Bigard et al., 2019). Moreover, in most previous studies, the effect of S/S on the quantity of metabolites accumulated at plant level has not been assessed which prevents the quantitative estimation of the losses of metabolites induced by thinning practices. Finally, few studies addressed the accumulation of glycosylated aroma precursors

3

(GAPs), a family of compounds which, due to their sugar moieties, potentially dependent on plant trophic balance. To characterize the metabolic variations induced by the manipulation of S/S, we have studied the effect of severe bunch thinning on the accumulation of primary metabolites and GAPs at the arrest of solute imports in the grapevine fruit for an aromatic and a non-aromatic white wine grape variety.

2. Materials and methods

2.1. Plant material and growing conditions

Experiments were performed during the period 2015-2017 at the INRAe Centre of Pech Rouge, South of France (43°8'35.180" N, 3°7'57.442" E) with two varieties of *V. vinifera*: cv. Chardonnay planted in 2000 and cv. Muscat à Petits Grains planted in 1997, both varieties grafted with 140 Ruggeri. These 2 genotypes were selected because of their different origins and biological behaviors (http://plantgrape.plantnet-project.org/en). Chardonnay, originated from Burgundy region (France) is described as an early ripening variety with a low vegetative vigor and yield (small bunches and berries) and moderate levels of sugar in the ripe fruits. Muscat à Petit Grains, originated from Greece, is described as a mid-season ripening variety with a moderate vegetative vigor and yield (mid-size berries) and high level of sugar in the ripe fruits. Chardonnay and Muscat are known to present different levels of GAPs in the fruits and contrasted distributions of GAP aglycons (Agosin et al., 2000). Experimental blocks were drip-irrigated and managed through standard cultivation practices, i.e. weed control by tilling and canopy management by vertical shoot positioning. Petiolar analyses carried out in 2014 in both plots did not show any mineral deficiency for either macro and micro elements (data not shown). NPK (60 kg/ha of N, 60 kg/ha of P and 60 kg ha of K) and urea (92 kg/ha of N) fertilizers were supplied to both plots in 2016 and 2017 respectively. Watering was managed to avoid severe water deficit maintaining leaf water potential (ψ_b)> -0.6 MPa. Temperature, rainfall and PET (potential evapo-transpiration) were recorded during all crop cycles (**Figure S1**).

2.2. S/S treatments and determination of the fruit sampling date

4

Each variety was experimented in separated plots; cv. Chardonnay was studied in 2015 and 2016 and cv. Muscat à Petit Grains in 2016 and 2017. Three rows from the border of the experimental plot and 3 plants from the beginning of each row were excluded from the experiments to avoid border-effects. For each treatment, 3 blocks of 3 plants were randomly selected. The controls corresponded to the plots managed through standard practices to target a production of 6.0-9.0 t/ha fresh grapes (1.5-2.5 kg/vine). This yield corresponds to the production threshold allowed by local PDO (Protected Designation of Origin) and PGI (Protected Geographical Indication) regulations. To obtain lower S/S than the control, bunches were removed at the phenological stage of pea size (Low 1) or at the onset of fruit ripening (Low 2) to 50% in 2015 and 2017 and 70 % in 2016. S/S balances were estimated through the calculation of the Ravaz index (Ravaz, 1912), i.e. fresh fruit weight to fresh winter pruning biomass ratio. Fruit sampling strategy was designed to target the end of phloem unloading, a transitory stage when berry volume reaches a maximum (Bigard et al., 2019). For each variety and year, from 2-3 weeks after the onset of ripening, the volume of 2 reference bunches was weekly and non-destructively monitored by Archimedes' method (Torregrosa, Pradal, Souquet, Rambert, Gunata & Tesniere, 2008). This allowed the anticipation of the slowing down of berry growth (Bigard et al., 2018) for a precise determination of the time of the maximum fruit contents in water and solutes. The date of sampling was then adjusted for variety and year (**Table S2**). At the sampling date, all the fruits of each repetition by treatment were collected separately. Then, within each batch, 200 berries were randomly sampled for primary metabolite analysis and the rest were stored at -20 ° C until GAPs analysis.

2.3. Primary metabolites

Each sample was grinded at room temperature with a domestic blender (Waring, New Hartford, USA) for 2 minutes. An aliquot of 2 ml of the clear juice was prepared and analysed for main sugars (glucose and fructose) and organic acids (tartaric and malic acids). Sugars and acids were quantified by high-performance liquid chromatography using a Bio-Rad® Aminex HPX-87H column (Bio-Rad, USA) as described in Bigard et al. (2019). The contents in primary metabolites

were expressed in mass, moles or equivalents of moles of carbon (C) per volume, organ or plant, considering hexoses and organic acids with 6 and 4 atoms of carbon respectively.

2.4. GAPs

GAPs were quantified according to the method described in Schneider, Razungles, Augier & Baumes, 2001. Briefly, 500g of defrosted berries (one night at 4°C) were crushed with a domestic blender (same type as mentioned above) at room temperature and centrifuged at 7000 rpm (20 min, 10°C). Three hundred and fifty ml of clear supernatant were sampled and added with 17.5 g of polyvinylpolypyrrolidone (PVPP, Supelco, France, 100 µm size particle). After filtration, the sample was aliquoted in 3x100 ml to constitute analytical triplicates. Glycosidic fraction was extracted using 500 mg C18 cartridges (Strata, Phenomenex, France). The bound glycosidic fraction was recovered by a final elution with 10 ml methanol (Sigma-Aldrich France, HPLC grade). The glycosidic fraction was dried with air flux in a water bath (40°C), and then hydrolysed in a phosphate/citrate buffer (sodium hydrogen phosphate 0.2 M, citric acid 0.1 M, pH 5.0) using a glycosidase enzyme preparation (Rapidase Revelation Aroma, Oenobrands, France). The aglycons released were then extracted using pentane/dichloromethane (2/1; v/v) (Sigma-Aldrich, France, >99,9% GC and HPLC PLUS grade respectively).

After concentration and addition of 200 µl of 4-nonanol (16mg/l, Sigma-Aldric, France, >96,5% GC) as internal standard, aglycone extract was analyzed with a Hewlett-Packard (HP) 5890 Series II GC system coupled to a HP 5989 A MS. The samples were injected in splitless mode (injector port temperature 245°C; purge on time 0.5-min) onto a DB-Wax column [30 m × 0.25 mm id, 0.25 µm film thickness (Agilent Technologies, USA)]. Compounds were separated using helium carrier gas at 1 ml/min. The temperature program started with an isotherm at 60°C for 3 min. The temperature of the oven was then raised by 3°C/min to 245°C and held for 10 min. The transfer line was held at 250°C, and compounds were detected with the source held at 150°C by ionisation by electronic impact generated at 70 eV. Full scan mass spectra were recorded between 29 and 350 m/z. Data was acquired and treated with the HP 5989 B.05.02 MS Chemstation. The compounds

6

were identified using NIST library and our own library. They were semi-quantified using 4-nonanol as an internal standard, and classified into 4 families: terpenes, alcohols, phenols and norisoprenoids (**Table S3**).

GAPs were expressed in mass, moles and equivalents of moles of C per volume, organ or plant. To convert GAPs into equivalents of moles of C, the molecular mass of each type of GAPs was weighted according to their aglycone and glycoside structure. For GAPs displaying both mono- and di-glycosylated structures, an average of the number of C atoms per molecule was used. All the calculations were performed at the molecule and molecule's familie level: alcohols, C_{13}-norisoprenoids, phenols and terpenes.

2.5. Data analyses and graphic representations - Experiments were carried on a randomized block design, with three repetitions for each treatment. All statistical analyses were performed using the INFOSTAT® (University of Cordoba, Argentina) software package. The data was subjected to analysis of variance (ANOVA). Mean comparisons were performed using Fisher's least significant difference (LSD) test and significance was set at $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***). Principal component analysis (PCA) was performed with variables expressed in concentration in the fruit and the quantity accumulated per plant.

3. Results and discussion

3.1. Plant growth and yield

Previous reports on the effects of the source/sink balance on grapevine development have not shown a clear threshold but a gradual effect whose scaling depended on complex interactions between pedo-climatic factors and cultivation practices. In this study, control plots have been set to reach the maximum yield allowed by local regulations. Then severe bunch thinning was performed during 2 years at 2 dates of the crop cycle for each variety. In general, the rate of bunch thinning in wine grape management is around 30-50%. Therefore, we have experimented the effect of 50% bunch thinning in 2015 for cv. Chardonnay and in 2017 for cv. Muscat à Petit Grains and performed

a more severe bunch thinning (70%) in 2016 for both varieties. This allowed the study of the response of the plants grown under a very comfortable S/S scenario.

Genotypic differences in the fruit development were obvious with a higher yield (**Table 1**) and an earlier ripening in Muscat than in Chardonnay, as shown in 2016, the common year of experimentation (**Table S2**). Practices to regulate vine development of the controls reduced inter-annual fluctuations of yield and canopy biomass but without completely normalizing them (**Table 1**, **Figure S4A and B**). Plant developmental fluctuations depend on environmental conditions, i.e. temperature, light and water regime which regulate bud fruitfulness, flowering set, fruit and shoot development. Environmental fluctuations are one of the main limitations when performing in-field experiments because of Genotype x Environment interactions (GxE). According to the classification of the wine growing regions, the climatic conditions during experiments corresponded to a Mediterranean hot and semi-arid region. Both indices, Huglin (1978) and PET were rather stable during the 3 years of experiment (**Figure S1, Table S2**), with slight temperature and rainfall fluctuations. Indeed, in the summer of 2015, temperature and rainfall were significantly higher than in 2016 and 2017.

Whatever the date and intensity of the treatment, bunch thinning resulted in a strong reduction of fruit yield (**Table1**, **Figure S4A**). Actually, in Chardonnay, in relation to the controls, fruit yields in Low 1 and Low 2 treatments decreased by 46% and 68% and 37% and 64% in 2015 and 2016 respectively. These crop reductions were in line with the intensity of thinning, i.e. 50% in 2015 and 70% in 2016. In Muscat, fruit yields in Low 1 and Low 2 treatments decreased respectively by 60% and 70% in 2016, and 60% and 59% in 2017, in comparison with the controls. This yield reduction beyond the level expected in 2017 for Muscat could result from an interaction between the treatment and the year and its effects on other components of the yield, such as the number of berries per bunch, that the experimental design does not allow to be explored. In any case, during the 2 years of experimentation on Muscat, bunch thinning resulted in contrasting S/S in comparison with the controls whatever the date of treatment (**Table1**, **Figure S4C**).

