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Hubert Alem

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HAL Id: tel-02542663

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**THÈSE POUR OBTENIR LE GRADE DE DOCTEUR
DE MONTPELLIER SUPAGRO**

En AGRONOMIE

**École doctorale GAIA – Biodiversité, Agriculture, Alimentation, Environnement, Terre, Eau
Portée par l'Université de Montpellier**

Unité de recherche Amélioration Génétique et Adaptation des Plantes (UMR 108-AGAP)

**Impact of the carbon source/sink balance on
glycosylated aroma precursors accumulation in
grapevine fruit (*Vitis vinifera* L.)**

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Le 17 décembre 2018

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**UNIVERSITÉ
DE MONTPELLIER**



Acknowledgements

First, I would like to deeply thank Hernán Ojeda for all his invaluable collaboration along these years. The day to day work with Hernán was really enriching for my professional training. No doubt this work could only be done thanks to his continuous support and dedication. I also thank him, as director of the UR Pech Rouge, for receiving me in this unit to carry out the Thesis and all the field and laboratory experiments. But above all, I want to thank him for his friendship.

I would like to thank my thesis director, Laurent Torregrosa, from whom I had continuous support and following-up during all my research. I have significantly learned from his scientific knowledge, which was invaluable for my Thesis. I really thank him for always being present for my questions.

I really want to thank Remi Schneider, my co-director of Thesis. His contributions at the scientific level were fundamental to my research. I thank him for training me in the techniques for the chemical determinations of the Thesis. I would not have been able to overcome some difficult situations in the laboratory, except for his knowledge and support.

I want to give a great thank you to the entire staff of the UR Pech Rouge for their continuous collaboration, without which it would be impossible to perform an investigation of this magnitude. Especially to laboratory staff and field staff: laboratory technicians Yanick Sire and Mélanie Veyert, and vineyard technicians Jean Noël Lacapere, Frédéric Caumette, Robert Fabien, Jean Jacques Regadera, Jérôme Cerutti, Jérôme Degroise, and Emmanuelle Garcia.

I want to thank the directors and staff of the laboratories of Nyseos and the SPO at Montpellier, and specially to Peggy Rigou, for having allowed me to work temporarily in their facilities and for their support and dedication.

Thesis Hubert J. Alem

I want to thank the Faculty of Agronomy of the University of Buenos Aires for having financed my stay in France during the whole period of the Thesis.

I greatly appreciate the invaluable collaboration of the trainees who have participated in this investigation: Silvina Van Houten, Miguel Delucchi, Lautaro García and Lucía Rolón.

I want to highlight the invaluable support from my father and my mother, my sisters and my whole family in Argentina.

And above all, I want to thank my wife and my children, for having supported me throughout this period.

This work is dedicated to my wife Valeria and to my children, Facundo and Joaquina.

Congresses and publications

Part of the present work was presented orally in the: **20th GiESCO International Meeting Mendoza, Argentina, November 5th – 10th 2017.**

The worked presented was entitled: “*Yield impacts on the concentration of aroma precursors in grapevine berries (Vitis vinifera L.)*”.

As part of this Thesis, a review was accepted for publication the 24th August 2018, in the Journal of the Science of Food and Agriculture. (*J Sci Food Agric* 99: 975–985, 2019)

The title of the review is: *Impact of agronomic practices on grape aroma composition: a review*

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Summary

Aroma compounds are secondary metabolites that play a key role in grape quality. Terpenes, C₁₃-norisoprenoids, phenols and non-terpenic alcohols are the most important aroma compounds in grapes and can be accumulated as free volatile or glycoconjugated (bound) molecules. The non-volatile glycosylated aroma precursors (GAP) group is the largest one, and it is present in all varieties of *Vitis vinifera* (L.), the most widely-used species for wine production. The GAP represents the 80-90% of the aromatic potential of grape, depending on the cultivar. Agronomic practices such as irrigation, training systems, leaf removal and bunch thinning can impact the plant and fruit development. The modification of the source/sink relationship (S/S) with the scope of increasing the grape quality, is very common between viticulturists. These practices include bunch thinning, pruning, and the election of the number of buds/plant. Bunch thinning, a very extended practice in viticulture and which directly impacts on S/S, is one of the less researched practice regarding GAP. In many cases, DOC and IGT production protocols include a limit in the fruit yield per hectare. Then, viticulturists regulate yield by managing number of buds/hectare and/or by fruit thinning.

The main objective of our work was to analyze the impact of the modifications of S/S balance on the biosynthesis of GAP. GAP are chosen in this research because: 1) they are present in every cultivar of *Vitis vinifera*, 2) they represent the biggest source of potential aromatic molecules, and 3) because these molecules incorporate glycosyl groups, their accumulation depends on the supply of carbohydrates and potentially on the carbon balance of the plant.

The main objective of our work concerned the study of the impact of the S/S ratio on the biosynthesis of GAP, and its possible modulation depending on the genotype. Five questions were addressed: 1) Influence of the genotype on in the biosynthesis of GAP and its

accumulation. The objective is to analyze the variability of GPA concentration at a given maturity stage among genotypes, including a set of varieties of *V. vinifera* (Marselan, Grenache, Muscat à petits grains blancs - Muscat from now onwards, Cabernet-Sauvignon, Syrah and Chardonnay) and hybrids. *V. vinifera* x *Muscadinia rotundifolia* (G5). 2) Influence of the year on S/S balance and GAP concentration. 3) The impact of the S/S balance on the biosynthesis of GAP expressed in concentration ($\mu\text{g/L}$) and in Content ($\mu\text{g/berry}$) as a function of grape development. 4) The relationship between primary and secondary metabolism (GAP and anthocyanins) and their modulation as a function of S/S balance. 5) Influence of the thinning date on the dynamics of GAP biosynthesis.

The results showed that levels of glycosylated aromatic compounds varied according to genotype. Varieties whose grapes contain terpenic compounds (Muscat and the *V. vinifera* x *Muscadinia rotundifolia* G5 hybrid) showed the highest levels of GAP in both concentration and amount per fruit. These genotypes showed the highest values of GAP/sugar ratio. In general, genotypes producing non-colored berries had higher GAP/sugar ratios than colored berries. Despite strong inter-annual variation, the impact of the S/S ratio on GAP biosynthesis was found to be genotype-dependent.

Thus, the GAP concentration was not affected during the modification of S/S in Cabernet-Sauvignon, while Muscat and Syrah showed large variations in GAP/berry contents as a function of the S/S ratio. The bunch thinning date was also an important modulating factor in the increase of GAP but varies according to genotype. In general, a significant decrease in the amount of primary metabolites accumulated in grapes is required to significantly increase the biosynthesis of secondary metabolites. This gain is very notable for anthocyanins, which are the most abundant carbon compounds after the primary metabolites (sugars and organic acids)

in grapes. With regard to aromatic precursors, the impact is more moderate regardless of the family of glycosylated compounds.

Résumé

Impact de l'équilibre source/puit de carbone sur l'accumulation de précurseurs aromatique glycosylés dans les fruits de la vigne (*Vitis vinifera* L.)

Les composés aromatiques sont des métabolites secondaires qui jouent un rôle clé dans la qualité du raisin. Les terpènes, les C-13 norisoprénoïdes, les phénols et les alcools non terpéniques sont les composés aromatiques les plus importants dans les raisins et peuvent être accumulés sous forme de molécules libres volatiles ou glyco-conjugués. Le groupe des précurseurs aromatiques glycosylés (GAP) est le plus important et il est présent dans toutes les variétés de *Vitis vinifera* (L.), l'espèce la plus largement utilisée pour la production de vin. Les GAP représentent 80 à 90% du potentiel aromatique du raisin selon le cultivar. Les pratiques agronomiques telles que l'irrigation, les systèmes de conduite, l'effeuillage et l'éclaircissage peuvent avoir un impact sur le développement de la plante et des fruits. La modification de la relation source/puit (S/P) dans le but d'augmenter la qualité des raisins est une pratique très courante en viticulture. Ces pratiques comprennent l'éclaircissage, l'écimage et le contrôle du nombre de bourgeons par plante. L'éclaircissage, est une pratique très répandue en viticulture et ayant un impact direct sur le rapport S/P, alors qu'il n'y a pas beaucoup de travaux sur l'effet réel de l'éclaircissage sur l'accumulation des GAP. Dans de nombreux cas, les cahiers des charges des AOP et d'IGP prévoient une limite du rendement en fruits par hectare. Ensuite, les viticulteurs régulent les rendements en gérant le nombre de bourgeons/hectare et/ou en éclaircissant les fruits.

L'objectif principal de nos travaux était d'analyser l'impact des modifications du rapport S/P sur la biosynthèse des GAP. Les GAP ont été choisis car : i) ils sont présents dans tous les cultivars de *Vitis vinifera*, ii) ils représentent la plus grande source de molécules aromatiques

potentielles et iii) car ces molécules incorporant des groupes glycosylés, leur accumulation dépend de la fourniture en hydrates de carbone donc potentiellement de la balance carbonée de la plante.

L'objectif principal de nos travaux a concerné l'étude de l'impact du rapport source/puit sur la biosynthèse des GAP, et sa modulation éventuelle en fonction du génotype. Cinq questions ont été abordées : 1) L'influence du génotype sur la biosynthèse des GAP et son accumulation à un stade de maturité donné. 2) L'influence de l'année sur la croissance du raisin et l'accumulation des GAP. 3) L'impact de l'équilibre S/P sur la biosynthèse des GAP exprimé en concentration ($\mu\text{g/L}$) et en quantité ($\mu\text{g/baie}$) en fonction du développement du raisin. 4) La relation entre les métabolismes primaire et secondaire (GAP et anthocyanes) et leur modulation en fonction de l'équilibre S/P. 5) Influence de la date d'éclaircissage sur la dynamique de biosynthèse des GAP.

Les résultats ont montré que les teneurs en composés aromatiques glycosylés variaient en fonction du génotype. Les variétés dont les raisins contiennent des composés terpéniques (Muscat à petits grains blancs and l'hybride *V. vinifera* x *Muscadinia rotundifolia* G5) ont présenté les plus grandes teneurs en GAP aussi bien en concentration qu'en quantité par fruit. Ces mêmes génotypes ont montré les valeurs les plus élevées du rapport GAP/sucre. En général, les génotypes produisant des baies non colorées ont présenté des ratios GAP/sucre plus élevés que les variétés à baies colorées.

Malgré de fortes variations interannuelles, l'impact du rapport S/P sur la biosynthèse de GAP s'est révélé être dépendant du génotype. Ainsi, la concentration en GAP n'a pas été affectée lors de la modification du S/P dans le Cabernet-Sauvignon, alors que Muscat et Syrah ont présenté de fortes variations de teneurs en GAP/baies en fonction du rapport S/P. La date d'éclaircissage s'est également révélée un facteur de modulation important de l'accroissement des GAP, mais variable en fonction du génotype. D'une façon générale, une diminution

importante de la quantité de métabolites primaires accumulés dans le raisin est nécessaire pour augmenter significativement la biosynthèse de métabolites secondaires. Ce gain est très notable pour ce qui concerne les anthocyanes, qui sont les composés carbonés les plus abondants après les métabolites primaires (sucres et acides organiques) dans le raisin. Pour ce qui concerne les précurseurs aromatiques, l'impact est plus modéré quelle que soit la famille de composés glycosylés.

1. Chapter I: Study context

1.1 Introduction

1.1.1 Grapevine origin

Grapevine is a perennial crop, clonally propagated and domesticated since approximately 8000 years ago in the Near East, as stated in archeological and genetic data (Yongfeng Zhou et al., 2017). Transcaucasia (Georgia, Armenia, and Azerbaijan) and Eastern Anatolia are the regions where *Vitis vinifera sylvestris* was domesticated and where the first wines were produced (Stefan et al., 2015). A single grape species (*Vitis vinifera* L. subsp. *sylvestris*) was the origin of 99% of the domesticated grapes that are cultivated nowadays all around the world (Stefan et al., 2015). Along centuries during the domestication process, grape was selected to become hermaphrodite, to have an increased sugar content and a bigger berry size. In this process, white cultivars were also selected. During the Neolithic period, from about 8500 to 4000 B.C., viticulture was developed and has been innovating since then (Mc Govern et al. 1995). A pottery jar containing grapevine wine residuals was found in Northern Zagros (Iran), dating from 5500 BC. This finding coincides with the human settlement, agriculture development and pottery making period (Mc Govern et al. 1996; Jackson, 2008, Stefan et al., 2015). Evidences demonstrates that wine was elaborated from a domesticated grapevine and not from the wild type. Later, it took centuries (table 1) to spread the viticulture and viniculture all around the world (Chambers and Pretorius, 2010).

Table I.1: Grapevine chronology

(Adapted from Chambers and Pretorius, 2010)

| | |
|------------------------------|--|
| 2000 AD - 1000 AD | Australia, New Zealand, Japan, South Africa, California Mexico, Peru, Chile, Argentina |
| 1000 AD - AD Birth of Christ | Eastern Europe, Germany |
| 1000 BC - AD Birth of Christ | Northern France |
| 2000 BC -1000 BC | Southern France, Spain, Portugal, India, China, Morocco |
| 3000 BC - 2000 BC | Sicily, mainland Italy |
| 4000 BC - 3000 BC | Italy, Crete, Greece, Phoenicia (ancient Canaan Lebanon, Syria, Palestine, Israel) |
| 5000 BC - 4000 BC | Egypt |
| 6000 BC - 5000 BC | Other areas of Asia minor, Iran, Iraq, Turkey, Caucasus (Georgia) and Mesopotamia (Ancient Persia) |
| 6000 BC - 7000 BC | Region between the Black and Caspian Seas |

1.1.2 Crop importance

Nowadays, grapevine is one of the most economically and culturally important crop in the world (Migicovsky et al. 2017). It represents the biggest fruit cultivar worldwide, with 7.5 million of hectares, and 5 countries concentrates approximately the 50% of the surface: Spain, China, France, Italy and Turkey. Approximately the 50% of the surface of the mentioned countries is dedicated to wine grapevine vineyards, although this percentage depends on the country. For example, to compare two opposite situations, China dedicates 83% of grapevine

production to fresh grapes, whereas in France 99% is used to produce wine. According to the OIV definition, “Wine is the beverage resulting exclusively from the partial or complete alcoholic fermentation of fresh grapes, whether crushed or not, or of grape must. Its actual alcohol content shall not be less than 8.5% vol. Nevertheless, considering climate, soil, vine variety, special qualitative factors or traditions specific to certain vineyards, the minimum total alcohol content may be able to be reduced to 7% vol. by legislation particular to the region considered¹.”