8

Whatever the treatment, Chardonnay displayed smaller berries (1.24 +/- 0.06 ml) than Muscat (1.87 +/- 0.16 ml). The effect of S/S on the berry volume was found statistically significant only in Chardonnay (data not shown), but with little variations (from +4.1 to +7.9 %) and no consistency between the dates of bunch thinning. These data indicate that crop reduction has not induced significant compensation effects on berry growth. Several authors (Carbonneau, Leclair, Dumartin, Cordeau & Roussel, 1977; Dokoozlian & Hirschfelt, 1995) showed that only early and high levels of bunch thinning (30 to 70% of crop removal) modify berry development. As observed in other studies (Rescic, Mikulic-Petkovsek, Stampar, Zupan & Rusjan, 2015; Bogicevic et al., 2015; Wang et al., 2018), data showed a significant reduction of the yield as a result of bunch thinning, regardless of the level and the date of S/S manipulation. This probably resulted from the period of thinning, which was performed after the completion of cell division in the berry (Ojeda, Deloire, Carbonneau, Ageorges & Romieu, 1999). Generally, varieties used for wine production display smaller berries than for table grapes. Smaller berries have a higher proportion of skins in comparison to flesh, which potentially results in higher amounts of phenolic compounds, terpenes, volatile acids, acetate esters and polysaccharides in the wines (Gil Cortiella, Úbeda, del Barrio-Galánc, & Peña-Neirac, 2019). In the wine viticulture sector, to limit the dilution of the metabolites during ripening growth (Bigard et al., 2019), practices aim to avoid excessive berry growth. Conversely, in most other perennial crops, obtaining large fruits is critical as the volume of the fruit size is an important qualitative parameter (Costa & Vizzotto, 2000; Guardiola & Garcia-Luis, 2000). Belhassine et al. (2019) showed that the link between crop load and fruit size is not linear. Indeed, below a crop load threshold, the effect of fruit thinning on fruit growth is attenuated. In the case of the wine viticulture, as the yields are far from the maximum fruitfulness, it is therefore not excluded that downward modulation of S/S may have only little effects on fruit growth.

The effect of bunch thinning on annual shoot biomass was also rather moderate and inconsistent depending on the year and the variety (**Table 1**, **Figure S4B**). In Chardonnay, in relation to the controls, annual vegetative biomass in Low 1 and Low 2 treatments varied from +11% and +41%

9

and -32% and +1% in 2015 and 2016 respectively. In Muscat, in relation to the controls, annual vegetative biomass in Low 1 and Low 2 treatments varied from +2% and -12% and -20% and -27% in 2016 and 2017 respectively. Differences between control and bunch-thinned plants were only statistically significant in 2017, but with an unexpected reduction of the shoot biomass at low S/S. The S/S balance was appreciated through the Ravaz index (Ravaz, 1912). In grapevine, vegetative vigor and lateral branching are dependent on the number of developing shoots and the fruit load. The pruning wood weight, measured at the end of the crop cycle, was found correlated with canopy biomass developed during the season (Smart, Dick, Gravett & Fisher, 1990).

The manipulation of crop load resulted in contrasted levels of S/S (**Table 1, Figure S4C**). For Chardonnay, control S/S were 4 in 2015 and 3.2 in 2016, 1.8 in 2015 (Low 2) and 0.8 in 2016 (Low 1). For Muscat, control S/S were 5.8 in 2016 and 5.6 in 2017, 1.9 in 2016 (Low 2) and 3.1 in 2017 (Low 2). To analyse the trophic competition for carbon, it is also relevant to compare dry matters, especially when organs to be compared display very contrasted water contents. At the arrest of phloem unloading, grapevine fruit contains 20% of dry matter, with hexoses and tricarboxylic acids being the main fractions of the non-structural C (Bigard et al., 2019). Fresh lignified shoots contain 50% of dry matter (DM), with starch and Ca oxalate being the major parts of the non-structural C (Bouard, 1966). We estimated dry matter S/S from the data of table 1, using the rates of 20% DM for fruits and 50% DM for pruning wood. Using this calculation, S/S were 1.6 in 2015 and 1.3 in 2016 in the Chardonnay controls, 0.7 in 2015 to 0.3 in 2016, in low S/S treatments. For Muscat, control S/S were 2.4 in 2016 and 2.2 in 2017, 0.8 in 2016 to 1.2 in 2017, in low S/S treatments. Therefore, variations of dry matter balance between vegetative and reproductive organs induced bunch thinning in this study is significant, i.e. 110% (2015) to 310% (2016) in Chardonnay and 300% (2016) to 180% (2017) in Muscat.

3.2. Accumulation of primary metabolites

Sugar concentrations recorded in this study were slightly different from the ones usually observed in commercial vineyards. For instance, Chardonnay grapes are generally harvested with 220 g/l

10

(1.22 mol/l) of sugars while sugar concentrations of the control fruits (Table 1) ranged from 198 g/l (1.10 mol/l) to 215 g/l (1.19 mol/l). Muscat grapes are commonly harvested beyond 270 g/l (1.5 mol/l) of sugars while sugar concentrations of the control fruits ranged from 193 g/l (1.07 mol/l) to 209 g/l (1.16 mol/l). These differences are directly related with the strategy of sampling to target the stage of the arrest of phloem unloading in the fruits. In commercial vineyards, to concentrate secondary metabolites and/or to obtain less astringent tannins, wine grapes are systematically harvested during the shriveling period after the phloem unloading has stopped (Bigard et al., 2019). Here, fruits were harvested at their maximum volume, i.e when phloem unloading stopped and the quantities of solutes per fruit were maximal (Bigard et al., 2018). The concentrations of primary metabolites in the control samples, i.e. around 1 mol/l of sugars and 60-70 mmol/l of tartaric and malic acids are representative of grapes sampled at this the end of physiological ripening (Bigard et al., 2019). Glucose/fructose ratios were around 1, as classically observed in *V. vinifera* ripe *f*ruit (Bigard et al., 2019).

Grape composition and quantity of primary metabolites accumulated per plant varied depending on the year, the genotype and the treatment (**Table 1**). For both varieties, control samples presented lower quantities of sugars in the fruits per plant in 2016. Fruit acidity and quantity of organic acid accumulated per plant were lower in 2016 than 2015 for Chardonnay controls. Muscat controls showed no variation between the 2 years of experiment for the concentration of organic acids in the fruit, and a lower quantity of organic acids per plant in 2016. These fluctuations confirmed the influence of environment (E) on the accumulation of metabolites in the ripe grape (Blancquaert, Oberholster, Ricardo-da-Silva & Deloire, 2019). The reduction of S/S tended to increase the concentration of sugars in ripe grapes with statistically significant effects of the treatment for both varieties and both years of experiments. At low S/S, sugar concentrations increased by 15% (2015) and 3 % (2016) in Chardonnay, and by 11% (2016) and 7% (2017) and Muscat. These results are consistent with other reports (Carbonneau, Leclair, Dumartin, Cordeau & Roussel, 1977; Rescic, Mikulic-Petkovsek, Stampar, Zupan & Rusjan, 2015; Wang et al., 2018), but disagree with Song,

Wang, Xie, Zhang and Zhen-We (2018), who have not observed any effect of bunch thinning on the concentration of sugars in Cabernet Sauvignon grapes. These discrepancies could be due to differences in sampling strategy, as precise physiological stages have not always been well defined in previous studies. Indeed, when phloem unloading stops at physiological ripe stage, the main driver of fruit solute concentration shifts from importation to shriveling and accumulation and concentration can be easily confused. Another source of experimental noise results from GxE specificities. Depending on experimental conditions, the fluctuations of the level of photosynthetically active radiation (PAR) and water supply can result in a range of C assimilation and partition statutes. The differences in sugar concentrations observed here regardless of the berry growth highlight an interesting biological feature. Indeed, sugars, which shift from less than 0.1 mol/l in the green berry to 1 mol/l in the fruit at physiological ripe stage, are the main contributors of berry osmotic potential during ripening (Bigard et al., 2019). The variations of sugar concentrations induced by S/S modulation independently of the fruit volume suggests some plasticity between fruit growth and osmotic potential. When expressed in quantity of sugars in the fruits per plant (**Table 1**), we observed the reverse as for the concentrations in the fruits. In comparison with the controls, the loss of sugars reached 65% in 2015 and 66% in 2016 for Chardonnay and 70% in 2016 and 60% in 2017 for Muscat, in low S/S treatments.

No consistent links were observed between S/S and acidity level of the ripe fruit. Indeed, organic acid concentrations were similar for all treatments in 2015 for Chardonnay, and slightly higher at low S/S in 2016. In Muscat, the concentrations of organic acids were lower at low S/S in 2016 and variable in 2017. Both major organic acids, i.e. tartaric and malic acids are accumulated during the first growing phase to a peak just before the onset of ripening (Bigard et al., 2019). During green berry growth, there is a small competition for photo-assimilates because organic acids are only accumulated to a few hundred mmol/l while sugars remain below 100 mmol/l. During ripening, both organic acids are diluted by fruit growth, while malic acid is also rapidly metabolized at the onset of sugar phloem unloading (Rienth, Torregrosa, Gauthier, Ardisson, Brillouet & Romieu,

12

2016). These observations are consistent with those reported by Song et al. (2018) who observed, depending on the year and the variety, very small or no effect of bunch thinning on organic acid concentrations. On the other hand, the effects of S/S on the level of organic acids accumulated in the fruits per plant were very significant. In comparison with the controls, the quantities of organic acids accumulated in the fruits at plant level decreased from 58% in 2015 to 66% in 2016 for Chardonnay and from 72% in 2016 to 62%, in 2017 for Muscat in low S/S treatments. The correlation between the variations in sugars and in organic acids was low (-0.2, p-value 0.002) for variables expressed in concentration in the fruits but very high (0.98, p-value 0.052) when expressed in total quantity in the fruits per plant, showing a strong overall effect of thinning on the accumulation of primary metabolites in the fruits at plant level.