Nowadays, the main wine producing countries are: Italy, France, Spain, USA, Australia, Argentina, China, South Africa and Chile, although the order of countries changes every year depending on each country harvest due to climate conditions².

The main wine exporting countries are France, Italy, Spain, USA, Chile and Germany. The EU is the world's leading producer with over 45% of the area planted with vines (around 3.4 million hectares) and concentrates the 60% of wine production in the world. The EU also the first consumer, with around 60% of world consumption and the world's largest exporter and importer. The world wine consumption for 2016 was 241 million hectoliters, which represents an increase from year 2000, but it is a huge decrease compared to the maximum point reached in the 1970s (approximately 350 million hectoliters). 50% of the world consumption is concentrated in 5 countries: USA, France, Italy, Germany and China. In the last 20 years China has triplicated its wine consumption. Interestingly, while wine trade volume has increased from 60 to 105 million of hectoliters between year 2000 and 2016, the value of the world wine commerce has largely increased from 12 to 29 billion Euro in the same period³.

1.1.3 Importance of aromas in wine quality

Wine is a highly chemically complex product. The aroma of wine has a profile of more than a thousand volatile compounds. The aroma is one of the most important factors in determining wine character and quality. But only some of them plays an outstanding role in the sensory perception of each wine (Rocha et al. 2010). One part of those molecules is biosynthesized in berries, and the other part is the result of winemaking and ageing. Reactions like alcoholic and malolactic fermentations, and chemical and enzymatic processes, produce a very complex aroma profile. Many authors have studied the influence of grape aroma on wine quality (among others: Gunata et al. 1985; Wilson et al. 1986; Voirin, et al. 1992a; Voirin et al. 1992b; Ugliano et al. 2006; Ugliano and Moio, 2008). San Juan et al. (2012) investigated the influence of aroma on wine quality concerning the final price of the product. They stated that the more expensive wines were richer in wood-related compounds, ethyl phenols, cysteinyl-derived mercaptans, volatile sulphur compounds, ethyl esters of branched acids, methional, and phenylacetaldehyde. Instead, the cheapest wines had an opposite profile, being richer in E-2-nonenal, E-2-hexenal, Z-3-hexenol, acetoin, and ethyl lactate. Luckic et al. (2007) showed the relationship between quality and aroma profile of Malvazija istarska wines. They found that isoamyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, hexanoic acid, octanoic acid and decanoic acid were important to distinguish the highest quality wines. Wines evaluated with higher amounts of isoamyl alcohol and isobutanol showed lower sensory evaluation scores.

1.1.4 Origin and classification of aroma molecules

The aroma of wine is the result of the interaction of barely a thousand volatile compounds of different nature and chemical origin, and its concentration can vary between a few ng/L and some μ /L. The threshold of perception, typical of each compound, determines its olfactory impact, and there is a complex relationship between them, evidenced in the synergistic and masking phenomena.

Many authors make a classification of the aromas of grapes and wine based on the origin of its formation and transformation. This classification is arbitrary, and in some cases the limit between each category is not precise. Therefore, aromas can be classified in primary, secondary and tertiary aroma compounds. The primary or varietal aromas are those present in the berry, originated by their own metabolism. The biosynthesis and concentration of this aroma compounds are determined by genetic and environmental variables, and also by the different agronomic practices. The aromas classified as secondary, are originated during winemaking as a result of alcoholic and malolactic fermentations performed by yeast and bacteria. The tertiary aromas are generated after fermentation as a result of the biochemical reactions occurring during wine ageing, either in barrels, tanks and even bottles. There are varieties of *Vitis vinifera* considered as aromatic, such as Muscat of Alexandria, Muscat of Frontignan, Gewuztraminer, Italian Muscat (between others), due to the high content of free terpenes present in the berry (up to 6 mg/L), which characterize these types of cultivars. Varieties with a content between 1-4 mg/L are considered as non-Muscat or intermediate aromatic (Traminer, Huxel, Kerner, Muller-Thurgau, Riesling, Schurebe, Wurzer, etc.). Non-aromatic or neutral wines with a low content of terpenes are the most abundant cultivars, for

example Pinot Noir, Chardonnay, Sauvignon, Cabernet-Sauvignon, Merlot, Chenin blanc, etc. (Mateo et al., 2000).

1.1.5 Bibliography review

The bibliography review presented here, is focus on the effect of agronomic practices on grape aroma compounds. It was published the 24th August 2018 in the Journal of the Science of Food and Agriculture and is titled as follows: *Impact of agronomic practices on grape aroma composition: a review*.

This review shows the state-of-the-art involving the effects of vineyard agronomic management on the biosynthesis of grape aroma compounds. The first part describes the molecules involved in the aroma of grapes intended for winemaking. In the following part the agronomic practices like leaf removal, canopy training systems and pruning, irrigation, exogenous compounds application, foliar fertilization and bunch thinning, are reviewed and analyzed.

The review gives the background to the thesis, showing which are the physiological mechanisms involved in the aroma compounds biosynthesis that are known and which remain unknown. Thus, it shows the gaps in research literature regarding the aroma compounds and vineyard management.

Note: The references of the review are shown at the end of this Chapter.

Impact of agronomic practices on grape aroma composition: a review

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Abstract

Aroma compounds are secondary metabolites that play a key role in grape quality for enological purpose. Terpenes, C₁₃-norisoprenoids, phenols and non-terpenic alcohols are the most important aroma compounds in grapes and can be found as free volatile or glycoconjugated (bound) molecules. The non-volatile glycosylated group is the largest one, and it is present in all varieties of *Vitis vinifera* (L.), the most widely-used species for wine production. These aroma precursors represent the reserve of aroma molecules that can be released during winemaking. Their relative and absolute concentrations at fruit ripening determines the organoleptic value of the final product. A large range of biotic and abiotic factors can influence their biosynthesis in several ways. Agronomic practices such as irrigation, training systems, leaf removal and bunch thinning can impact at plant level. The spraying of stimulatory compounds on fruit at different developmental stages has also been shown to modify metabolic pathway at fruit level with some impact on the aroma composition of the grapevine fruit. Viticulturists could act to promote aroma precursors in order to improve the aromatic profile of grapes and the wine ultimately produced. However, agronomic practices do not always have uniform results. The metabolic and physiological changes resulting from the agronomic practices are unknown, because there is not sufficient research to date. This review presents the state-of-the-art regarding the influences of vineyard agronomic management on the biosynthesis of grape aroma compounds. Although literature regarding the topic is abundant, there are still many unknown biological mechanisms involved and/or studied deeply. Therefore, the aim of this work is to find the gaps in scientific literature so that future investigations can focus on them.

Key words: *Vitis vinifera*, grapevine, fruit quality, aroma compounds, agronomic practices

INTRODUCTION

Wine, which results from the fermentation of crushed grapes, is a chemically complex product with an aromatic profile involving more than a thousand volatile compounds. Aroma is one of the most important factors in determining wine character and quality¹ and the concentration of aroma molecules can vary between a few ng L⁻¹, some µg L⁻¹, or even mg L⁻¹ (table 1).

| Molecule group | Compound | Typical conc. | Variety | Reference |
|----------------|--------------------|-------------------------------|--------------------|---|
| Norisoprenoids | β-damascenone | 10 (µg L ⁻¹) | Riesling | Kwasniewski et al. (2010) ⁷¹ |
| | TDN | 71 (µg L ⁻¹) | Riesling | Kwasniewski et al. (2010) ⁷¹ |
| | α-ionone | 17 (µg L ⁻¹) | Cabernet-Sauvignon | Bindon et al. (2007) ¹⁰⁹ |
| C6 compounds | Total glycosylated | 357.71 (µg Kg ⁻¹) | Merlot | Song et al. (2012) ¹¹³ |
| | Hexanol | 338.57 (µg Kg ⁻¹) | Merlot | Song et al. (2012) ¹¹³ |
| Thiols | (S) 3MHCys | 5-9 (µg L ⁻¹) | Riesling | Roland et al. (2010) ⁵⁸ |
| | (R) 3MHCys | 4-6 (µg L ⁻¹) | Riesling | Roland et al. (2010) ⁵⁸ |
| | (S) 3MHGlu | 97–119 (µg L ⁻¹) | Riesling | Roland et al. (2010) ⁵⁸ |
| | (R) 3MHGlu | 35–38 (µg L ⁻¹) | Riesling | Roland et al. (2010) ⁵⁸ |
| Terpenes | Total glycosylated | 1899 (µg L ⁻¹) | Riesling | Friedel et al. (2016) ⁸⁴ |
| | Linalool | 862 (µg L ⁻¹) | Riesling | Friedel et al. (2016) ⁸⁴ |
| | Geraniol | 121 (µg L ⁻¹) | Riesling | Friedel et al. (2016) ⁸⁴ |

Table 1 – Aroma compounds concentrations in grape

Part of those molecules are biosynthesized in berries, and the other part results from winemaking and aging²⁻⁶. Many authors have studied the influence of grape aroma on wine

quality⁷⁻¹⁹. Understanding how these factors impact grapes' composition is critical to developing strategies for adapting viticultural practices to climate change²⁰⁻²².

Plant volatile compounds (VOCs), which are determined by the variety and may be influenced by vineyard management and biotic or abiotic stresses, have a key role in grape quality. VOCs are secondary metabolites, and as already shown in previous research on phenolic compounds²³, agronomic practices can influence the content and profile of secondary metabolites in two different ways. Directly, where biosynthesis of the molecules changes as a result of the agronomic practice, and indirectly as a consequence of a variation in the concentration of the molecules, due to changes in the fruit volume and weight²³. While these influences have been recorded in phenolic compounds, it is possible to extrapolate this response could occur in aroma VOCs.

Aroma compounds in plants are typically found both as “free” or “bound” (glycosylated) to a sugar moiety. When bound, these compounds have little or no active odor; however, upon hydrolysis of the glycosides, these compounds may then be volatilized, becoming active odor molecules. In grapes and wine, a large proportion of volatile aroma compounds are found in the bound form²⁴.

Objectives and limits of this review

This review is mainly concentrated on the effects of agronomic practices on *Vitis vinifera* (L.) grape aroma molecules (free and glycosylated aroma precursors) and illustrates the gaps in scientific knowledge so that future investigations can focus on them. Volatile molecules, which are produced during winemaking (fermentation and aging), are not considered here,

although some research is reported where otherwise relevant. The effect of climate and soil features (including soil fertilizers) is also not analyzed in this review.

Grape aroma molecules

Terpenes

Terpenes (also called isoprenoids) represent one of the biggest groups of secondary metabolites in plants²⁵. They are present in the grape berry in free and bound (glycosylated) forms²⁶. Terpenes have an important role in plant resistance to diseases caused by fungi and bacteria²⁷. They are primarily found in the berry's skin, and in minor proportions in the flesh. Glycosylated terpenes are non-volatile and non-odor producing molecules and constitute a large source of potential volatile molecules once fermentation occurs. Glycosylated terpenes are more abundant than free terpenes, depending on cultivar. For example, in Muscat of Alexandria the total glycosylated terpenes concentration is more than 2 times that of free terpenes (ranges 1500 and 4000 $\mu\text{g L}^{-1}$, respectively)⁷. They are usually conjugated to glucose, arabinose, rhamnose and apiose⁹. In a free form, terpenes are very volatile and odorous compounds, and they are mainly present as monoterpenes (C_{10} molecules) and sesquiterpenes (C_{15} molecules). Terpenes represent the most important and analyzed group of VOCs. Notably, some terpenes are also considered as primary metabolites²⁸. In the plant kingdom, terpenes include hemiterpenes (C_5), monoterpenes (C_{10}), sesquiterpenes (C_{15}), diterpenes (C_{20}) and tetraterpenes (C_{40})²⁹. Terpenes are composed of one or more five carbons molecules

(isopentyl diphosphate - IPP), which are the basic units of terpenes. Two alternative pathways for terpenes' biosynthesis are hypothesized at this time (figure 1).