3.3. Accumulation of GAPs

The total amounts of GAPs accumulated in the ripe grape and also the proportion of the families were dependent on the genotype and the year (**Table 1**). In the controls, GAPs fluctuated up to 20% in fruit's concentration and up to 37% in the quantity accumulated in the fruits per plant. In the controls, the effect of the year was statistically significant in concentration in the fruits and in total quantity per berry or per plant in Chardonnay, but not in Muscat despite marked differences. Whatever the level or the date of bunch thinning, Muscat accumulated 3 times more GAPs in concentration in the fruits than Chardonnay. In quantity of GAPs in the fruits per plant, the differences were five-times higher than in fruit's concentration, partially due to a bigger volume of the Muscat berries compared to Chardonnay. As reviewed by Alem et al. (2018), the contribution of alcohols and terpenes was opposite in Chardonnay and Muscat varieties. Alcohols varied between 69 and 81% of the total GAPs in Chardonnay while in Muscat, terpenes were the most abundant GAP compounds, i.e. 60 to 79 % of the total of GAPs (**Table S5**). S/S modulation only resulted in little changes in the concentrations of GAPs in the physiologically ripe fruits. Indeed, in Chardonnay, S/S reduction did not impact fruit GAPs concentration in 2015 and curiously decreased the level of GAPs in 2016. In Muscat, an increase of GAP concentration in the fruits for

13

low S/S treatment was observed in 2016, but no effect in 2017. S/S modulation tremendously impacted the quantity of GAPs accumulated in the fruits at plant level, whatever the date of bunch thinning (**Table 1**). In Chardonnay, in comparison with controls, the quantities of GAPs in the fruits per plant were reduced from 46% in 2015 to 71%, in low S/S treatments with no statistical differences between the dates of bunch thinning. In Muscat, in comparison with controls, the quantities of GAPs in the fruits per plant were reduced from 55% in 2016 to 62% in 2017, in low S/S treatments with no statistical differences between the dates of treatment.

Analyzed across years and treatments, the variations of the families of GAPs were well related to the variations of the total of GAPs expressed either in concentrations in the fruits or in total quantities in the fruits per plant (**Table S6**). However, the major classes of each variety were not always well correlated with minor families. For instance, for Chardonnay, the non-terpenic alcohol variations were not linked with the 3 other families of GAPs. Similar observations could be done in Muscat for terpenes in concentration in the fruits and also, but to a lesser extent, in total quantity in the fruits per plant. At sub-family level (**Figure S7**), for Chardonnay, there were no clear links between the date of bunch thinning and the concentration of GAP sub-families in the ripe fruit. In 2015, Chardonnay fruits displayed a slight increase of most sub-families of GAPs with no statistical significant differences between treatments. In 2016, an unexpected decrease of shikimic derivatives in Low 1 and Low 2 treatments was observed, without statistical significance, and very small variations for other sub-families. In Muscat, in 2016, the reduction of S/S led to an increase of the concentration of all sub-families of GAPs in the fruits with higher levels for late bunch thinning (Low 2). In 2017, no differences were observed between modalities. Taken together, these data showed that, despite a strong impact on crop yield, the effects of S/S on total GAP concentrations in the fruits were very limited and/or inconsistent.

At compound levels, the effects of the year and of the S/S on the concentration in the fruits and in total quantity accumulated in the fruits per plant are presented in tables 2 (Non-terpenic alcohols), 3 (C_{13}-norisoprenoids), 4 (Phenols) and 5 (Terpenes). For non-terpenic alcohols (**Table 2**), benzyl

14

alcohol and 2-phenylethanol were the major compounds for both varieties. For Chardonnay, in the controls, the concentration of non-terpenic alcohols showed some stability despite annual yield variations. Muscat displayed more marked yearly variations for minor compounds, but a great stability for major compounds. Modulation of S/S resulted in inconsistent effects on GAP distribution in the fruits. For Chardonnay, S/S reduction decreased the concentrations of the two major compounds in 2016 but not in 2017. In Muscat, in comparison with the controls, low S/S resulted in an increase of the concentrations of benzyl alcohol and 2-phenylethanol in 2016 but the reverse was observed in 2017, whatever the date of bunch thinning. In quantities of alcohols accumulated in the fruits per plant, the decrease of S/S systematically reduced all compounds for both varieties regardless of the year and the date of treatment. For instance, bunch thinning resulted in a reduction of 72% for 2-phenylethanol in Chardonnay in 2015 at Low 1 and 95% for 1-octen-3-ol in Muscat in 2017 at Low 2 with respect to their respective controls.

For the other 3 families of GAPS, the abundance of the compounds and the proportion of each compound were highly dependent on the year and the variety (**Table 3, 4 and 5**). Modulation of S/S resulted in non-significant or erratic effects on the concentration of C_{13}-norisoprenoids in the ripe fruit for both varieties. Only in 2016, late bunch thinning (Low 2) could improve the concentration of C_{13}-norisoprenoids in Muscat fruits, but this effect was not confirmed in 2017. In total quantity accumulated in the fruits, the decrease of S/S reduced most of C_{13}-norisoprenoids for both varieties regardless of the year and the date of treatment. For instance, in comparison with the controls, bunch thinning resulted in a reduction of 86% of 3-hydroxy-7,8-dihydro-beta-ionol accumulated in the fruits per plant in Chardonnay in 2016 at Low 2 and 78% of the quantity of 3-hydroxy-beta-damascone in the fruits per plant in Muscat in 2017 at Low 2. For glycosylated phenols, depending on the year, the modulation of S/S resulted in non-significant or unpredictable effects for the concentration of these compounds in the fruits at physiological ripe stage in both varieties. In total quantity in the fruits per plant, the decrease of S/S resulted in a very significant reduction of most phenols in both varieties regardless of the year and the date of treatment. For instance, bunch

15

thinning resulted in a reduction of 82% of guaiacyl-propanol accumulated in the fruits per plant in Chardonnay in 2016 at Low 2 and 73% of the unknown 1 compound in Muscat in 2016 at Low 1 with respect to their respective controls.

For terpenes, nerol and geraniol hydrates were found the major compounds for Chardonnay, respectively 72% in 2015 and 56% in 2016 of the total of the family in the controls. In Muscat berry, these compounds were accumulated at the same range as in Chardonnay but other terpenic compounds were accumulated as well. Nerol and geraniol represented 38% in 2016 to 45% in 2017 of the total amounts of terpenes, but 3,7-dimethyl-1,5-octadien-3,7-diol and 2,6-dimethylocta-2,7-dien-1,6-diol compounds were also significantly accumulated. The modulation of S/S resulted in inconsistent effects on the concentrations of terpenes in the Chardonnay ripe fruits. Only 8-hydroxy-6,7-dihydro-linalool and 2,6-dimethylocta-2,7-dien-1,6-diol compounds showed a statistically significant increase of concentrations in the fruits in low S/S treatments. In Muscat, the effects of the modulation of S/S on terpene concentrations in the fruits were difficult to interpret as the response changed from year to year. In quantity accumulated in the fruits per plant, the decrease of S/S reduced most terpene compounds regardless of the year and the date of treatment. For instance, in comparison with the controls, bunch thinning resulted in a reduction of 68 % of the nerol and geraniol hydrates in 2016 in Chardonnay at Low 1 and of 67% for 2,6-dimethylocta-2,7-dien-1,6-diol compound in Muscat in 2017 at Low 2.

Many studies stated the influence of bunch thinning in wine aroma, but just a few focused their research in the berry aroma profile (Alem et al., 2018). In a single year experiment, Kok (2011) observed that Sauvignon Blanc's berries from bunch-thinned plants displayed higher free volatile and glycosylated terpene concentrations, particularly when thinning was performed just before the onset of ripening. Suklje et al. (2013) showed that cluster thinning can increase or decrease the concentration of volatile thiols in wines as a function of various parameters linked to the general balance of the plant, such as the total area of the canopy. Song et al. (2018) also reported variable effects of bunch thinning on the distribution of aroma molecules in the fruits depending on the year,

the variety and the compound. When expressed in total concentration of volatile compounds, S/S effects were not or only hardly statistically significant, with inconsistent variation trends. This highlights the difficulty to experiment with aroma compounds that are accumulated at a very low level and in strong interaction with environmental factors (Schmidtke, Antalick, Suklje, Blackman, Boccard & Deloire, 2020). Our results, obtained with two varieties and two dates of bunch thinning, showed that the concentration in the fruits of secondary metabolites varied in a limited and independent way in response to S/S modulation (**Figure S9A**). At plant level, the quantities of primary and secondary metabolites accumulated in the fruits were significantly decreased by the reduction of S/S regardless of the variety, the year and the date of bunch thinning (**Figure S9B**).

3.4. Effects of S/S on the balance between GAPs and vs primary metabolites

Analysing the impact of practices on the accumulation of fruit metabolites needs relevant variables to figure out the possible competition for photo-assimilates. As sugars, organic acids or secondary metabolites have different carbon structures, molar concentrations of each compound family were transformed in equivalent moles of C (**Table S8**). Sugars plus organic acids represent 6 to 7.5 moles of C per kg of fresh fruit at the arrest of phloem unloading (Bigard et al., 2019). Major primary metabolites are largely the main sink of photo-assimilates during grape ripening. In comparison to primary metabolites, GAPs represent a very small fraction of the non-structural C of the fruit. Indeed, GAPs/primary metabolites in the control fruits varied from 5.04 to 6.33 10^{-6} in the non-aromatic Chardonnay variety and from 16.43 to 17.98 10^{-6} in the aromatic Muscat variety. Decreasing S/S resulted in an unexpected diminution of this ratio in Chardonnay in both years of experiments with statistically significant effect in 2016. This was due to an increase of sugars on bunch-thinned plants while the concentration of GAPs in the fruits remained unaffected. In Muscat grape, the effect in 2016 and 2017 were opposite with a statistically significant increase in 2016 and a decrease of GAPs/primary metabolites in the ripe fruits in 2017, without statistical significance.