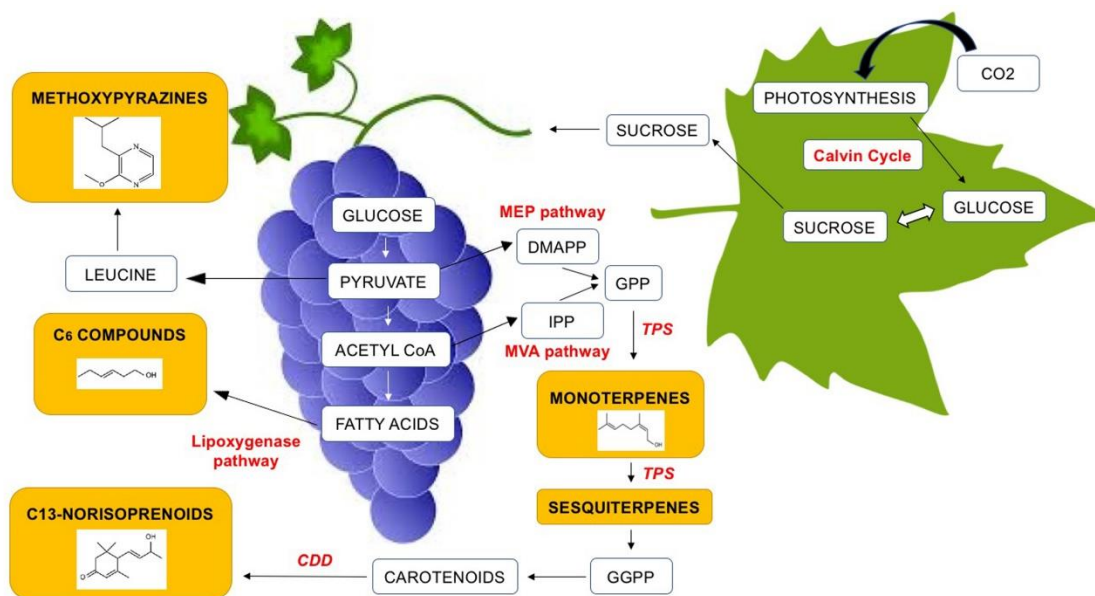


Figure 1 – Simplified VOCs metabolic pathways

Abbreviations: VOCs, volatile organic compounds; DMAPP, dimethylallyl pyrophosphate; IPP, isopentenyl pyrophosphate; GPP, geranyl diphosphate; GGPP, geranylgeranyl pyrophosphate; TPS, terpene synthase; CDD, carotenoid cleavage dioxygenase; MEP, methylerythritol phosphate; MVA, mevalonic acid

The first is the mevalonic acid (MVA) pathway, which originates with the acetyl-CoA located in the cytosol. The second pathway is known as the methylerythritol phosphate (MEP) pathway, which originates with the pyruvate and glyceraldehyde-3-phosphate, located in plastids³⁰⁻³¹. The condensation of these basic five carbon molecule units form molecules of ten, fifteen, twenty, thirty and forty carbon molecules. Monoterpenes (C₁₀) are typical of

aromatic varieties, like Muscat, Gewürztraminer and Malvasia. In non-aromatic varieties, monoterpenes are present but in a much lower concentration, thus below their olfactory odor threshold. Sesquiterpenes (C₁₅) are found in the berries' epicuticular wax. Within sesquiterpenes, the ketone rotundone is a key aroma compound for the peppery character of high-quality Syrah wines³².

Glycosylated terpenes can be freed after enzymatic or chemical hydrolysis and can thus contribute to an increase in odor intensity of wine³³. It is well established that both endogenous and exogenous enzymes exhibit a two-step hydrolysis mechanism. Firstly, α -rhamnosidase, α -arabinosidase and β -D-apiosidase cleave the terminal sugar, releasing rhamnose, arabinose and apiose. The aroma moiety is finally released through the action of β -D-glucosidase, which releases the bound sugar molecule³⁴. In terms of aroma moiety, generally monoterpenes are the most abundant and consist of hydrocarbons, aldehydes and alcohols. Alcohols such as linalool, terpenol, nerol, α -terpineol, geraniol and citronelol, are monoterpenes with a great olfactory impact and low perception thresholds. Many authors have reviewed the importance, metabolism and structure of terpenes in grape³⁵⁻⁴¹.

Norisoprenoids

Norisoprenoids are VOCs with a cyclic structure of 9, 10, 11 or 13 carbon atoms. The C₁₃-norisoprenoids, the most abundant norisoprenoid in grapes, is the most important for black and white wine aromas. Among these molecules, we can find: TCH (2,2,6-trimethylcyclohexanone), β -damascenone, β -ionone, vitispirane, actinidiol, TDN (1,1,6-trimethyl-1,2-dihydronaphthalene) and Riesling acetal⁴². They are derived from the

biodegradation of carotenoids, followed initially by enzymatic conversion to the aroma precursor, and secondarily by the acid-catalysed conversion to the aroma-active compounds⁴³. C₁₃-norisoprenoids are among the most important aroma molecules in wine and provide floral and fruity attributes⁴⁴. There are two main groups of norisoprenoids: the megastigmane forms and the non-megastigmane forms. The megastigmanes skeleton is characterized by a benzene cycle substituted on C₁, C₅ and C₆⁴⁵. β -damascenone and β -ionone belong to this group and influence the aromatic profile of grapes and wine. Carotenoids in nature can be degraded by a chemical, photochemical and/or oxidative reaction. Carotenoids are located principally in the berries' skin²⁴. It has been established that carotenoid cleavage is not regiospecific, and as a result, is responsible for the formation of C₉, C₁₀, C₁₁, C₁₃ and C₁₅ atoms⁴⁶. Nevertheless, several studies have shown that there is a regiospecific reaction in the berry⁴⁷. In another study, a gene (VVCCD1) was identified, which gene was involved in the formation of the carotenoid cleavage dioxygenase (CCD) enzyme, responsible for cleavage of the carotenoid in the berry⁴⁸. The enzyme catalyses the symmetrical cleavage of zeaxanthin and lutein to generate 3-hydroxy- β -ionone and 4,9-dimethyldodeca-2,4,6,8,10-pentaene-1,12-dialdehyde. Interestingly, the gene is induced at the early stages of the grape berry's development. Almost all C₁₃-norisoprenoids found in wine and grape juice are derived from their glycosylated precursors^{47,49}. They are found also in the leaves of *Vitis vinifera* Riesling, with a concentration between ten and a hundred times greater than that found in grapes alone, but with very similar molecular structures in both organs⁵⁰. Furthermore, the concentration of carotenoids in the berry is very low, as opposed to the carotenoid concentration in the leaves⁵¹. Although leaves seem to be the most important source of reserve for glycosylated C₁₃-norisoprenoids, there is evidence that there is not an important translocation from the leaves to the berries²⁴.

Methoxypyrazines

Methoxypyrazines (MPs) are free VOCs which highly influence the aromatic profile of wine, reminiscent of a bell pepper and asparagus-like aroma in Sauvignon, Chardonnay, Semillon, and Riesling varieties⁵². These flavors can be desirable or non-desirable in Cabernet-Sauvignon, Merlot, Cabernet franc and Carmènere, depending on concentration. These volatile N-containing heterocycles derive from the metabolism of amino acids, although the pathway of biosynthesis is not clear yet. Furthermore, it is not clear if they are synthesized in the leaves and then translocated and/or if they are synthesized *in situ*⁵³. Seven MPs have been detected. The most important MPs in grapes and wine are the 3-isobutyl-2-methoxypyrazine (IBMP), the 3-isopropyl-2-methoxypyrazine (IPMP), and the 3-sec-butyl-2-methoxypyrazine (SBMP)⁵³. MPs are located principally in stems (79.2%); in berries, most of the IBMP molecules are in the skin (72%). The seeds contain 23.8% of the IBMP and the pulp contains a very little amount of IBMP (4.2%)⁵⁴. Concentrations of MPs are very low in grape and wine (approximately 2–30 ng L⁻¹)⁵³. The odor perception threshold is very low, with values between 1 and 16 ng L⁻¹ in wine and 1–2 ng L⁻¹ in water⁵⁵.

C₆ alcohols and aldehydes

Many C₆ compounds are derived from fatty acid molecules throughout oxidation by the lipoxygenase (LOX) pathway and are partly responsible for the green aromas in grapes and wine. Besides alcohols and aldehydes, there are other C₆ compounds involved, which are ketones, acids, esters, and lactones⁴³.

Thiols

Volatile thiols are sulfur compounds which are very important to the wine aromatic profile⁵⁶. Thiols are found in grapes in a bound form. Grape thiols are originated from fatty acids and are usually bound with cysteine or glutathione and are odorless until enzymatic release. In winemaking, during fermentation thiols are freed. This occurs when the enzyme carbon-sulfur (C-S) lyase, from some yeast strains release the thiols. The C-S lyase enzyme has been determined to be responsible for the cleavage of cysteine-S-conjugated forms of the 3-mercaptohexyl acetate (3MH) and the 4-mercapto-4-methylpentan-2-one (4MMP) into free thiols. Researchers have selected the most effective yeast strains to improve the release of free thiols, thus increasing wine aroma⁵⁷. The conversion of these cysteinylated precursors into their corresponding free thiols is accepted to be very limited, typically less than 5%⁵⁶. Also called mercaptans, thiols can give noxious odors to wine, but in low concentrations thiols can provide desirable blackcurrant, citrus and passion fruit aroma to wines. Notably, thiols' perception threshold is very low, from 0.8 to 60 ng L⁻¹, depending on the kind of thiol molecule involved. There are 3 thiols which have notable effects on wine aroma: the 4MMP thiol,

provides blackcurrant and guava aromas with a perception threshold of 0.8 ng l^{-1} . The 3MHA thiol provides a passion fruit aroma with a perception threshold of 9 ng L^{-1} and the 3-mercaptohexan-1-ol, (3MH) provides grapefruit aroma with a perception threshold 50 ng L^{-1} . Ripe Sauvignon blanc and Cabernet-Sauvignon berries are characterized by thiol molecules. In many cultivars, “passion fruit” and “grapefruit” aromas are produced by thiols as well⁵⁸.

AGRONOMIC PRACTICES

Agronomic practices like leaf removal, canopy training systems, foliar fertilization, irrigation, spraying of exogenous compounds and bunch thinning, can have an impact on primary and secondary metabolism. In some cases, these practices can also have a direct impact on the aroma pathways of the fruit. However, in most cases, primary metabolism is first affected, and this can indirectly modify the aromatic profile (figure 2).

All the interactions between primary metabolism involved in the regulation of aroma compounds are not completely understood. There is still a lack of sufficient information on how agronomic practices can modify aroma metabolic pathways.

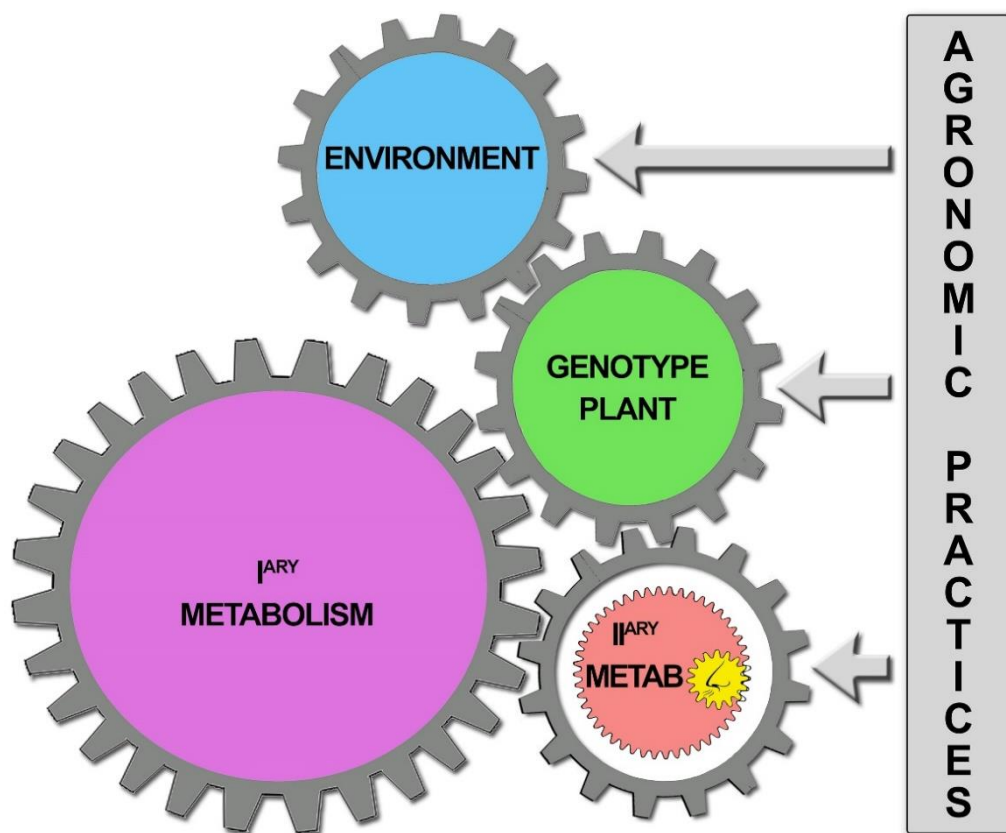


Figure 2 - Relationship between agronomic practices and primary and secondary metabolism

Leaf removal

It is well known that plants microclimate can influence the final fruit composition, regarding primary and secondary metabolites⁴³. Sunlight composition and intensity are the most important parameters influencing the microclimate of bunches, often in conjunction with the effects of temperature. It is not clear if the impact of sunlight on berry composition is the result just of sunlight, of temperature, or both, since it is difficult to separate their effects when trials are performed in vineyards conditions⁵⁹. Trials performed at the gene level, however, have demonstrated that temperature and sunlight have separate effects on the concentration of a berry's flavonoids⁶⁰. Sunlight quality and intensity which reaches a bunch may be altered by many external factors that viticulturists can manipulate. The slope of the vineyard and row orientation can greatly influence the sunlight's effect. Vineyards with rows facing south in the north hemisphere are always more illuminated. Further, in mountainous regions, slopes facing south are always more exposed to sunlight⁵⁹. Vine vigour is also a key parameter because it determines the level of shading affecting the grapes. Vine vigour can be manipulated by rootstock and cultivar selection, and by many viticulture practices like pruning, irrigation, fertilization, etc.⁶¹⁻⁶⁷.