Conclusion

17

Wine viticulture has some specificities in comparison with other perennial fruit crops, including table grapes, because the size of the fruit is not the first qualitative target. A range of viticulture practices, such as bunch thinning, are empirically implemented to tune S/S with the purpose to improve grape composition. These approaches are based on the hypothesis that the balance between C assimilation and crop load is limiting for the accumulation of organic molecules of interest. However, in previous reports the effects of bunch thinning on grape composition remained uncertain because fruit phenotyping was mostly assessed in solute concentration without precise physiological landmarks. In this study, fruit sampling strategy was designed to distinguish the accumulation of organic solutes, whose maximum occurs at the arrest of phloem unloading, from concentration fluctuations induced by fruit shriveling. Fruit removal systematically decreased the quantity of sugars, organic acids and glycosylated aroma compounds accumulated in the fruits at plant level. S/S manipulation could not de-correlate the balance between GAPs and primary metabolites either in non-aromatic or aromatic grapes. Considering the labor cost required for fruit thinning, i.e. 20-40 h per ha, and the huge loss of metabolites of interest accumulated in the fruits per plant, these practices should be performed with circumspection. Other cultivation practices, which potentially influence metabolic pathways without impairing plant C balance or yield performance, would be more profitable to improve grape composition. For instance, the manipulation of bunch's microclimate (Bureau, Baumes & Razungles, 2000; Asproudi, Petrozziello, Cavalletto, & Guidoni, 2016) or plant water status (Kondouras, Marinos, Gkoulioti, Kotseridis & van Leeuwen, 2006) has long been shown to be effective increasing aroma compounds of the grape.

Literature cited

Agosin, E., Belancic, A., Ibacache, A., Baumes, R., Bordeu, E., Crawford, A., & Bayonove, C. (2000) Aromatic potential of certain Muscat grape varieties important for Pisco production in Chile. *American Journal of Enology and Viticulture*, 51, 404–408.

Alem, H., Rigou, P., Schneider, R., Ojeda, H., & Torregrosa, L. (2018) Impact of agronomic practices on grape aroma composition: a review. *Journal of the Science of Food and Agriculture*, 99, 975–985, https//doi: 10.1002/jsfa.9327.

Allen, M.S., & Lacey, M.J. (1998) Methoxypyrazines of grapes and wines. *ACS Symposium Series*, 714: 31–38. In Waterhouse AL, Ebeler S (Eds), *Chemistry of Wine Flavor*, Oxford University Press, Washington DC, USA.

Asproudi, A., Petrozziello, M., Cavalletto, S., & Guidoni, S. (2016) Grape aroma precursors in cv. Nebbiolo is affected by vine microclimate. *Food Chemistry*, 211, 947–956, https://doi.org/10.1016/j.foodchem.2016.05.070.

Belhassine, F., Martinez, S., Bluy, S., Fumey, D., Kelner, J.J., Costes, E., & Pallas, B. (2019) Impact of within-tree organ distances on floral induction and fruit growth in apple tree: Implication of carbohydrate and gibberellin organ contents. *Frontiers in Plant Science*, 10, 1233, https//doi:10.3389/fpls.2019.01233.

Bernizzoni, F., Civardi, S, Van Zeller, M., Gatti, M., & Poni, S. (2011) Shoot thinning effects on seasonal whole-canopy photosynthesis and vine performance in *Vitis vinifera* L. cv. Barbera. *Australian Journal of Grape and Wine Research*, 17, 351–357, https://doi.org/10.1111/j.1755-0238.2011.00159.x.

19

Bigard, A., Berhe, D.T., Maoddi, E., Sire, Y., Boursiquot, J.M., Ojeda, H., Péros, J.P., Doligez, A., Romieu, C., & Torregrosa, L. (2018) *Vitis vinifera* L. fruit diversity to breed varieties anticipating climate changes. *Frontiers in Plant Science*, 9, 405, https//doi:10.3389/fpls.2018.00455.

Bigard, A., Romieu, C., Sire, Y., Veyret, M., Ojeda, H., & Torregrosa, L. (2019) Grape ripening revisited through berry density sorting. *OenoOne*, *4*, 719–724, https//doi:10.20870/oeno-one.2019.53.4.2224.

Blancquaert, E.H., Oberholster, A., Ricardo-da-Silva, J.M., & Deloire, A.J. (2019) Grape flavonoid evolution and composition under altered light and temperature conditions in Cabernet Sauvignon (*Vitis vinifera* L.). *Frontiers in Plant Science*, 10, 1062, https://doi.org/10.3389/fpls.2019.01062.

Bogicevic, M., Maras, V., Mugosa, M., Kodzulovic, V., Raicevic, J., Sucur, S., & Failla, O. (2015) The effects of early leaf removal and cluster thinning treatments on berry growth and grape composition in cultivars Vranac and Cabernet Sauvignon. Chemical and Biological Technologies in Agriculture, 2, 13, https//doi10.1186/s40538-015-0037-1.

Bouard, J. (1966) Recherches physiologiques sur la vigne et en particulier sur l'aoûtement des sarments. PhD of the University of Bordeaux, France, 398 p.

Bureau, S.M., Baumes, R.L., & Razungles, A.J. (2000) Effects of vine or bunch shading on the glycosylated flavor precursors in grapes of *Vitis vinifera* L. Cv. Syrah. *Journal of Agriculture and Food Chemistry*, 48, 1290–1297, https//doi.org/10.1021/jf990507x.

Carbonneau, A., Leclair, P., Dumartin, P., Cordeau, J., & Roussel, C. (1977) Etude de l'influence chez la vigne du rapport « partie végétative / partie productrice » sur la production et la qualité des raisins. *Journal International des Sciences de la Vigne et du Vin*, 11, https//doi.org/10.20870/oeno-one.1977.11.2.1437.

Costa, G., & Vizzotto, G. (2000) Fruit thinning of peach trees. *Plant Growth Regulation*, 31, 113–119, https://doi.org/10.1023/A:1006387605042.

20

Di Profio, F., Reynolds, A.G., & Kasimos, A. (2011) Canopy management and enzyme impacts on Merlot, Cabernet franc, and Cabernet sauvignon. I. Yield and berry composition. *American Journal of Enology and Viticulture*, 62, 139–151.

Dokoozlian, N.K., & Hirschfelt, D.J. (1995) The influence of cluster thinning at various stages of fruit development on flame seedless table grapes. *American Journal of Enology and Viticulture*, 46, 429–436.

Gil, M., Esteruelas, M., Gonzáles, E., Kontoudakis, N., Jiménez, J., Fort, F., Canals, J.M., Hermosín-Gutiérrez, I., & Zamora, F. (2013) Effect of two different treatments for reducing grape yield in *Vitis vinifera* cv Syrah on wine composition and quality: berry thinning versus cluster thinning. *Journal of Agriculture and Food Chemistry*, 61, 4968–4978, https://doi.org/10.1021/jf400722z.

Gil Cortiella, M., Úbeda, C., del Barrio-Galánc, R., & Peña-Neirac A. (2019) Impact of berry size at harvest on red wine composition: a winemaker's approach. *Journal of the Science of Food and Agriculture*, 100, https//doi.10.1002/jsfa.10095.

Greer, D.H, & Weedon, M.M. (2012) Modelling photosynthetic responses to temperature of grapevine (*Vitis vinifera* cv. Semillon) leaves on vines grown in a hot climate. *Plant Cell and Environment*, 35, https//doi: 10.1111/j.1365-3040.2011.02471.x.

Guardiola, J.L., & Garcia-Luis, A. (2000) Increasing fruit size in Citrus. Thinning and stimulation of fruit growth. *Plant Growth Regulation*, 31, 121–132, https://doi.org/10.1023/A:1006339721880.

Gunata, Z., Bayonove, C., Baumes, R., & Cordonnier, R. (1985) The aroma of grapes. Localisation and evolution of free and bound fractions of some grape aroma components c.v. Muscat during first development and maturation. *Journal of Agricultural and Food Chemistry*, 36, 857–862, https://doi.org/10.1002/jsfa.2740360915.

Huglin, P. (1978) Nouveau mode d'évaluation des possibilités héliothermiques d'un milieu viticole. In: Proc. Symposium Int. Ecologie de la Vigne, Constança, p.89–98.

Kok, D. (2011) Influences of pre- and post-veraison cluster thinning treatments on grape composition variables and monoterpene levels of *Vitis vinifera* L. cv. Sauvignon Blanc. *Journal of Food Agriculture and Environment*, 9, 22–26.

Kondouras, S., Marinos, V., Gkoulioti, Q., Kotseridis, Y., & van Leeuwen, C. (2006) Influence of vineyard location and vine water status on fruit maturation of non-irrigated Cv. Agiorgitiko (*Vitis vinifera* L.). Effects on wine phenolic and aroma components. *Journal of Agricultural and Food Chemistry*, 54, https://doi.org/10.1021/jf0605446.

Link, H. (2000) Significance of flower and fruit thinning on fruit quality. *Plant Growth Regulation*, 31, 17–26, https://doi:10.1023/A:1006334110068.

Ojeda, H., Deloire, A., Carbonneau, A., Ageorges, A., & Romieu, C. (1999) Berry development of grapevines: Relations between the growth of berries and their DNA content indicate cell multiplication and enlargement. *Vitis*, 38, 145–150.

Parker, M., Capne, D.L., Francis, L., & Herderich, M.J. (2018) Aroma precursors in grapes and wine: Flavor release during wine production and consumption. *Journal of Agricultural and Food Chemistry*, 66, https://doi.org/10.1021/acs.jafc.6b05255.

Ravaz, L. (1912) Taille hâtive ou taille tardive ? *Prog. Agric. Vitic.*, 386–389, 422–424. 453–454.

Razungles, A, Gunata, Z., Pinatel, S., Baumes, R., & Bayonove, C. (1993) Etude quantitative de composés terpéniques, norisoprénoïdiques et de leurs précurseurs dans diverses variétés de raisins. *Science des Aliments*, 13, 59–72.