One of the most important viticulture practices for enhancing the amount of sunlight which reaches the bunch is leaf removal around the fruit zone. This usually enhances aromas and avoids rot⁶⁸. In fact, this is the method used by most researchers to study the influence of sunlight on aroma precursors. Many authors have examined the influence on aroma molecules of light intensity and/or composition on the bunch zone. Most of them have focused their

research in the main aroma molecules: glycosylated aroma precursors, free aroma volatiles, C₁₃-norisoprenoids, terpenes and methoxypyrazines. Although the removal of basal leaves around a bunch is usually chosen to increase the exposure to light, some authors have used artificial shading (boxes e.g.) for reaching the scope⁶⁹⁻⁹².

Carotenoid derived C₁₃-norisoprenoids are well known for providing floral and fruity aromas to wine and for having a very low detection threshold. Some of these compounds, such as the 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), which are detected at very low thresholds (e.g. 20 µg/L in Riesling wines), provide a very distinctive flavor of kerosene-scent⁹³. Many authors^{20,59,93;69-72} have studied the influence of bunch exposure to sunlight on glycosylated and/or free C₁₃-norisoprenoids. The effect of sunlight and shade on the C₁₃-norisoprenoid levels found in Riesling and Chenin blanc berries has been reported⁶⁹. Sun-exposed bunches showed an increase in C₁₃-norisoprenoid concentration in berries and wine. Interestingly, β-damascenone concentration did not change between control plants and leaf removed plants, but an enhancement of TDN was observed⁶⁹. Therefore, too much shade can result in rot and low concentration of aroma precursors, but an overexposure to sun can also lead to an undesirable gain in TDN concentrations. Similar results were obtained in Riesling grapes and in Cabernet-Sauvignon^{59,70}. Most sun exposed bunches were richer in TDN and vitispirane⁵⁹. On the other hand, where no leaf removal occurred, C₁₃-norisoprenoid concentrations were enhanced, especially for β-damascenone⁶⁹.

The importance of the timing of leaf removal around a bunch has also been shown in cv Riesling⁷¹. Authors have found that when fruit zone leaf removal occurred 33 days after berry set (PBS), the concentration of TND was at a maximum level. In contrast, when leaf removal was performed earlier, 2 days PBS or later (68 days PBS), TDN concentrations were not increased. β-damascenone concentrations decreased after leaf removal treatment by 30%

compared to control plants, except for 33 days PBS where no differences were found⁷¹. These results show the importance of the timing of leaf removal dependent on the objective of the viticulturist. Such timing issues should be analyzed as related to regional agro-climatic conditions. Carotenoids, C₁₃-norisoprenoid and terpenol concentrations also increased in berries of Sauvignon blanc directly exposed to solar radiation after leaf removal²⁰.

The effect of canopy shading in glycoconjugate monoterpenes and C₁₃-norisoprenoids was studied in Sauvignon blanc grapes⁷³. The authors observed that the concentration of these molecules decreased when canopy density was higher, while IBPM concentrations increased⁷³. Similar results were obtained for Muscat à Petit Grains blancs, where concentrations of glycoconjugate phenolics, C₁₃-norisoprenoid and terpenol molecules decreased in shaded bunches⁷⁴. Melon grapes' glycoconjugates aroma precursors and Colombard wines derived thiols, 3-mercapto-hexanol and acetate were also studied⁷⁵. Treatments consisted of a combinations of bunch shading and training systems. Melon grapes from a trellised system, where leaves were thinned out, resulted in richer glycoconjugate aroma precursors concentrations than grapes from non-trellised systems. Leaf removal practices did not decrease the grape thiols in Colombard wines. Other results obtained in Riesling research, showed that free and bound aroma compounds like hexen-1-ol, linalool, α -terpineol, β -damascenone and geraniol were positively affected by sunlight exposure⁷⁶. Glycosylate terpenes and C₁₃-norisoprenoids concentrations increased when vines of Pinot noir were subjected to leaf removal^{72,75}.

The influence of light in the concentration of free and/or bound terpenols has been extensively studied^{74,78,79;81-84}. Most of the authors applied artificial shading to the bunches, but one of them applied a leaf removal treatment^{78,79}. Results were very similar for most of the authors, the more exposed the bunches, the more terpenol concentrations increased. By contrast, in a

study conducted in Sicily on the white cv. Grillo, shading increased concentrations of free and glycosylated terpenes, C₁₃-norisoprenoids, benzenoids and C₆-alcohols⁸². Possibly this result occurred due to the warm climate conditions and possible heat stress on the grapes. Although sugar content was decreased in shaded plants, the aroma potential was enhanced.

The 3-isobutyl-2-methoxypyrazine (IBMP) is a methoxypyrazine known for giving wine herbaceous, musty, and unripe aromas. IBMP could contribute positively to wine aroma, but excessive levels above the perception threshold (16 ng/L approx., depending on cultivar) are considered undesirable. Most researchers have found that exposure to sunlight decreases the content of IBMP; treatments consisted mostly of leaf removal⁸⁵⁻⁸⁸, or by artificially shading the berries with a box⁸⁹. In bunches of Cabernet franc exposed to sunlight in pre-onset of ripening, the IBPM concentrations were lower than results in the shaded bunches. The post-onset of ripening shading treatments had no impact on IBPM levels⁸⁵. In another research study⁸⁶, the authors studied the effect of basal leaf removal on IBPM concentrations in Cabernet franc and Merlot berries. Treatments consisted in either removing 50% or 100% of leaves from the fruiting zone at either 10 days after anthesis; 40 days after anthesis, or 60 days after anthesis. On Cabernet franc grapes, it was shown that only early (pre-onset of ripening) basal leaf removal treatments reduced the concentration of IBPM at harvest. In the 2007 trials, a reduction in IBPM concentrations in the treated plants resulted in a range between 46-88 % compared to the control plants, while in 2008 the reduction in the treated plants was between 34-60 %. In Merlot trials, all basal leaf removal treatments reduced IBPM concentration at harvest⁸⁶. In contrast, shaded Cabernet-Sauvignon berries showed an increased concentration of IBPM compared with the control plants berries; the expression of *VvOMT3* (a methyltransferase gene recently found to be responsible for methoxypyrazine production) also increased⁸⁹. Another research group found, that in Sauvignon blanc grapes, there were

differences in IBPM concentration but there was not an effect on the genic expression⁸⁸. However, some authors^{91,92} did not find differences in IBPM concentrations after leaf removal (before anthesis) in Cabernet-Sauvignon and Sauvignon blanc (after anthesis).

Canopy training systems

Canopy training combined with the pruning methods can also influence sunlight reception^{75,94,95}. Many researchers have studied the effects of canopy training systems on aroma precursors. A research group tested five different training systems for five years: alternate double crossarm (ADC), Lenz Moser (LM), low cordon (LC), low-V (LV) and pendelbogen (PB). They evaluated the concentrations of both, free and bound terpenes in Riesling berries. Results showed significant differences between training systems and that the ADC system produced the highest concentration of free and bound terpenes, although it is not clear if the results were a consequence of concentration/dilution or not. Authors concluded that in the ADC system, bunches receive more light, which resulted in the increase in terpenes⁹⁴. Three different training systems were also evaluated for Viognier: vertical shoot-positioned (VSP), Smart-Dyson up and down (SD up; SD down), and Geneva double curtain (GDC). Researchers found that the SD up and GDC methods resulted in the highest concentrations of total free and bound aroma molecules, although results were expressed in concentration ($\mu\text{g L}^{-1}$). More exposed bunches could be the reason for the increase in the concentration of aroma molecules⁹⁵. Another research study on Viognier compared two training systems during four seasons in the south of France: vertical shoot positioning (VSP) and minimal pruning (MP)⁹⁶. Glycosylated aroma precursor concentrations ($\mu\text{g L}^{-1}$) in MP

berries, were significantly higher than in VSP by the following amounts: C₆ compounds (+38%), aromatics alcohols (+46%) and C₁₃-norisoprenoids (+133%). Although there were differences between years, the effects of the training systems were always the same. Authors concluded that the MP training system provided a greater aroma potential for grapes than the VSP system. This result occurred mainly due to the positive effect on the concentration of aroma glycoside precursors by a reduction in berry size caused by the MP system. For instance, the content of aroma precursors on a berry basis (ng berry⁻¹) was similar for both training systems. But, the MP training system reduced berry weight and volume, increasing the concentrations of aroma precursors (expressed in ug L⁻¹ of must). There was no impact on the biosynthesis of aroma precursors, when analyzed on a berry basis⁹⁶.

Foliar fertilization

Foliar fertilization is an extended and worldwide agronomic practice. Literature regarding foliar fertilization usually focuses on aromatic profiles in wine, especially in white wines. Sulfur and nitrogen foliar fertilization can increase berry thiols, which then enhances the aroma quality of the wine after fermentation. These applications are extensively practiced with Sauvignon blanc⁹⁷⁻¹⁰¹. On the contrary, very little research is dedicated to the influence of foliar fertilization on the aromatic profile and metabolic pathways in berries. The foliar application of proline, phenylalanine, urea and nitrogen fertilizers at Tempranillo vineyards was studied in Spain¹⁰². Treatments consisted of the application of water solutions with a concentration of total N of 750 mg/L of proline, phenylalanine, urea and commercial nitrogen products. Control plants were sprayed with a water solution alone. Terpenes decreased and

norisoprenoids did not change with any of the treatments. Treatments with phenylalanine increased the concentrations of 2-phenylethanol and 2-phenylethanal, but decreased the C₆ compounds, compared to control plants. Further, phenylalanine applications were the sole treatment that improved the presence of some aroma molecules.

Copper (Cu) is largely used as an anti-cryptogamic in vineyards all around the world. It has been used for pest management since the 1880s and is known as the Bordeaux mixture¹⁰³. Copper is also one of the most important biopesticides used in organic farms, being found to be effective against many crops' pests, as fungicide and bactericide¹⁰⁴. Although the Bordeaux mixture application is a common vinicultural practice, we found no research regarding the effects of copper on the grape aroma molecules in berries. Some researchers, however, have studied the effect of copper on the aroma profile of wine. A research study performed in the Bordeaux area on Sauvignon blanc, Cabernet-Sauvignon and Merlot wine, from grapes sprayed with a copper solution, showed decreased thiol molecule concentrations compared with the control plants. The difference in thiol molecule concentrations occurred in the 3-mercaptohexanol in Sauvignon blanc, Merlot and Cabernet-Sauvignon wines and occurred in the 4-mercapto-4-methylpentan-2-one concentrations in Sauvignon blanc¹⁰⁵. In a study carried out in Portugal on wines from cv. Vinhão grapes treated with the Bordeaux mixture or on control plants treated with a triazole-based fungicide, the aromatic profile of the wine was altered in the treated grapes. Some molecules decreased: isobutanol, isoamyl alcohol, acetaldehyde, isovaleric acid, γ -butyrolactone, diacetyl, ethyl furoate, *m*-cresol, 4-allyl-2,6-dimethoxyphenol, vanillin, ethyl vanillate, acetovanillone and δ -decalactone. In other molecules the concentrations increased: ethyl acetate, acetic acid, acetoin, benzyl alcohol, syringaldehyde, ethyl propanoate, ethyl butyrate, ethyl hexanoate, ethyl octanoate, ethyl isobutyrate, isoamyl acetate, isobutyl acetate, γ -nonalactone and linalool¹⁰⁶. Although there is

not a sensory evaluation of wines in this research study, results evidence the great impact of Bordeaux mixture on the aroma profile of this wine.

Irrigation

Water has an important role in plant production, in regard to primary and secondary metabolism, and as a result many authors have studied the influence of water limitations on the concentrations of aroma compounds. This is a critically important issue because many viticulture regions in the world are becoming warmer and dryer due to climate change, and this can impact grape quality²¹. Besides negative effects of extreme lack of water, moderate water stress is often used for increasing grape quality and saving water. Water stress impacts the biosynthesis of aroma compounds in different ways depending on the molecule family concerned. One research shows that the concentrations of bound 3-hydroxy- β -damascenone, a precursor of β -damascenone, increased under water deficit treatments. On the other hand, water deficit did not affect the concentrations of free terpenes studied (limonene, linalool, α -terpineol and geranyl acetone), nor the linalool glycosides concentrations¹⁰⁷. The effect of water deficit in Sauvignon grapes on 3 cysteinylated thiol precursors has also been studied¹⁰⁸. The 4-mercapto-4-methylpentan-2-one (4MMP), 4-mercapto-4-methylpentan-2-ol (4MMPOH) and the 3-mercaptohexan-1-ol (3MH) concentrations were analyzed after plants were submitted to water stress treatments. Severe water stress (predawn leaf water potential reaching -1.0 MPa) negatively affected the aroma potential. In contrast, vines under mild water deficit showed the highest aroma potential¹⁰⁸. In another research, the effect of water

stress on three C₁₃-norisoprenoid molecules (β -damascenone, β -ionone, and 1,1,6-trimethyl-1,2-dihydronaphthalene) was studied in Cabernet-Sauvignon¹⁰⁹. Water stress was triggered by a partial root zone drying system (PDR), in which plants received 66% of the water received by the control plants. The concentrations of the three molecules (expressed in ng g⁻¹) were increased by water stress treatments over the two seasons studied. Nevertheless, when results were expressed in terms of ng berry⁻¹, the results showed no significant difference¹⁰⁹. Possibly, results expressed in concentration (ng g⁻¹) occurred due to changes in the volume and/or weight of the berry as a consequence of water limitation. Similar results were obtained by a different research group with Cabernet-Sauvignon¹¹⁰. This group found that water stressed plants, produced berries with a higher concentration of total glycosylated aroma precursors, measured by the phenol-free glycosyl glucose (PFGG) method¹¹⁰. However, analyzing results expressed in terms of precursors per berry, there were no significant differences. These results again show that the berries higher concentrations of aroma molecules in berries is due sometimes to the reduction of berry size induced by water stress.