Rescic, J., Mikulic-Petkovsek, M., Stampar, F., Zupan, A., & Rusjan, D. (2015) The impact of cluster thinning on fertility and berry and wine composition of 'Blauer Portugieser' (*Vitis vinifera* L.) grapevine variety. *Journal International des Sciences de la Vigne et du Vin*, 49, https://doi.org/10.20870/oeno-one.2015.49.4.16.

Reynolds, A.G. (1989) Impact of pruning strategy, cluster thinning, and shoot removal on growth, yield, and fruit composition of low-vigor De Chaunac vines. *Canadian Journal of Plant Science*, 69, 269–275, https://doi.org/10.4141/cjps89-036.

Rienth, M., Torregrosa, L., Gauthier, S., Ardisson, M., Brillouet, J.L., & Romieu, C. (2016) Temperature desynchronizes sugar and organic acid metabolism in ripening grapevine fruits and remodels its transcriptome. *BMC Plant Biol.*, 164, https://doi.10.1186/s12870-016-0850-0.

Schmidtke, L.M., Antalick, G., Suklje, K., Blackman, J.W., Boccard, J., & Deloire, A. (2020) Cultivar, site and harvest date: the gordian knot of wine terroir. *Metabolomics*, 16, https://doi.org/10.1007/s11306-020-01673-3.

Schneider, R., Charrier, F., Moutounet, M., & Baumes R. (2004) Rapid analysis of grape aroma glycoconjugates using Fourier-transform infrared spectrometry and chemometric techniques. *Analytica Chimica Acta*, 513, 91–96, https://doi.org/10.1016/j.aca.2003.11.082.

Schneider, R., Razungles, A., Augier, C., & Baumes, R. (2001) Monoterpenic and norisoprenoidic glycoconjugates of *Vitis vinifera* L. cv. Melon B. as precursors of odorants in Muscadet wines. *Journal of Chromatography*, 936, 145–157, https://doi.org/10.1016/S0021-9673(01)01150-5.

Smart, R.E., Dick, J.K., Gravett, I.M., & Fisher, B.M. (1990) Canopy management to improve grape yield and wine quality - principles and practices. *South African Journal of Enology and Viticulture*, 11, https://doi: 10.21548/11-1-2232.

Song, C.Z, Wang, C., Xie, S., Zhang, N., & Zhen-We, G. (2018) Effects of leaf removal and cluster thinning on berry quality of *Vitis vinifera* cultivars in the region of Weibei dryland in China. *Journal of Integrative Agriculture*, 1, 1620–1630, https://doi.org/10.1016/S2095-3119(18)61990-2.

Suklje, K., Cesnik, H. B., Janes, L., Kmecl, V., Vanzo, A., Deloire, A., Sivilotti, P., & Lisjak, K. (2013) The effect of leaf area to yield ratio on secondary metabolites in grapes and wines of *Vitis vinifera* L. Cv. Sauvignon blanc. Journal International des Sciences de la Vigne et du Vin, 47, 83-97.

Torregrosa, L., Pradal, M., Souquet, J. M., Rambert, M., Gunata, Z., & Tesniere, C. (2008) Manipulation of *VvAdh* to investigate its function in grapevine berry development. *Plant Science*, 174, https://doi: 10.1016/j.plantsci.2007.10.006.

Wang, Y., He, Y.N., Chen, W.K., He, F., Chen, W., Cai, X-D., Duan, C.Q., & Wang, J. (2018) Effects of cluster thinning on vine photosynthesis, berry ripeness and flavonoid composition of Cabernet Sauvignon. *Food Chemistry*, 248, 101–110, https://doi.org/10.1016/j.foodchem.2017.12.021.

Table captions

Table 1 - Effect of bunch thinning on fresh fruit yield, annual shoot biomass, S/S balance and the accumulation of sugars, organic acids and GAPs in the ripe grape of *V. vinifera* cv. Chardonnay and cv. Muscat à Petit Grains. Control, no thinning, Low 1, early bunch thinning and Low 2, late bunch thinning. Metabolite contents are expressed in concentration in the fruits and in total quantity in the fruits per plant. Means +/- standard deviation (SD). (ns) non-significant.

Table 2 - Effect of the S/S balance the accumulation of the glycosylated non-terpenic alcohols in the ripe grape of *V. vinifera* cv. Chardonnay and cv. Muscat à Petit Grains. S/S were modulated through bunch thinning: Control, no thinning, Low 1, early bunch thinning and Low 2, late bunch thinning. Chardonnay and Muscat à Petit Grains. Metabolite contents are expressed in concentration in the fruits and in total quantity in the fruits per plant. Means +/- standard deviation (SD). (ns) non-significant.

Table 3 - Effect of the S/S balance the accumulation of the glycosylated C_{13}-norisoprenoids in the ripe grape of *V. vinifera* cv. Chardonnay and cv. Muscat à Petit Grains. S/S were modulated through bunch thinning: Control, no thinning, Low 1, early bunch thinning and Low 2, late bunch thinning. Metabolite contents are expressed in concentration in the fruits and in total quantity in the fruits per plant. Means +/-standard deviation (SD). (ns) non-significant, (nd) not detected.

Table 4 - Effect of the S/S balance the accumulation of the glycosylated phenols in the ripe grape of *V. vinifera* cv. Chardonnay and cv. Muscat à Petit Grains. S/S were modulated through bunch thinning: Control, no thinning, Low 1, early bunch thinning and Low 2, late bunch thinning. Metabolite contents are expressed in concentration in the fruits and in total quantity in the fruits per plant. Means +/- standard deviation (SD). (ns) non-significant, (nd) not detected.

Table 5 - Effect of the S/S balance the accumulation of the glycosylated terpenes in the ripe grape of *V. vinifera* cv. Chardonnay and cv. Muscat à Petit Grains. S/S were modulated through bunch thinning: Control, no thinning, Low 1, early bunch thinning and Low 2, late bunch

25

thinning.Metabolite contents are expressed in concentration in the fruits and in total quantity in the fruits per plant. Means +/- standard deviation (SD). (ns) non-significant, (nd) not detected.

Table 1

		Treatments									
		Control				**Low 1**			**Low 2**		
Chardonnay	Year	Mean	SD	Y		Mean	SD	Y	Mean	SD	Y	S/S
Fruit yield	2 015	1500	241	ns		810	274	***	956	125	***	a b b**
g/plant	2 016	1245	64			400	106		445	175		a b b***
Shoot biomass	2 015	375	55	ns		419	97	ns	532	23	*	ns
g/plant	2 016	391	52			517	38		392	48		b a b*
S/S	2 015	4.06	0,59	ns		2.01	0.50	*	1.78	0.10	*	a b b***
Ravaz Index	2 016	3.19	0.40			0.77	0.06		1.13	0.14		a b b***
Sugars	2 015	1.10	0.01	***		1.22	0.02	ns	1.27	0.02	**	c b a***
mol/l	2 016	1.19	< 0.01			1.22	< 0.01		1.21	< 0.01		c a b ***
Sugars	2 015	1.43	0.01	***		0.86	0.01	***	1.04	0.02	***	a c b***
mol/plant	2 016	1.34	< 0.01			0.45	< 0.01		0.49	< 0.01		a c b***
Organic acids	2 015	73	2	**		72	2	*	73	1	*	ns
mmol/l	2 016	66	< 1			69	< 1		71	< 1		c b a***
Organic acids	2 015	95	3	***		51	1	***	60	1	***	a c b***
mmol/plant	2 016	74	< 1			25	< 1		29	< 1		a c b***
GAPs	2 015	964	32	*		1056	118	*	954	203	ns	ns
µg/l	2 016	816	1			740	14		677	51		a ab b*
GAPs	2 015	1257	42	**		743	83	**	782	166	*	a b b*
mg/plant	2 016	918	1			271	5		275	21		a b b***
Muscat												
Fruit yield	2 016	2212	114	ns		785	49	*	636	138	ns	a b b***
g/plant	2 017	2562	253			1027	272		1047	123		a b b***
Shoot biomass	2 016	379	41	ns		386	34	ns	328	65	ns	ns
g/plant	2 017	459	51			363	34		333	28		a b b*
S/S	2 016	5.84	0,60	ns		2.03	0.19	*	1.94	0.44		a b b***
Ravaz Index	2 017	5.59	0.65			2.83	0.26		3.15	0.27		a b b***
Sugars	2 016	1.07	0.03	*		1.19	0.03	ns	1.12	0.02	*	b a b**
mol/l	2 017	1.16	0.02			1.23	0.02		1.24	0.06		b ab a***
Sugars	2 016	2.18	0.07	***		0.85	0.02	***	0.65	0.01	*	a b c***
mol/plant	2 017	2.75	0.04			1.16	0.02		1.10	0.05		a b b**
Organic acids	2 016	68	< 1	ns		64	1	ns	64	< 1	*	a b b***
mmol/l	2 017	67	1			65	1		68	2		ab b a***
Organic acids	2 016	138	1	***		46	< 1	***	38	< 1	*	a b c***
mmol/plant	2 017	159	3			61	1		60	2		a b b***
GAPs	2 016	2455	124	ns		3276	114	ns	3875	134	ns	c b a***
µg/l	2 017	2952	322			2888	332		2980	674		ns
GAPs	2 016	5081	215	ns		2352	136	ns	2309	108	ns	a b b***
mg/plant	2 017	6986	761			2724	313		2626	594		a b b**

Table 2

Alcohols		Contents in concentration (µg/L)										Quantity per plant (µg/plant)									
		Control			Low 1			Low 2				Control			Low 1			Low 2			
Chardonnay	Year	Mean	SD	Y	Mean	SD	Y	Mean	SD	Y	S/S	Mean	SD	Y	Mean	SD	Y	Mean	SD	Y	S/S
hexanol	2015	11.3	0.6	ns	11.9	4.1	ns	15.3	2.2	ns	ns	14.7	0.8	ns	8.4	2.9	ns	12.5	1.8	**	a b ab*
	2016	13.6	1.2		11.4	<0.1		10.9	1.4		a ab b*	15.3	1.3		4.2	<0.1		4.6	0.5		a b b***
3-hexen-1-ol cis	2015	1.7	<0.1	**	1.8	0.6	ns	1.1	1.2	ns	ns	2.2	<0.1	ns	1.3	0.4	*	0.9	1.0	**	a b ab*
	2016	1.9	<0.1		1.5	0.2		1.8	0.5		ns	2.2	<0.1		0.5	<0.1		0.7	0.1		a b b***
2-hexen-1-ol trans	2015	2.5	0.3	ns	2.2	0.8	ns	1.5	1.7	ns	ns	3.3	0.4	ns	1.5	0.6	ns	1.3	1.4	***	a b ab*
	2016	3.0	1.0		4.3	3.7		1.3	0.2		ns	3.4	1.2		1.9	1.1		0.5	0.1		a ab b*
1-octen-3-ol	2015	1.0	0.2	ns	1.0	0.2	ns	1.8	0.1	***	b b a*	1.4	0.2	ns	0.7	0.1	**	1.5	0.1	***	a b c*
	2016	0.9	<0.1		0.9	<0.1		0.9	0.2		ns	1.1	<0.1		0.3	<0.1		0.4	<0.1		a b b***
benzyl alcohol	2015	400	39	ns	385	90	ns	427	58	ns	ns	521.9	51	ns	271.0	64	*	350	47	**	a b b**
	2016	392	6		347	4		314	42		a b b*	441.3	7		126.7	1		127	12		a b b***
2-phenylethanol	2015	234	18	ns	249	60	ns	270	38	ns	ns	305.8	24	ns	175.5	42	*	221	31	**	a b b*
	2016	253	2		225	16		205	26		a ab b*	284.8	3		80.8	5		84	8		a b b***
Total alcohols	2015	651	57	ns	651	154	ns	717	96	ns	ns	849.3	74	ns	458.4	109	*	587	79	**	a b b*
	2016	665	6		590	16		534	70		a b b*	748.1	7		214.3	5		217	20		a b b***
Muscat																					
hexanol	2016	10.5	1.1	ns	15.9	1.0	**	20.1	2.7	*	c b a**	21.4	2.2	ns	11.4	0.7	***	11.8	1.6	*	a b b**
	2017	30.5	9.4		24.3	0.8		43.7	4.7		ns	72.1	22.3		23.0	0.8		45.7	4.9		a c b*
3-hexen-1-ol cis	2016	5.3	0.4	ns	6.9	1.1	ns	9.9	0.2	*	b b a*	10.8	0.9	ns	4.9	0.8	*	5.8	0.1	*	a b b**
	2017	14.0	4.0		8.4	0.7		15.8	1.3		ns	33.1	9.5		7.9	0.7		16.6	1.4		a c b**
2-hexen-1-ol trans	2016	5.2	0.5	*	6.8	1.4	*	7.2	1.0	*	ns	10.5	0.9	*	4.9	1.0	*	4.2	0.6	*	a b b**
	2017	17.8	3.4		2.3	0.7		2.4	0.3		a b b**	42.1	8.1		2.1	0.7		2.5	0.3		a b b**
1-octen-3-ol	2016	1.2	0.1	**	1.6	0.3	*	2.4	0.4	ns	b ab a*	2.4	0.2	ns	1.1	0.2	**	1.4	0.3	*	a b ab *
	2017	20.4	28.9		5.1	1.6		2.1	0.4		a b b**	48.3	68.3		4.8	1.5		2.2	0.4		a b b***
benzyl alcohol	2016	399	16	ns	458	21	*	567	13	**	c b a**	810	32	ns	328	15	ns	332	8	ns	a b b***
	2017	350	42		291	50		283	38		ns	827	100		275	47		296	40		a b b**
2-phenylethanol	2016	209	12	ns	255	9	*	307	2	*	c b a***	424	25	*	183	6	ns	180	1	ns	a b b***
	2017	215	<1		185	24		195	32		ns	509	0.4		174	23		204	33		ab c***
Total alcohols	2016	630	30	ns	745	29	*	914	19	*	c b a***	1279	62	ns	534	21	ns	534	11	ns	a b b***
	2017	647	31		516	79		542	64		ns	1532	73		487	74		567	67		a b b***

Table 3

| C₁₃-Norisoprenoids | | Contents in concentration (µg/L) | | | | | | | | | Quantity per plant (µg/plant) | | | | | | | | | |
| | | Control | | | Low 1 | | | Low 2 | | | Control | | | Low 1 | | | Low 2 | | | |
Chardonnay	Year	Mean	SD	Y	Mean	SD	Y	Mean	SD	Y	S/S	Mean	SD	Y	Mean	SD	Y	Mean	SD	Y	S/S
3-hydroxy-beta-damascone	2015	29.7	3.1	*	29.0	1.6	***	39.3	6.1	*	ns	38.7	4.0	*	20.4	1.1	***	32.2	5.0	**	a c b**
	2016	19.6	0.8		18.6	0.5		15.9	0.6		ns	22.0	0.9		6.9	0.2		6.9	0.9		a b b***
3-hydroxy-7,8-dihydro-beta-ionol	2015	16.4	0.1	ns	14.9	1.2	**	18.4	2.3	**	ns	21.4	0.1	ns	10.5	0.9	***	15.0	1.9	***	a c b***
	2016	5.9	5.7		9.2	0.4		7.8	0.6		ns	6.6	6.4		3.3	0.1		3.4	0.5		ns
3-hydroxy-7,8-dehydro-beta-ionol	2015	2.2	0.4	*	2.3	0.3	**	2.9	0.3	***	b ab a*	2.9	0.5	**	1.6	0.2	**	2.4	0.3	***	a b a*
	2016	0.6	0.1		0.4	0.5		0.5	<0.1		ns	0.7	0.2		0.2	0.2		0.1	0.1		a b b*
3-hydroxy-7,8-dihydro-beta-ionone	2015	10.6	0.4	ns	9.0	0.9	*	12.4	1.7	*	ab b a*	13.8	0.5	*	6.3	0.6	***	10.1	1.4	**	a c b**
	2016	9.2	0.4		7.1	0.5		6.7	0.2		a b ab*	10.4	0.4		2.5	0.2		3.0	0.5		a b b***
3-hydroxy-beta-ionone	2015	1.4	2.0	ns	2.6	0.7	ns	3.2	0.1	***	ns	1.9	2.7	ns	1.9	0.5	***	2.6	0.1	**	ns
	2016	2.3	0.4		2.5	0.1		1.8	0.2		ab a b*	2.6	0.5		0.9	0.0		0.8	0.1		a b b***
3-oxo-7,8-dihydro-alpha-ionol	2015	45.5	0.7	**	35.0	2.5	**	36.4	6.8	**	a ab b*	59.4	1.0	***	24.6	1.8	***	29.8	5.6	**	a b b***
	2016	7.8	0.1		5.4	7.0		7.5	0.8		ns	8.8	0.1		2.8	2.2		2.0	1.8		a b b*
3-oxo-alpha-retroionol	2015	3.0	0.4	ns	2.4	0.4	ns	3.9	0.8	*	ab b a*	3.9	0.6	*	1.7	0.2	**	3.2	0.7	**	a b a*
	2016	1.6	0.3		1.6	0.1		1.3	0.0		ns	1.8	0.3		0.6	0.0		0.6	0.1		a b b***
3-oxo-retro-alpha-ionol	2015	2.7	0.5	ns	2.5	0.2	**	3.9	0.6	**	ab b a*	3.5	0.7	ns	1.8	0.1	***	3.2	0.5	**	a b a*
	2016	1.7	0.1		2.0	0.2		1.2	0.2		ns	1.9	0.2		0.6	0.2		0.5	0.1		a b b***
4-oxo-beta-ionol	2015	5.1	0.1	**	4.3	0.4	***	4.7	0.5	**	ns	6.7	0.1	**	3.0	0.2	***	3.9	0.4	***	a b b***
	2016	1.3	0.4		1.7	<0.1		1.4	0.6		ns	1.5	0.5		0.7	0.1		0.6	0.2		a b b*
4,5-dihydrovomifoliol	2015	1.1	0.1	ns	0.3	0.6	ns	1.6	0.6	ns	ns	1.4	0.1	*	0.2	0.4	ns	1.3	0.5	ns	a b ab*
	2016	0.6	0.2		1.1	0.7		0.6	<0.1		ns	0.7	0.3		0.3	0.2		0.3	0.1		ns
3,4-dihydro-3-oxo-actinidol I	2015	3.5	0.9	ns	1.2	2.1	ns	-	-	nd	ns	4.6	1.2	ns	0.8	1.5	*	0.0	0.0	nd	-
	2016	2.3	0.2		2.0	0.2		0.5	0.1		a b b***	2.6	0.2		0.8	0.1		0.2	0.1		a b c***
3.4-dihydro-3-oxo-actinidol II	2015	1.9	<0.1	**	1.9	0.5	*	2.3	0.4	**	ns	2.5	<0.1	***	1.4	0.3	**	1.9	0.3	**	a b b*
	2016	1.1	<0.1		1.1	0.1		0.7	0.0		ns	1.3	<0.1		0.4	0.1		0.4	0.1		a b b***
3,4-dihydro-3-oxo-actinidol III	2015	2.5	0.5	*	2.0	0.2	*	2.6	0.6	*	ns	3.3	0.7	ns	1.4	0.1	***	2.2	0.5	**	a b b*
	2016	1.5	0.1		1.4	0.1		1.0	<0.1		ns	1.7	0.1		0.5	<0.1		0.5	0.1		a b b***
vomifoliol	2015	30	7	ns	29	4	**	38	9	*	ns	39	9	ns	20	3	***	31	8	**	a b ab*
	2016	13	1		10	1		9	1		ns	15	1		4	<0.1		4	1		a b b***
Total C₁₃-norisoprenoids	2015	155	11	**	136	13	***	170	29	**	ns	203	14	**	96	9	***	139	24	**	a b b**
	2016	68	5		64	6		56	0		ns	77	6		24	2		24	2		a b b***
Muscat																					
3-hydroxy-beta-damascone	2016	27.1	2.5	ns	27.4	<0.1	ns	42.6	0.7	ns	b b a***	55.1	5.0	ns	19.6	<0.1	*	24.9	0.4	ns	a b b***
	2017	43.0	18.8		33.8	5.9		21.6	11.0		ns	102	44		31.9	5.6		22.6	11.5		a ab b*
3-oxo-alpha-ionol	2016	53.5	4.5	-	54.9	1.3	-	84.8	1.6	-	b b a***	108.5	9.1	-	39.4	0.9	-	49.6	1.0	-	a b b***
	2017	nd			nd			nd			-	nd			nd			nd			-
3-hydroxy-beta-ionone	2016	25.8	0.5	-	27.2	2.4	-	36.5	0.4	-	b b a***	52.3	1.1	-	19.5	1.7	-	21.3	0.3	-	a b b***
	2017	nd			nd			nd			-	nd			nd			nd			-
3-hydroxy-7,8-dihydro-beta-ionone	2016	31.7	1.4	**	36.4	1.8	***	63.7	3.6	**	b b a***	64.4	2.9	**	26.1	1.3	***	37.2	2.1	**	a c b***
	2017	3.2	2.4		1.6	<0.1		2.2	1.3		ns	7.5	5.7		1.5	<0.1		2.3	1.4		ns
3-oxo-alpha-retroionol	2016	3.6	0.2	-	2.8	0.4	-	5.7	0.7	-	b b a**	7.4	0.3	-	2.0	0.3	-	3.3	0.4	-	a c b***
	2017	nd			nd			nd			-	nd			nd			nd			-
3-hydroxy-7,8-dihydro-beta-ionol	2016	15.4	1.1	ns	14.4	0.7	ns	23.5	0.4	*	b b a***	31.2	2.2	ns	10.3	0.5	ns	13.7	0.2	*	a c b***
	2017	14.1	3.8		13.3	5.7		6.9	4.2		ns	33.4	9.0		12.6	5.3		7.2	4.4		a b b**
4-oxo-beta-ionol	2016	4.7	0.6	-	3.3	0.5	-	4.7	1.5	-	ns	9.5	1.2	-	2.4	0.4	-	2.8	0.9	-	a b b***
	2017	nd			nd			nd			-	nd			nd			nd			-
4,5-dihydrovomifoliol	2016	1.9	2.7	-	5.5	2.3	-	5.5	2.5	-	ns	3.9	5.6	-	3.9	1.6	-	3.2	1.4	-	ns
	2017	nd			nd			nd			-	nd			nd			nd			-
vomifoliol	2016	34.2	2.4	-	26.7	2.3	-	53.6	4.8	-	b b a**	69.3	4.9	-	19.1	1.6	-	31.3	2.8	-	a c b***