The influence of water stress at a metabolite level was studied by integrated transcript and metabolite profile methods¹¹¹. Studies were carried out in a red and in a white genotype: Cabernet-Sauvignon and Chardonnay. In control plants, water was provided by drip irrigation as soon as stem water potential reached -0.6 Mpa; in stressed plants water was provided at -1.2 Mpa. Water deficit affected mostly the phenylpropanoid, ABA, isoprenoid, carotenoid, amino acid and fatty acid metabolic pathways. Although the study did not provide aroma precursors analysis, it provides important information about the enzymatic activity, showing that water deficit, impacted positively on the abundance of enzymes involved in aroma precursors production. For example, the transcript abundance of terpenoid synthetase increased significantly in Chardonnay at maturity. Furthermore, water deficit increased the

transcript abundance of carotenoid cleavage dioxygenase (CCD) in both cultivars. CCD cleaves zeaxanthin into a C₁₃-norisoprenoid and a C₁₄-dialdehyde, both volatile compounds¹¹¹. Although the literature shows clear effects of water deficit on fruity aromas in both red and white wines, it is not possible to directly relate enzyme activity and volatile production¹¹².

The influence of deficit irrigation on the free and bound aroma molecules in Merlot was investigated¹¹³. The authors observed that the concentrations of C₆ compounds, known for giving non-desirable aromas to wine, was decreased in berries from water stressed plants, but there was no effect on free terpene molecules. On the other hand, water stress increased the concentration of some bound terpenols as nerol and geraniol, as well as the C₁₃-norisoprenoids and β -damascenone (in both free and bound forms). In conclusion, water stressed plants increased aroma compound concentrations and decreased non-desirable aroma compound concentrations in berries¹¹³.

The effects of irrigation on the volatile aroma profile of Muscat blanc (*Vitis vinifera* L.) grapes grown in the north-west region of Italy was also investigated¹¹⁴. Three water regimes were compared: standard irrigation (pre-dawn water potential levels above -0.2 MPa), moderate irrigation (pre-dawn water potential levels above -0.2 MPa until the onset of ripening and -0.2 to -0.4 Mpa after onset of ripening) and drought (no irrigation). Free linalool and geraniol concentrations were higher in the standard irrigated plants than in the drought regime, with 78% more free linalool and 73% more free geraniol than the drought plants¹¹⁴. The impact of water stress on dimethyl sulfur potential (DMSP) during maturation of berries was studied in the south of France¹¹⁵. In that research, there were well irrigated control plants, plus plants exposed to three levels of water stress. Authors found that water stressed plants produced berries and wine with an elevated concentration of DMSP. At maturity, the DMSP content of

berries was also higher for water stressed plant treatments than for control plants. Furthermore, in wines originated from water stressed plants grapes, the DMSP concentration showed also higher concentrations of DMSP¹¹⁵.

Methoxypyrazines (MPs) are aroma molecules derived from the condensation of NH₃ with an amino acid, such as valine or leucine. These molecules are synthesized in the berry and are known for providing wines with detrimental herbaceous flavors¹¹⁶. The olfactory detection threshold of MPs is as low as 2 ng mL⁻¹ and becomes undesirable above this threshold¹¹⁷. An increase of berry MPs was reported (analyzed at metabolite and molecular levels) when Carmènere plants were exposed to water stress and lateral shoot removal¹¹⁸. An increase of MPs was also seen in wine produced from treated berries. Although it is not possible to separate water stress from leaf removal effects, both practices (often used to reduce MPs herbaceous aroma), seemed to negatively impact the wine's quality when used together.

In 2016 research, the effects of water deficiency on secondary metabolism of white grapes was studied using large scale metabolite and transcript profiling methods¹¹⁹. This detailed and extensive study analyzed most of the secondary metabolism compounds. The research was performed in two different water regimes with Tocai Friulano, and plants were irrigated when ψ_{stem} reached -0.8 and -1.5 Mpa in control plants and stressed plants respectively. The authors studied the effect of water deficiency on phenolic, carotenoid, tocopherol and free aroma VOCs metabolites and transcriptomes at 6 different stages of berry development. Results showed that 12 of 37 free VOCs had concentrations and transcripts that were increased in the berries of treated plants, sampled at the late ripening period. Monoterpenes (hotrienol, linalool, nerol and α -terpineol) concentrations (μ kg⁻¹) were found to be increased at this period. Water deficit also modulated the expression of several genes in the MPE and MVA terpene pathways. Some genes (1-deoxy-D-xylulose-5-phosphate synthase and 1-deoxy-D-

xylulose-5-phosphate reductoisomerase; VviDXS and VviDXR respectively) were down-regulated by water deficiency in an early stage (41 DDA), while another VviDXS was down-regulated later at 68 days after anthesis DDA and up-regulated 93 DDA. In addition, the research showed that 7 terpene synthases genes were up-regulated mostly at 93 DDA. Water stress also impacted the gene expression profile; two terpene synthases (VviTPSs) (VIT_12s0134g00030 and VIT_19s0014g04930) were up-regulated at 82 DAA. These results suggest that the over-production of monoterpenes is part of the fruit's response to drought. C₁₃-norisoprenoids like β -ionone and β -damascenone, are key molecules for wine quality. The authors of the study also found that water stress up-regulated the expression of genes related to the norisoprenoids synthesis enzymes, the (9,10) (9',10') cleavage dioxygenase (VviCCD4b) at 68 DAA and down-regulated the expression of VviCCD4a at 93 DAA. Besides, a higher degradation of carotenoids was observed under water deficit. Nevertheless, there was no modulation observed in C₁₃-norisoprenoids concentrations. The authors conclude that many VOCs are accumulated in the early stages of grape development but when water stress continues for an extended period, several VOCs accumulate in later stages¹¹⁹.

Although literature regarding the topic is abundant, results are sometimes expressed solely in terms of concentration ($\mu\text{g g}^{-1}$), lacking the information about the content on a berry basis, which is the only way to evaluate the modulation of the abundance through biosynthesis or through water accumulation into the fruit. For instance, in many research studies, water limitation significantly decreased berry weight by 20 to 30%. Thus, it is unknown if there is a direct influence from water stress in the biosynthesis of aroma compounds or if it is due to the concentration of the molecules. In the future it would be desirable for authors to express results in $\mu\text{g L}^{-1}$ and $\mu\text{g berry}^{-1}$. The information necessary to calculate this is clearly known (weight or volume of the berry).

Exogenous product applications

Volatile molecules impacting vines can also be absorbed by the leaves and translocated to the berries, and as a consequence change the berries aromatic profile¹²⁰. Many authors have studied the impact on grape quality of smoke released from fire surrounding vineyards (for review, see Krstic et al., 2015)¹²⁰. The impact of spraying oak extracts onto grapes has also been also extensively studied¹²¹⁻¹²⁴. Molecules studied were cis-oak lactone, trans-oak lactone, furfural, 5-methylfurfural, eugenol, 6-methoxyeugenol, guaiacol, 4-vinylguaiacol, 4-ethylguaiacol, 4-ethylphenol, syringol, vanillin, acetovanillone, 3-methyl-4-hydroxyoctanoic and methyl-vanillate. Oak extract sprayed onto grapes increased oak aroma in the berries, and some of the aforementioned compounds were also found in the wine produced from the treated grapes. Interestingly, in this process, aroma volatiles were absorbed and glycosylated in the plant, and then liberated during winemaking. In a recent research study¹²⁵, it was shown that an aqueous extract of toasted Airen (*V. vinifera*) lignified canes sprayed onto microvine shoots, increased total glycosylated aroma precursors by more than 3 times, from 126.27 to 434.05 μL^{-1} . This demonstrates that it could be possible to manipulate grape aroma composition by spraying different molecules onto the plant. When plant growth regulators or elicitors, such as abscisic acid (ABA) or methyl jasmonate (MeJA) were applied to plants, a positive response on the secondary metabolism was observed^{126,127}. An increase of C₆ compounds in Cabernet-Sauvignon berries after application of ABA and MeJA at the onset of ripening, combined with an increase in the LOX activity, was also observed¹²⁶. Applications of ABA and gibberellic acid in Malbec plants resulted in an increase in the level of monoterpenes and sesquiterpenes in the berries¹²⁷.

Bunch thinning: modification of source/sink balance

As in other fruit crops, fruit removal is a worldwide practice to regulate the source/sink ratio to increase the accumulation of secondary metabolites. Despite the fact that there is much research about the effects of bunch thinning on wine composition, there are just a few studies concerning the bunch thinning effects of VOCs found in berries. A research showed that Cabernet-Sauvignon wine aromas and flavors responded positively to yield manipulation when yield was altered early in fruit development¹¹². Also, the effect of bunch removal on the wine aroma showed an enhancement of sensory attributes in Grenache wines, and a reduction in sensory attributes in Tempranillo wines¹²⁸. The concentrations of free volatile terpenes and glycosylated terpenes in Sauvignon blanc berries, were significantly increased in bunch thinned plants, and concentrations were the highest when bunch thinning was performed one week before the onset of ripening¹²⁹. In another research performed on Cabernet-Sauvignon, IBPM concentrations were increased 40% in bunch-thinned plants (on a concentration basis), or up to 80% (on a per berry basis)⁸⁹. In 2017 a research showed that the modification of the source/sink ratio (50% of bunch thinning), influenced both the primary and secondary metabolism and that the true impact of bunch thinning was genotype and timing dependent¹³⁰. The glycosylated aroma precursors (expressed on a per berry basis - $\mu\text{g berry}^{-1}$) were increased when bunch thinning was performed before the onset of ripening with Syrah and at the onset of ripening with Muscat. In contrast, in Cabernet-Sauvignon, bunch thinning did not have an impact on the aromatic profile. Thus, bunch thinning was useless for enhancing the aromatic potential of Cabernet-Sauvignon grapes.

Despite the importance of this topic, there is little information about how plants regulate the accumulation of aroma compounds in relation to C balance changes. Furthermore, most articles showed the effects of bunch thinning on the quality of the grapes and/or the resultant wines, but few researchers considered the effects of bunch thinning on the entire aromatic profile.

CONCLUDING REMARKS

Literature regarding grape aroma compounds is abundant and results show that agronomic practices can modify primary and secondary metabolite concentrations in the berry in a genotype-dependent way. Nevertheless, the physiological mechanisms and interactions at different plant/organ levels are poorly understood. Most studies have focused on changes in VOCs concentrations at a single sampling date with no interpretation of the biosynthesis pathway modulation. In addition, the research mostly focuses on free or bound aroma precursors, or a single category of molecules (terpenes, phenols, etc.) but not the whole grape aroma profile. This provides only a partial analysis because sometimes for example, when a molecule group is increased by a certain treatment, another molecule group is decreased, thus creating an aromatic profile imbalance. Furthermore, some practices could increase non-desirable molecules such as IBPM (methoxypyrazine). We can also observe a lack of interpretation in the research to date regarding the timing of treatments which can affect different groups of molecules, because the different molecules are synthesized at different times during the reproductive cycle. Furthermore, in most studies the results were only reported in terms of metabolite concentrations with no information about the dynamic of the berries' growth. This reveals a strong limitation in the research, because, with no information provided on the timing of phloem loading and water uptake, metabolic effects can be confused with dilution/concentration effects. This is especially critical for all practices that potentially impact primary metabolism and fruit expansion, such as water deficit or source/sink ratio manipulations.

Agronomic practices used to increase the accumulation of secondary metabolites in berries remain empirically performed. Available research provides some information, but results

cannot be extrapolated from year to year and generalized to different agro-climatic situations. Are all practices applicable to every genotype or vineyard situation? Does the timing of performing or the intensity of a treatment affect the different molecule groups in the same way? Another important issue is related to the cost/benefit analysis of practices that actually reduce the grape yield (e.g. water limitation, source/sink manipulations). Indeed, to understand the gain resulting from such practices, viticulturists must be able to compare the effect of secondary metabolite concentrations versus the net loss in biomass and metabolites yield.

ACKNOWLEDGEMENTS

This research was supported by the Institut National de la Recherche Agronomique (INRA)-UEPR Pech Rouge, France, the Centre d'Etudes Supérieures Agronomiques de Montpellier (Montpellier SupAgro), France and the Unité Mixte de Recherches Sciences Pour l'Œnologie (UMR SPO), France.

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1.2 Objectives and hypothesis

1.2.1 Introduction

Grape quality for enological purpose, depends (among other parameters) on the concentration and variability of glycosylated aroma precursors (GAP). GAP represent the 80-90% of the aromatic potential of grape, depending on the cultivar (Park et al. 1991).

Many agronomic practices which are performed for enhancing the berry quality, involve metabolic processes that are not completely understood. Most of the practices are performed in an empirical way. The modification of the source/sink relationship (S/S) is very common between viticulturists, and includes practices like bunch thinning, pruning, and the selection of the trailing system and number of buds/hectare.

In the bibliographic review publication entitled “*Impact of agronomic practices on grape aroma composition: a review*” (Alem et al., 2018; published on 24th August 2018 in the *Journal of the Science of Food and Agriculture*), conclusions show that there are few publications concerning the impact of the source/sink (S/S) balance on GAP biosynthesis. Bunch thinning, a very extended practice in viticulture and which directly impacts on S/S, is one of the less researched practice regarding GAP. In many cases, DOC and IGT production protocols include a limit in the fruit yield per hectare. Then, viticulturists regulate yield by managing number of buds/hectare and/or by fruit bunch thinning.