	Year																				
	2017	nd			nd			nd			-	nd			nd			nd			-
3,4-dihydro-3-oxo-actinidol I	2016	**3.6**	0.4	-	**3.1**	0.3	-	**4.7**	0.1	-	b b a**	**7.3**	0.7	-	**2.2**	0.2	-	**2.8**	<0.1	-	a c b***
	2017	nd			nd			nd			-	nd			nd			nd			-
3,4-dihydro-3-oxo-actinidol II	2016	**5.6**	1.7	-	**6.3**	0.3	-	**8.0**	0.9	-	ns	**11.3**	3.4	-	**4.5**	0.2	-	**4.7**	0.5	-	a b b*
	2017	nd			nd			nd			-	nd			nd			nd			-
Total C$_{13}$ norisoprenoids	2016	**207**	15	**	**208**	5	***	**333**	6	***	b b a***	**420**	31	*	**149**	4	***	**195**	3	**	a c b***
	2017	**60**	25		**49**	12		**31**	17		ns	**143**	59		**46**	11		**32**	17		a ab b*

Table 4

| Phenols / Chardonnay | Year | Contents in concentration (µg/L) | | | | | | | | | | Quantity per plant (µg/plant) | | | | | | | | | |
| | | Control | | | Low 1 | | | Low 2 | | | S/S | Control | | | Low 1 | | | Low 2 | | | S/S |
		Mean	SD	Y	Mean	SD	Y	Mean	SD	Y		Mean	SD	Y	Mean	SD	Y	Mean	SD	Y	
eugenol	2015	7.2	1.1	ns	7.2	1.7	ns	8.5	1.9	*	ns	9.4	1.5	ns	5.0	1.2	**	7.0	1.5	**	a b b*
	2016	4.3	0.4		4.9	0.4		3.5	0.3		ab a b*	4.9	0.4		1.7	0.1		1.5	0.1		a b b***
phenol	2015	9.8	2.9	ns	13.6	2.7	**	13.1	7.6	ns	ns	12.8	3.7	ns	9.6	1.9	**	10.7	6.2	ns	ns
	2016	5.1	0.8		4.2	1.6		4.5	1.3		ns	5.7	0.9		1.4	0.5		2.0	0.5		a b b***
vanillin	2015	5.7	0.4	*	7.1	1.8	*	9.1	0.5	***	b ab a*	7.4	0.5	*	5.0	1.2	**	7.5	0.4	***	a b a*
	2016	2.9	0.4		3.3	0.2		2.0	0.1		ab a b*	3.2	0.5		1.2	0.1		0.9	0.1		a b b***
unknown 1	2015	0.9	<0.1	**	1.2	0.3	ns	3.6	0.3	***	b b a**	1.2	<0.1	*	0.9	0.2	**	2.9	0.3	***	b b a***
	2016	0.6	<0.1		0.7	0.2		0.6	0.2		ns	0.7	<0.1		0.3	0.1		0.3	0.1		a b b***
methyl vanillate	2015	2.2	0.3	ns	1.4	0.3	ns	1.9	0.6	ns	ns	2.9	0.5	ns	1.0	0.2	**	1.5	0.5	*	a b b**
	2016	0.5	0.7		0.9	0.5		0.7	<0.1		ns	0.5	0.7		0.3	0.1		0.3	<0.1		ns
unknown 2	2015	13.7	0.9	*	11.1	0.7	***	15.9	2.2	***	ab b a*	17.9	1.1	*	7.8	0.5	***	13.0	1.8	***	a c b**
	2016	1.5	2.1		4.1	0.3		2.6	<0.1		ns	1.7	2.4		1.5	0.1		0.8	0.4		ns
zingerone	2015	1.5	0.5	ns	1.4	0.1	ns	1.9	0.3	**	ns	2.0	0.7	ns	1.0	0.1	**	1.5	0.3	**	a b ab*
	2016	0.8	0.1		1.3	0.1		0.6	0.1		ns	0.9	0.1		0.4	0.2		0.3	0.1		a b b**
guayacol-propanol	2015	3.4	0.8	ns	3.9	0.9	ns	3.9	1.9	**	ns	4.4	1.0	ns	2.8	0.6	n	3.2	1.5	ns	ns
	2016	1.5	0.2		6.2	6.6		1.0	0.1		ns	1.7	0.2		1.7	2.0		0.3	0.2		ns
Total phenols	2015	44.3	4.4	*	46.9	6.2	*	57.8	14.8	*	ns	57.8	5.7	*	33.1	4.4	**	47.3	12.1	**	a b ab*
	2016	17.1	1.6		25.5	9.5		15.3	1.2		ns	19.2	1.9		8.5	2.9		6.2	0.4		a b b**

Muscat

| Phenols | Year | Control | | | Low 1 | | | Low 2 | | | S/S | Control | | | Low 1 | | | Low 2 | | | S/S |
		Mean	SD	Y	Mean	SD	Y	Mean	SD	Y		Mean	SD	Y	Mean	SD	Y	Mean	SD	Y	
eugenol	2016	9.2	0.3	-	11	0.8	-	12	0.6	-	b b a**	19	0.7	-	8	0.6	-	7	0.3	-	a b b***
	2017	nd			nd			nd			-	nd			nd			nd			-
phenol	2016	79	7.3	-	65	6.5	-	141	0.1	-	b b a***	160	15	-	47	4.6	-	82	0.1	-	a c b***
	2017	nd			nd			nd			-	nd			nd			nd			-
vanillin	2016	27	0.7	-	26	5.9	-	41	7.0	-	ns	55	1.4	-	19	4.2	-	24	4.1	-	a b b***
	2017	nd			nd			nd			-	nd			nd			nd			-
unknown 1	2016	8.3	3.7	ns	6.3	2.7	***	6.1	1.2	ns	ns	16.9	7.5	ns	4.5	1.9	**	3.6	0.7	**	a b b*
	2017	68	30		73	21		39	25		ns	161	70		69	20		35	22		a ab b*
methyl vanillate	2016	6.0	0.4	-	5.1	0.7	-	8.7	0.7	-	b b a*	12	0.9	-	4	1	-	5.1	0.4	-	a b b*
	2017	nd			nd			nd			-	nd			nd			nd			-
unknown 2	2016	11.3	3.8	-	5.7	1.5	ns	13	1	-	ab b a*	22.9	7.8	-	4.1	1.1	-	7.8	0.5	-	a b b***
	2017	nd			8.4	11.9		nd			-	nd			nd			nd			-
guayacol-propanol	2016	9.9	0.4	-	9.3	0.4	-	18	<0.1	-	b b a***	20.2	0.8	-	6.7	0.3	-	10.3	0.1	-	a c b***
	2017	nd			nd			nd			-	nd			nd			nd			-
Total phenols	2016	150	16	ns	128	8.3	ns	239	4.9	**	b b a***	305	33	ns	92	6	ns	140	3	***	a c b***
	2017	68	30		82	33		39	25		ns	161	70		77	31		35	22		a ab b*