The main objective of this research is to analyze the impact of the modifications of S/S balance on the biosynthesis of GAP. GAP are chosen in this research because:

- they are present in every cultivar of *Vitis vinifera*,
- they represent the biggest source of potential aromatic molecules

- the fact of being bound to carbohydrates molecules, indicates a possible link with the primary metabolism.

Then, a principal research hypothesis is postulated:

1.2.2 Hypothesis:

The changes of the source/sink level impact on the biosynthesis of glycosylated aroma precursors, and this impact is genotype and timing-dependent.

(Definition: source/sink, is the relationship between leaves and fruits)

Based on this hypothesis, five main objectives are proposed:

1.2.3 Main objectives

1) Influence of genotype in GAP biosynthesis

The goal is to analyze the variability between genotypes, including a set of *V. vinifera* varieties (Marselan, Grenache, Muscat, Cabernet-Sauvignon, Syrah and Chardonnay) and the *V. vinifera* x *Muscadinia rotundifolia* G5 hybrid (Ojeda et al., 2017) regarding the biosynthesis of GAP. For this objective, 8 genotypes were explored in 2015 (one of them in 2016). GAP contents ($\mu\text{g}/\text{berry}$) and concentrations ($\mu\text{g}/\text{L}$) were analyzed from berries sampled at physiological maturity (methodology shown in Chapter II.2.6, Materials and methods). Some of them were also sampled and analyzed before, during and after physiological maturity. The results are presented in Chapter III.3.1, pg. 94.

2) Influence of the year on fresh fruit weight and GAP concentration

A comparison between years will be analyzed in this point, to evaluate how does the effect “year” impacts on aroma precursors and fresh fruit weight. Results show the concentration ($\mu\text{g/L}$) and content of GAP ($\mu\text{g/berry}$) and fresh fruit weight (kg/plant) along two or three years in Muscat, Cabernet-Sauvignon, Syrah and Chardonnay. Results are shown in Chapter II.3.2, pg. 104.

3) Impact of S/S balance on GAP biosynthesis

The aim is to analyze the concentration ($\mu\text{g/L}$) and content ($\mu\text{g/berry}$) of GAP for three levels of S/S. Evaluations were made for the 8 cultivars during 2015, 2016 and 2017, and treatments consisted in the modification of the S/S by different practices explained in Chapter II (Materials and methods). Results are shown for berries in physiological maturity, and can be seen in Chapter III.3.3, pg 107.

4) Relationship between primary and secondary metabolism

The scope is answering the following questions:

- Is there a link or relationship between primary and secondary metabolism of grapes when S/S is modified?
- Does GAP and sugar content change when of S/S is modified?
- Are anthocyanins and GAP impacted the same way or not?
- Does the level of S/S affect in the same way the eight cultivars studied in this research?

Then, 2015, 2016 and 2017 results are analyzed to identify possible relationships between both metabolisms. Results are shown in Chapter III.3.4, pg. 113.

5) Influence of bunch thinning timing on GAP biosynthesis

This part of the research is focused on the timing of bunch thinning as a practice to modify S/S balance. Therefore, trials consisted in bunch thinning the grape bunch in two periods of the growing cycle. First, 2-3 weeks before onset of ripening, when green berries grow at high rate (RGS, rapid growth stage), and the second one at the onset of ripening (OOR). Bunch thinning levels were 50% and 70% depending on the year (details are explained in Chapter II- Materials and methods). Results are shown in Chapter III.3.5, pg. 120.

1.2.4 Secondary objectives

This research has also the scope of evaluating the effect of S/S balance on GAP concentrations from a productive point of view. For this purpose, two secondary objectives are proposed:

1) Quantify the balance between the loss in sugar and the gain in GAP when source/sink is modified

The aim is to analyze how much sugar content at plant level is lost by decreasing S/S, to obtain an increase in GAP concentration. This is analyzed at plant level and is important from a productive point of view. The results are shown in Chapter III.3.6, pg. 125.

2) The economic balance between reducing yield (kg/ha) and increasing the potential of grape quality

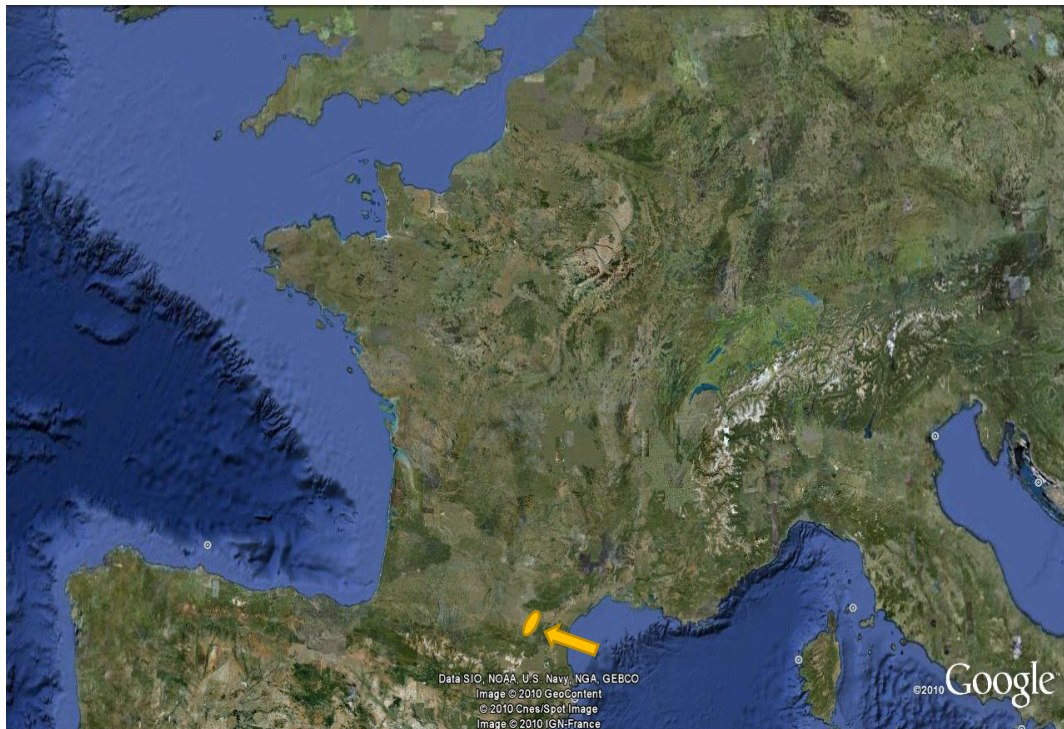
A simulation was made to reflect viticulturists incomes per hectare when yield is reduced in order to increase quality. Results are shown in Chapter III.3.7, pg. 127.

2. Chapter II: Materials and methods

2.1 Location

Experiments were held during the growing seasons of 2015, 2016 and 2017, at the UE INRA Pech Rouge, located in the South of France, in the Occitanie region (coordinates: Lat = $43^{\circ}8'35.180''$ - Lng = $3^{\circ}7'57.442''$), close to the Mediterranean Sea.

Figure II.1.1: Geographic location of Pech Rouge



The UE INRA Pech Rouge has approximately 38 hectares of well managed vineyards. There are approximately 20 different varieties, the most representatives of the Occitanie area, and 250 genotypes in evaluation at the site. The plots for the experiments were chosen from those with uniform soil, management, conducting system and rootstock. At least three rows of the

border of the plot were let to avoid border influence, and three plants from the beginning of the row were also let.

2.2 Climate

Temperature during the growing season was similar during the three years of experiment, although there is a shift of the curve in 2016.

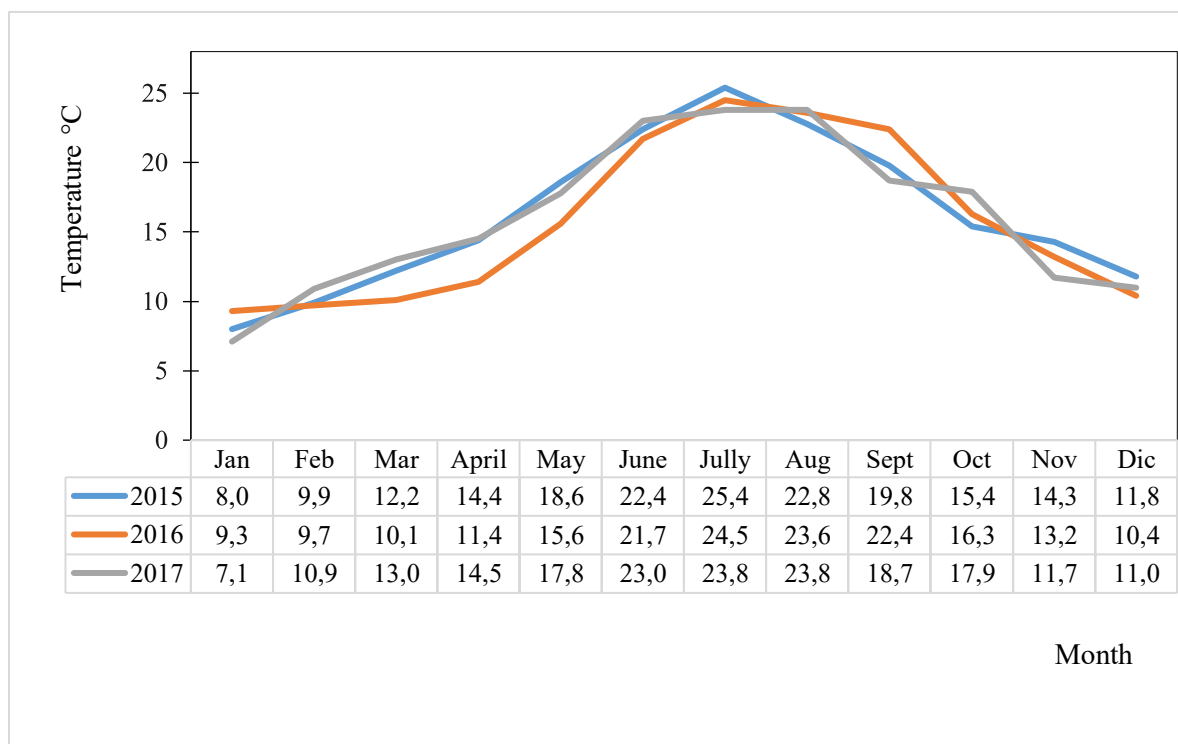


Figure II.2.1: Monthly temperature average (°C) during 2015, 2016 and 2017. (Source: adapted from INRA Pech Rouge data base)

Precipitation were higher during 2016 (429,5 mm), although most of the rainfall occurred during October and November (113,0 and 68;0 mm respectively), out of the growing season.

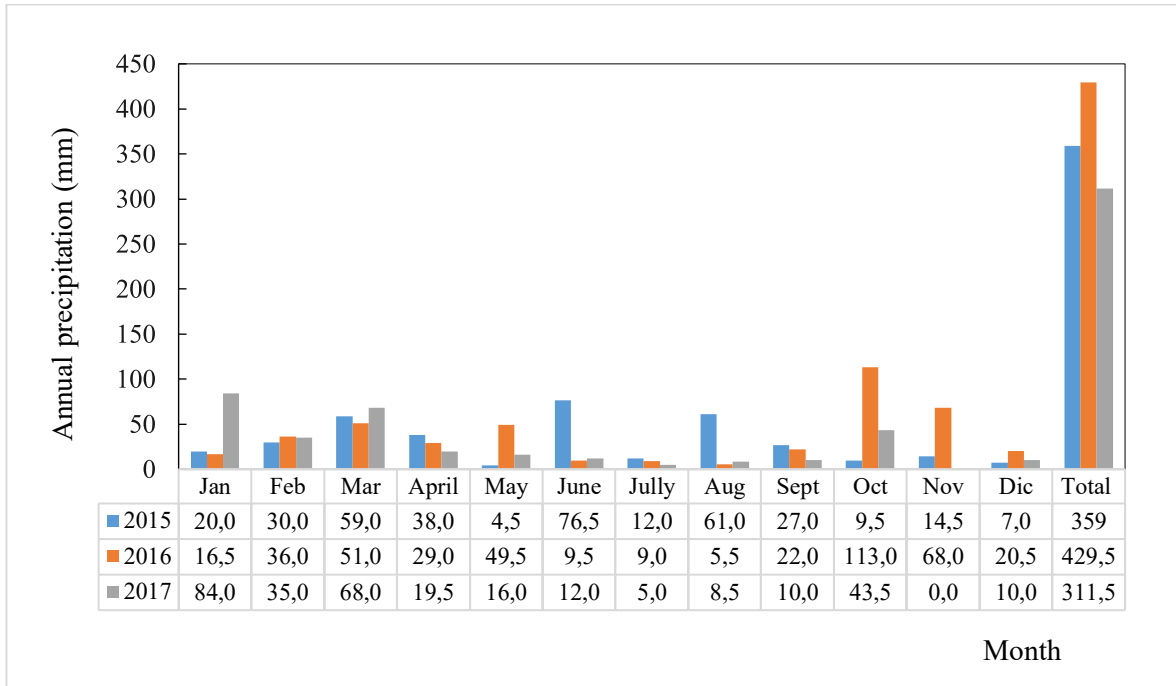


Figure II.2.2: Annual precipitation distribution during 2015, 2016 and 2017.

Potential evapotranspiration (ETp) was very similar during the three years.

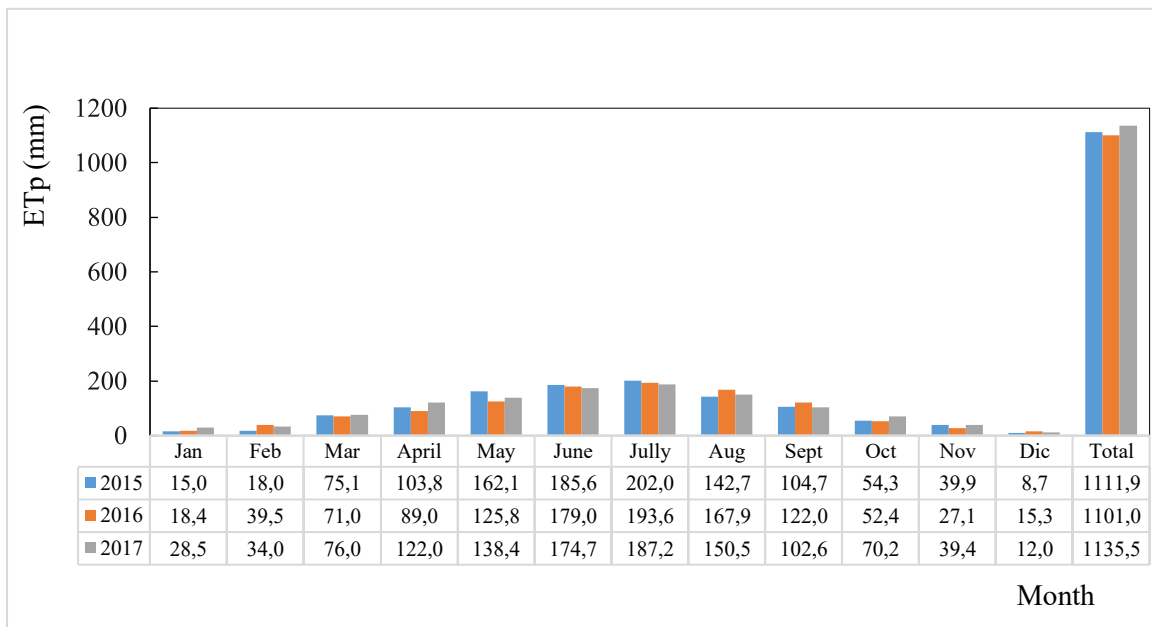


Figure II.2.3: ETp (mm) during 2015, 2016 and 2017.

Table II.2.1: Cool night index

| | 2015 | | 2016 | | 2017 | |
|--|---------------|---------|---------------|-------|---------------|-------|
| | Sampling date | T min * | Sampling date | T min | Sampling date | T min |
| Syrah | 19-8 | 19,66 | 29-8 | 18,43 | 22-8 | 19,38 |
| | 2-9 | 18,80 | 13-9 | 17,35 | 15-9 | 17,43 |
| | 14-9 | 17,21 | | | | |
| Cab. Sauv | 24-8 | 18,92 | 1-9 | 17,35 | 11-9 | 17,98 |
| | 4-9 | 18,66 | 19-9 | 17,02 | 25-9 | 17,98 |
| | 28-9 | 15,95 | | | | |
| Chardonnay | 19-8 | 19,66 | 22-8 | 19,08 | | |
| G5 | 7-9 | 17,99 | | | | |
| Marselan | 27-8 | 18,44 | | | | |
| Grenache | 21-8 | 19,29 | | | | |
| | 3-9 | 18,69 | | | | |
| Viognier | 18-8 | 19,81 | | | | |
| Muscat | | | 19-8 | 19,66 | 7-8 | 20,39 |
| | | | 24-8 | 18,92 | 17-8 | 19,74 |
| | | | 29-8 | 18,43 | 28-8 | 19,57 |
| | | | 2-9 | 18,80 | | |
| | | | 7-9 | 17,99 | | |
| | | | | | | |
| | | | | | | |
| | | | | | | |
| T min * : average of minimal temperatures since 30 days before sampling date | | | | | | |
| Dates in red indicates the maximum grape volume (Vmax) | | | | | | |

Table II.2.3: Long term average data

| Year | Pech Rouge climate index | | |
|------|--------------------------|-----------------------------|-------------------------|
| | Dryness index | Huglin's heliothermal index | Cool night index |
| 2007 | -27 | 2333 | 14,7 |
| 2008 | -66 | 2098 | 13,5 |
| 2009 | -89 | 2285 | 15,0 |
| 2010 | -47 | 2178 | 14,2 |
| 2011 | -44 | 2254 | 16,4 |
| 2012 | -44 | 2128 | 15,0 |
| 2013 | -33 | 2098 | 15,4 |
| 2014 | -10 | 2367 | 15,4 |
| 2015 | -77 (moderately dry) | 2414 (temperate warm) | 15,2 (temperate nights) |
| 2016 | -111(very dry) | 2327 (temperate warm) | 17,7 (temperate nights) |
| 2017 | -141 (very dry) | 2370 (temperate warm) | 13,8 (fresh nights) |

2.3 Cultivars

The genotypes chosen for the experiments are 8: Viognier, Chardonnay, Grenache, Marselan, Syrah, Cabernet-Sauvignon, Muscat à petits grains blancs (from now onwards, Muscat) and the G5, a genotype with specific metabolic and pests resistance features.

The G5 is a new cultivar displaying a significant reduction of sugar accumulation (-30%) during berry ripening and resistance to powdery and downy mildew (Salmon et al., 2018).

The origin of the G5 (among other selected clones) began with a hybrid, a F1 originally obtained in USA (1900-1916) by hybridation of *Muscadinia rotundifolia* and *Vitis vinifera*.

The breeding program continued in France in the 1970s (A. Bouquet, INRA Montpellier), including up to 6 successive backcrossing with different varieties of *V. vinifera* (Grenache, Chasan, Merlot, Cabernet-Sauvignon, Fer Servadou, Marselan, Pinot Noir, Ugni-blanc, etc.).

Since 2005 several finalized programs led by INRA with the support of the IGP Sud de France and the CIVL (Conseil Interprofessionnel des Vins du Languedoc) have selected 20 genotypes. These genotypes have been assessed 5 to 10 years at INRA Pech-Rouge, for several criteria: tolerance levels to downy and powdery mildew, susceptibility to secondary diseases and pests, agronomic performances in various management systems, adaptation to the southern climate in relation with the control of acidity, pH and alcohol contents in wines.

This group of hybrids shows up to 99 % of *V. vinifera*, keeping a high enological quality, and still conserving the pest resistance from *Muscadinia rotundifolia*. These new varieties are genetically, morphologically and qualitatively very close to *V. vinifera* and are characterized by a resistance hypothetically "monogenic" or more precisely "monocus" (Ojeda et al. 2017).

These cultivars will be very important in a future, characterized by a changing climate with increasing temperatures, resulting in higher contents of sugar/berry, which originates more alcoholic wines (in a market with consumers looking for low alcohol wines). The resistance to pests is also very significant, due to governments looking for less inputs-dependent products, as well as consumers.

The significant reduction of the sugar biomass accumulation into the fruit during ripening of G5 genotype, could be associated to some peculiarities in the rules of C partitioning between primary and secondary metabolisms. Therefore, the inclusion of the G5 in the present research has the scope of characterizing its aroma profile and evaluate the response to the modification of the source/sink balance for first time.

2.4 Agronomic factors

Treatments consisted in obtaining plants with different fresh fruit weight/vegetative biomass balances. Ravaz Index ($RI = \text{fresh fruit weight} / \text{winter pruning biomass}$) was used to quantify the level of this balance. Fruit fresh weight is correlated to the fraction of the C sink dedicated to reproductive organs. Fresh weight of the canes removed by winter pruning weight is correlated to the biomass allocated to vegetative development. Different fresh fruit weight levels were managed by bunch thinning and/or special winter pruning. Plants were thinned up to 50% of clusters compared with control plants in 2015 and up to 70 % in 2016, remaining just the 30 % of them. Bunch thinning was performed at two fruit development stages (rapid growth stage; RGS from now onwards), approximately 30 days before onset of ripening and the other one at onset of ripening (*veraison*; OOR from now onwards).

During 2016 and 2017 season a reduced number of genotypes were explored. These genotypes were selected according to previous data, to confirm or extend observations. In accordance with this statement, genotypes chosen in 2017 were Syrah, Cabernet-Sauvignon and Muscat. Based on the main objective of the thesis and on literature review, the aim for the 2017 season was to increase the RI in Cabernet-Sauvignon and Syrah plants, and evaluate how the aroma precursor's biosynthesis is affected when the plant cultivated at very high fruit load. It is assumed that a higher fresh fruit weight level will impact in the C balance of the plant and this could have consequences in the concentration of glycosylated molecules of grapes. To reach this scope, some plants were specially pruned in winter 2017 to have an increased number of fertile shoots by plant. Then, plants that were in a Guyot trailing system were converted into a double Guyot trailing system, with a doubled number of fertile shoots.



Figure II.2.4: Simple and double Guyot

In 2017 there were three treatments: control plants (C), plants thinned at the rapid growth stage (RGS) and plants with high RI or fruit over-charge (FOC). For Muscat, treatments were the same as in 2016. During 2016 and 2017, G5 was not explored due to

Table II.2.3: Treatments summary

| 2015 | 2016 | 2017 | Abbreviation |
|---|---|---|--------------|
| Control plants | Control plants | Control plants | C |
| Bunch thinning at rapid growth stage (50%) | Bunch thinning at rapid growth stage (70%) | Bunch thinning at rapid growth stage (50%) | RGS |
| Bunch thinning veraison (50%) | Bunch thinning veraison (70%) | Bunch thinning veraison (50%) – (Only for Muscat) | OOR |
| - | - | Fruit over-charge (Only for Syrah and Cabernet-Sauvignon) | FOC |
| Varieties involved | | | |
| Viognier Chardonnay Grenache Marselan Syrah Cabernet-Sauvignon G5 | Chardonnay Syrah Cabernet-Sauvignon Muscat | Syrah Cabernet-Sauvignon Muscat | |

2.5 Plant water status

In all experimental plots, plant water status was measured weekly during the growing period, with a pressure chamber (Schölander, 1965) to determine pre-dawn leaf water potential.

According to the scores, drip irrigation was adapted to avoid water stress during the three growing seasons. Then, to maintain an adequate water status of the plant, i.e. a moderate drought during ripening, plots were irrigated keeping a predawn leaf water potential of $\psi_b > = -0.7$ Mpa for red cultivars and of $\psi_b > = -0.6$ Mpa for white cultivars. Measurements of vine water status were generally performed every 10-15 days. A summary of the levels of measured leaf water potential is shown in next table. Plots were irrigated from 0 to 60 mm depending on water status.

Table II.2.4: Predawn water potential (Mpa)

| * average of 4-6 measurements | 2015 | | 2016 | | 2017 | |
|-------------------------------|------------|---------------------|------------|---------------------|------------|---------------------|
| | Date | Predawn potential * | Date | Predawn potential * | Date | Predawn potential * |
| Cabernet sauvignon | 16/06/2015 | -0,3 | | | 1-8-17 | -0,28 |
| | 23/06/2015 | -0,23 | | | | |
| | 07/07/2015 | -0,22 | | | | |
| | 21/07/2015 | -0,36 | | | | |
| | 05/08/2015 | -0,39 | | | | |
| | 18/08/2015 | -0,34 | | | | |
| G5 | 30/06/2015 | -0,22 | | | | |
| | 17/06/2015 | -0,44 | | | | |
| | 16/07/2015 | -0,46 | | | | |
| | 28/07/2015 | -0,5 | | | | |
| | 11/08/2015 | -0,33 | | | | |
| | 29/08/2015 | -0,51 | | | | |
| Grenache | 16/06/2015 | -0,2 | | | | |
| | 30/06/2015 | -0,21 | | | | |
| | 16/07/2015 | -0,24 | | | | |
| | 28/07/2015 | -0,32 | | | | |
| | 11/08/2015 | -0,26 | | | | |
| | 25/08/2015 | -0,42 | | | | |
| Marselan | 17/06/2015 | -0,26 | | | | |
| | 08/07/2015 | -0,24 | | | | |
| | 22/07/2015 | -0,44 | | | | |
| | 06/08/2015 | -0,41 | | | | |
| | 19/08/2015 | -0,31 | | | | |
| Chardonnay | 17/06/2015 | -0,35 | 25/06/2016 | -0,35 | | |
| | 08/07/2015 | -0,35 | 07/07/2016 | -0,5 | | |
| | 22/07/2015 | -0,5 | 27/07/2016 | -0,68 | | |
| | 06/08/2015 | -0,57 | | | | |
| | 19/08/2015 | -0,44 | | | | |
| Syrah | 17/06/2015 | -0,25 | 09/08/2016 | -0,72 | 04/07/2017 | -0,37 |
| | 08/07/2015 | -0,3 | 13/07/2016 | -0,54 | 21/07/2017 | -0,23 |
| | 22/07/2015 | -0,71 | 09/08/2016 | -0,77 | 01/08/2017 | -0,64 |
| | 06/08/2015 | -0,49 | | | 24/08/2017 | -0,45 |
| | 19/08/2015 | -0,36 | | | | |
| Muscat | | | 13/07/2016 | -0,47 | 23/06/2017 | -0,21 |
| | | | 27/07/2016 | -0,37 | 04/07/2017 | -0,37 |
| | | | 09/08/2016 | -0,59 | 21/07/2017 | -0,23 |
| | | | | | 01/08/2017 | -0,38 |

2.6 Sampling

Grape sampling was performed at 1 to 5 periods of grape ripening. In all cases, there were three field repetitions with at least three plants by treatment and berries from the three field repetitions were mixed to avoid field differences. The targeted berry development stage was the maximum average volume of the berries. According to Shahood (2017), this point corresponds to the arrest of phloem unloading at single berry level. However, even this point makes sense a single fruit level, is not affordable at bunch level. Indeed, at bunch or population level, the stage when the average volume of the berry stops increasing corresponds to a balance between berries still growing up taking water and shriveling berries losing water by dehydration (Shahood, 2017, Bigard et al. 2018).