Table 5

Terpenes		Contents in concentration (µg/L)										Contents in concentration (µg/L)									
		Control			Low 1			Low 2			S/S	Control			Low 1			Low 2			S/S
Chardonnay	Year	Mean	SD	Y	Mean	SD	Y	Mean	SD	Y		Mean	SD	Y	Mean	SD	Y	Mean	SD	Y	
linalol oxide *trans*	2 015	0.9	<0.1	***	1.4	0.4	*	1.3	0.2	**	ns	1.2	<0.1	**	1.0	0.3	ns	1.1	0.1	ns	ns
	2 016	2.7	0.1		2.4	0.2		2.0	0.2		a a b**	3.0	0.1		0.9	<0.1		0.8	0.1		a b b***
linalol	2 015	1.4	0.1	*	2.6	0.6	ns	2.3	0.3	ns	b a a*	1.8	0.2	*	1.8	0.4	**	1.9	0.2	*	ns
	2 016	2.5	0.2		2.3	0.3		2.5	0.6		ns	2.8	0.2		0.8	0.1		1.1	0.2		a b b***
HO-trienol	2 015	2.3	<0.1	**	2.2	0.1	ns	2.2	0.4	ns	ns	3.0	<0.1	*	1.6	0.1	**	1.8	0.3	**	a b b***
	2 016	2.1	<0.1		1.8	0.5		2.0	0.1		ns	2.3	<0.1		0.7	0.2		0.8	0.1		a b b***
trans-pyran linalool oxide	2 015	1.1	0.1	ns	1.6	0.3	ns	1.7	0.1	ns	ns	1.5	0.2	ns	1.2	0.2	**	1.4	0.1	***	ns
	2 016	1.4	0.1		1.3	0.0		1.3	0.2		ns	1.5	0.1		0.5	<0.1		0.6	0.1		a b b***
cis-pyran linalool oxide	2 015	0.7	0.1	*	0.8	0.1	**	0.7	<0.1	*	ns	0.9	0.1	*	0.6	0.1	**	0.6	<0.1	ns	a b b**
	2 016	1.1	0.1		1.2	<0.1		1.1	0.2		ns	1.3	0.2		0.4	<0.1		0.5	0.1		a b b***
citronellol	2 015	0.4	<0.1	ns	0.4	0.1	ns	0.5	0.1	ns	ns	0.5	<0.1	ns	0.3	<0.1	ns	0.4	0.1	ns	a b b*
	2 016	0.4	<0.1		0.4	0.0		0.4	0.0		ns	0.5	<0.1		0.2	<0.1		0.2	<0.1		a b b***
nerol	2 015	3.0	0.6	ns	2.4	0.5	ns	2.7	0.3	*	ns	3.9	0.8	ns	1.7	0.4	*	2.3	0.2	***	a b b**
	2 016	2.2	0.1		2.0	0.2		1.8	0.4		ns	2.5	0.2		0.7	0.1		0.8	0.1		a b b***
geraniol	2 015	6.1	0.5	ns	6.1	1.6	ns	7.1	0.8	*	ns	7.9	0.6	ns	4.3	1.1	*	5.8	0.7	***	a b b*
	2 016	5.6	0.7		4.2	0.2		3.9	0.6		a a b*	6.3	0.8		1.5	0.1		1.7	0.3		a b b***
nerol hydrate + geraniol hydrate	2 015	81	6.8	*	87	5.9	***	95	14	*	ns	106	8.9	*	61	4	***	78	12	**	a b b**
	2 016	38	0.9		37	2.3		37	0.1		ns	42	1.0		14	0.6		16	2.1		a b b***
geranic acid	2 015	1.5	<0.1	**	0.9	1.5	ns	nd		-	ns	2.0	<0.1	**	0.6	1.1	**	nd		-	ns
	2 016	0.5	0.1		0.6	0.0		1.8	0.2		b b a***	0.6	0.1		0.2	<0.1		0.8	0.1		b c a***
8-hydroxydihydrolinalool	2 015	1.9	0.2	ns	3.8	0.3	**	4.9	0.9	*	b a a*	2.5	0.3	ns	2.7	0.2	***	4.0	0.8	**	ns
	2 016	1.9	0.2		2.6	0.1		2.3	0.2		b a a*	2.1	0.2		0.9	0.1		0.9	<0.1		a b b***
2,6-dimethylocto-2,7-dien-1,6-diol	2 015	10.7	1.4	ns	15.3	1.1	***	19.0	3.1	**	b ab a*	13.9	1.9	*	10.8	0.8	***	15.6	2.5	**	ns
	2 016	6.7	<0.1		7.9	0.1		7.0	0.4		ns	7.6	0.0		2.8	0.2		3.0	0.2		a b b***
p-menth-1-ene-7,8 diol	2 015	1.7	0.5	ns	1.8	0.1	**	3.0	0.2	***	b b a*	2.2	0.6	ns	1.3	0.1	***	2.5	0.2	***	a b a*
	2 016	0.7	<0.1		0.8	0.3		0.7	0.3		ns	0.8	<0.1		0.3	0.1		0.3	0.1		a b b***
Total terpenes	2 015	113.0	9.1	*	126.7	11.6	***	140.2	20.0	**	ns	147.4	11.9	**	89.2	8.2	**	114.8	16.4	**	a b b**
	2 016	65.5	0.2		64.9	3.1		64.0	0.8		ns	73.7	0.2		23.5	0.9		27.6	2.8		a c b***
Muscat																					
linalol oxide *trans*	2 016	37.3	4.4	ns	63.4	10.0	*	87.2	15.9	ns	b ab a*	75.7	8.9	ns	45.5	7.2	*	51.0	9.3	ns	a b b*
	2 017	26.8	5.9		28.7	2.4		39.5	7.0		ns	63.5	13.9		27.1	2.3		41.3	7.3		a b ab*
linalol oxide *cis*	2 016	23.5	3.3	ns	35.1	11.6	ns	55.9	10.3	*	b ab a*	47.7	6.7	ns	25.2	8.4	ns	32.7	6.0	ns	a b ab*
	2 017	15.1	1.6		13.7	0.6		21.6	2.0		b b a**	35.7	3.7		12.9	0.6		22.6	2.1		a b ab*
linalol	2 016	5.3	1.0	*	7.3	1.4	***	8.3	0.0	**	b ab a*	10.7	2.1	**	5.2	1.0	***	4.8	<0.1	**	a b b*
	2 017	101.6	22.5		92.5	11.7		152.0	14.3		ab b a*	240.4	53.2		87.2	11.0		158.1	15		a b ab*
HO-trienol	2 016	0.6	0.1	-	0.7	0.2	-	1.1	0.2	**	ns	1.2	0.1	-	0.5	0.2	-	0.6	0.1	**	ns
	2 017	nd			nd			4.6	0.04		-	nd			nd			4.8	0		-
terpineol	2 016	10.7	1.2	ns	16.7	2.9	ns	21.6	2.2	*	b ab a*	21.8	2.3	ns	12.0	2.0	ns	12.7	1.3	ns	a b b**
	2 017	11.6	1.0		14.6	10.6		14.4	0.2		ns	27.5	2.4		13.8	10.0		15.1	0.2		ns
trans-pyran linalool oxide	2 016	78.3	7.9	ns	107.8	17.6	ns	132.8	13.3	ns	b ab a*	158.9	16.0	ns	77.3	12.6	ns	77.7	7.8	*	a b b**
	2 017	148.0	26.7		131.9	23.7		125.6	2.1		ns	350.3	63.2		124.4	22.3		131.5	2.2		a b b*
cis-pyran linalool oxide	2 016	12.4	0.6	ns	14.7	1.4	ns	22.2	2.2	ns	b b a**	25.2	1.3	*	10.6	1.0	*	13.0	1.3	*	a b b***
	2 017	17.7	2.2		24.1	7.2		22.0	3.0		ns	41.8	5.2		22.7	6.8		23.0	3.1		a b b*
citronellol	2 016	23.1	2.6	**	42.1	0.5	**	32.5	1.7	*	c a b***	46.9	5.3	**	30.2	0.4	***	19.0	1.0	**	a c b**
	2 017	60.0	4.2		52.1	2.0		65.5	8.5		ns	142.1	10.0		49.1	1.9		68.5	8.9		a b b*
nerol	2 016	315	25	ns	483	17	*	504	19	ns	b a a**	639	51	ns	347	12	***	295	11	**	a b b***
	2 017	557	128		546	16		624	120		ns	1319	303		515	15		653	126		a b b**

Compound	Year																				
geraniol	2016	244	24	ns	332	10	**	434	9	ns	c b a***	495	48	ns	238	7	***	254	5	*	a b b***
	2017	433	84		429	8		525	114		ns	1025	198		404	7		549	119		a b b**
nerol hydrate + geraniol hydrate	2016	nd		-	nd		-	nd		-	-	nd		-	nd		-	nd		-	-
	2017	47	17		45	19		30	16		ns	111	40		43	18		32	16		a b b*
geranic acid	2016	14	4	***	17	3	***	13	1.2	ns	ns	28	8.4	***	12	2.2	***	8	0.7	**	a b b*
	2017	320	7		249	12		255	118		ns	758	16		235	11		267	124		a b b**
exo-2-hydroxycineole	2016	6.9	0.4	-	6.9	0.1	-	11.8	1.3	-	b b a**	13.9	0.7	-	4.9	0.1	-	6.9	0.8	-	a c b***
	2017	nd			nd			nd			-	nd			nd			nd			-
3,7-dimethyl-1,5-octadien-3,7-diol	2016	292	12	ns	391	31	ns	423	21	ns	b b a*	593	24	ns	281	22	ns	247	12	ns	a b b***
	2017	168	165		318	127		246	75		ns	399	391		299	120		257	78		ns
Z-8-hydroxylinalol	2016	6.5	0.9	-	5.8	0.7	-	8.4	1.4	-	ab b a *	13.1	1.9	-	4.2	0.5	-	4.9	0.8	-	a b b***
	2017	nd			nd			nd			-	nd			nd			nd			-
8-hydroxydihydrolinalool	2016	17	1.2	**	20	2.9	ns	26	4	ns	b ab a*	34	2	**	14	2	*	15	2	*	a b b***
	2017	62	1.9		71	32		56	17		ns	146	5		67	30		58	18		a b b**
2,6-dimethylocto-2,7-dien-1,6-diol	2016	355	7.0	**	480	39	*	558	5	*	c b a**	721	14	*	344	28	*	326	3	*	a b b***
	2017	195	7.2		214	71		144	61		ns	461	17		201	67		151	63		a b b**
p-menth-1-ene-7,8 diol	2016	27	1.6	*	24	2.6	*	49	<0.1	-	b b a***	55	3	ns	17	2	ns	29	<0.1	-	a c b***
	2017	13	4.3		12	5.3		nd			b b a*	30	10		11	5		nd			ns
Total terpenes	2016	1468	97	ns	2048	72	ns	2389	104	ns	c b a***	2979	196	ns	1468	52	ns	1397	61	ns	a b b***
	2017	2177	346		2241	208		2346	517		ns	5150	818		2114	196		2457	541		a b b**