As analyses for GAP requires several hundreds of grams of fresh material, sampling cannot be based on single berry monitoring. To monitor sampling timing, the volume of reference grapes was non-destructively monitored with a method based in the Archimedes theory (Lang and Thorpe, 1989).

The method consisted in submerging the bunch grape in an Erlenmeyer containing water. A balance was previously positioned under the Erlenmeyer. Once the bunch is submerged in the water (and being careful that the bunch do not touch the recipient), the weight was noted. This measure is the weight of the volume of water displaced by the bunch (assuming a water density =1). The bunch remains always linked to the vine.

Bunch volume measurements were performed before sunrise to avoid variation of volume due to transpiration. Three bunches per treatment were previously selected and conditioned by removing zonal leaves. Volume measurements were always performed in the same group of

bunches. Sampling was always done after veraison, with one sampling point before the maximum average berry volume (Vmax), a sampling during the Vmax and one after Vmax.

The next figures show the evolution of the grape bunch volume in selected cultivars.

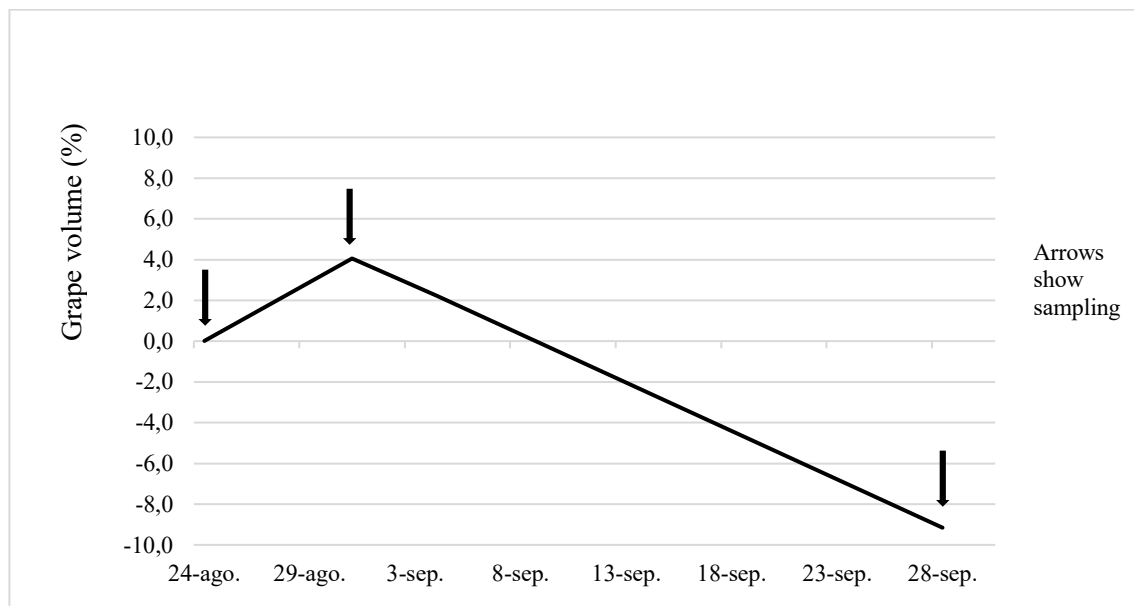


Figure II.6.1: Cabernet-Sauvignon 2017 grape volume (%)

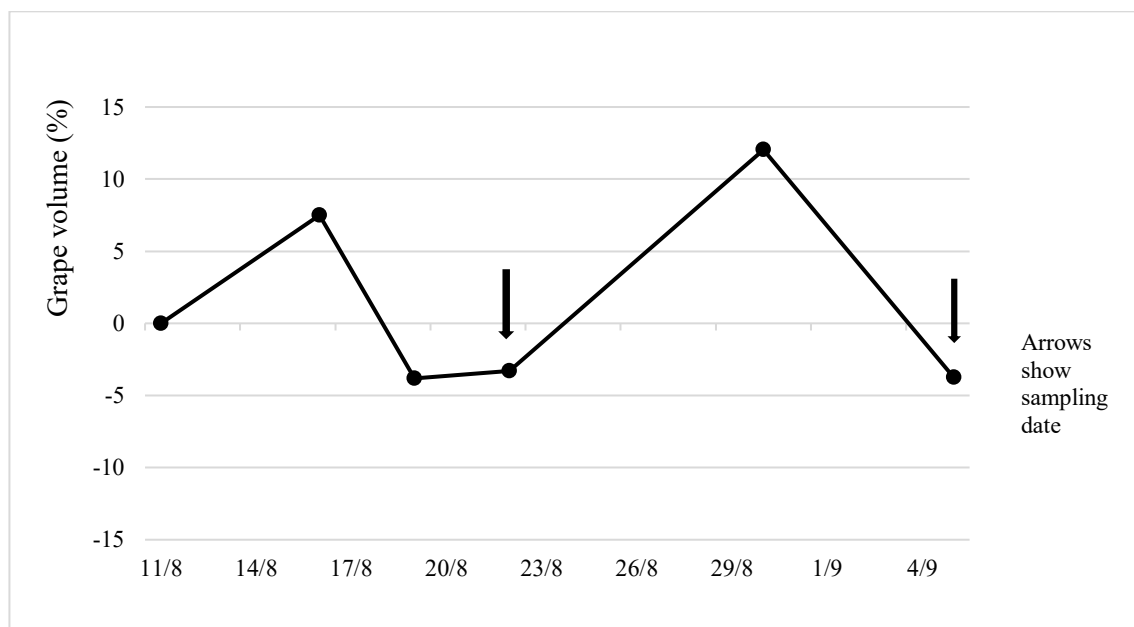


Figure II.6.2: Syrah 2017 grape volume (%)

In 2017, a sorting of the berries was performed as described by Rolle et al. (2013) to assess the impact of the treatment on berry heterogeneity and hence the contribution of this factor on the values of the different aromatic precursor compounds. Torchio, F. (2016) also stated that berry classification based on density could minimize the negative effects of the variability between and within clusters. Then, berries were divided in three or four groups regarding their density (in this case meaning sugar concentration) and only groups with the same sugar concentration were analyzed (a way to eliminate no representative berries). Berries were submerged in water with different NaCl concentration (140, 160, 180 and 200 g/L). Berries were separated in function of their floatability. Those densities correspond to °Brix ranges (18-19; 20-21; 22-23 and 24-25 respectively).

Then, comparisons were made between berries belonging to groups with similar sugar concentration. The selected sugar level group is the main one, representing approximately 60 % of the hole berries group. See details in Annex 10.

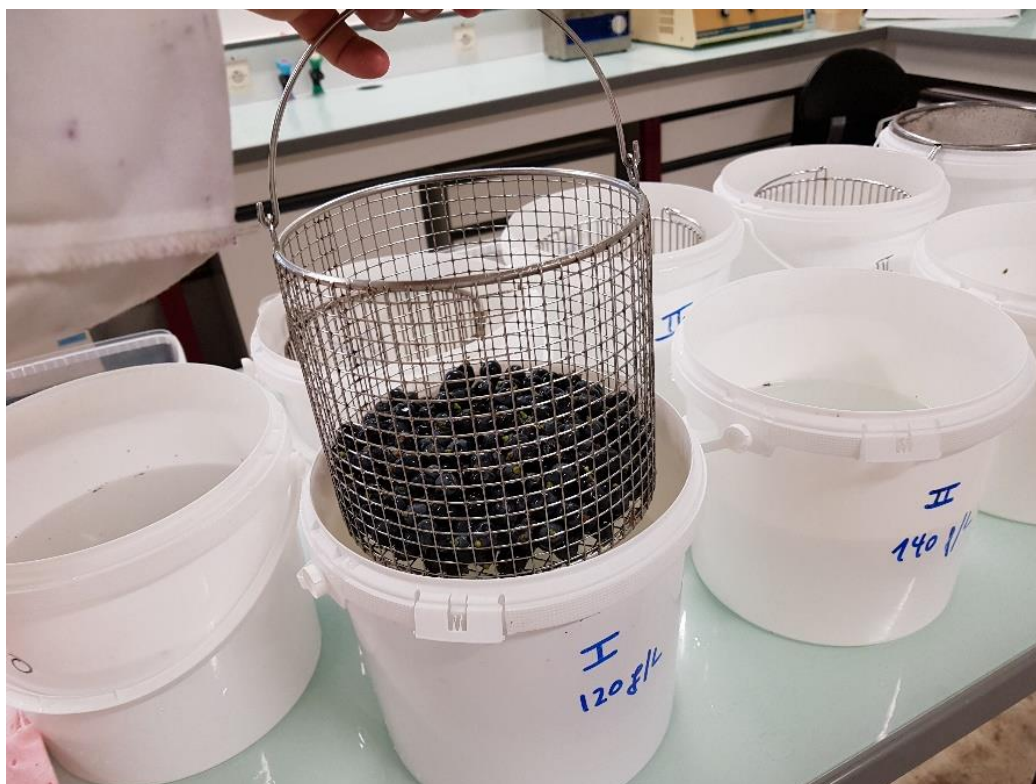


Figure II.6.3: Berry sorting by density



Figure II.6.4: Berry sorting by density bis

2.7 Analytic methods

For each treatment, an exhaustive analysis on the composition and aroma volatile precursors production of the grape berry was performed. Analysis consisted in measuring cluster and berries' weight and berries' volume. Primary metabolites: sugar and total acidity. Secondary metabolites: anthocyanins concentration (only in red genotypes), total polyphenols index (IPT, only in red genotypes). All the analyzes were performed at INRA Pech Rouge laboratories. The laboratories official protocol used in the research, is shown in annex 1.

Analyzes and quantification of GAP

GAP were quantified by gaseous chromatography (GC-MS), after extraction of the aglycone fraction (Bisotto et al., 2015).

Grape juice of each cultivar was extracted by crushing berries with a blender. After centrifugation (7000 rpm @ 20 min @ 10°C) and separation from the non-soluble residuum, grape juice (350 mL) was mixed with polyvinylpolypyrrolidone (PVPP) (1 g of PVPP/100 ml of juice in white cultivars and 5 g/100 ml in red cultivars). Once filtered, the solution was eluted through a XAD-2 column (Sigma-Aldrich Chimie, Lyon, France), then washed with water (100 mL). In the next step, pentane/dichloromethane (2/1 v/v, 10 mL) was used to remove the terpenes' free fraction. The XAD-2 column was previously conditioned with 10 ml of methanol and 10 ml of water. The bound glycosidic fraction was recovered by elution with 10 mL methanol.

The glycosidic fraction was dried with air flux in water bath (40°C), and the residuum was then solubilized in 2 mL of phosphate citrate buffer (sodium hydrogen phosphate 0.2 mol, citric acid 0.1 mol, pH 5.0). Then, 200 µL of an enzymatic preparation (AR2000 at 70 mg/mL; DSM Food Specialties, Heerlen, the Netherlands) in citrate phosphate buffer was added. After mixing, the solution was taken to an oven at 35°C for 16 h.

After the enzymatic hydrolysis, the volatile fraction was extracted by five times 1 mL of azeotrope, the pentane/dichloromethane (2/1; v/v). The organic extract was then dried on anhydrous sodium sulfate. As an internal standard, 100 µL of 4-Nonanol was used in a concentration of 16 mg/L. The extract was then concentrated to about 400 µL by partial

rectification at 35°C using a Dufton spiral column. The extract was conserved at -20°C until GC/MS analyzes.

The 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, Santa Clara, CA, USA)]. Compounds were separated using helium carrier gas at 1 mL/min. The temperature program began 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°C. Full scan mass spectra were recorded between 29 and 350 *m/z*. Data were acquired and treated with the HP 5989 B.05.02 MS Chemstation. The terpenes identified were semi-quantified using 4-nonanol as an internal standard.

The molecules analyzed corresponds to four glycosylated groups: terpenes, alcohols, phenols and norisoprenoids. A detailed list is shown in table II.7.1.

Table II.7.1: Glycosylated molecules list

| | | |
|---------------------|----|--|
| Alcohols | 1 | hexanol |
| | 2 | 3-hexen-1-ol cis |
| | 3 | 2-hexen-1-ol trans |
| | 4 | 1-octen-3-ol |
| | 5 | benzylic alcohol |
| | 6 | 2-phenylethanol (2PHEN) |
| C13-norisoprenoides | 1 | 3,4-dihydro-3-oxo-actinidol I (ACT I) |
| | 2 | 3,4-dihydro-3-oxo-actinidol II (ACT II) |
| | 3 | 3,4-dihydro-3-oxo-actinidol III (ACT III) |
| | 4 | 3-hydroxy-B-damascenone (DAM3L) + ACTI IV |
| | 5 | 3-hydroxy-7,8-dihydro-b-ionone (BIN3H) |
| | 6 | 3-hydroxy-b-ionone (BIN3O) |
| | 7 | 3-oxo-7,8-dihydro-a-ionol (3ODAOL) |
| | 8 | 3oxo-Ar-etroAionol (3ORAOL) |
| | 9 | 3-hydroxy-7,8-dihydro-b-ionol (BIL3D) |
| | 10 | 3-oxo-a-ionol (3OIOL) |
| | 11 | 3-oxo-A-retroionol (3OAROL) |
| | 12 | 3-hydroxy-7,8-dihydro-B-ionol (BIL3H) |
| | 13 | 4,5-dihydrovomifoliol (VOMBH) |
| | 14 | vomifoliol |
| Phenols | 1 | eugenol |
| | 2 | phenol |
| | 3 | unknown 52 |
| | 4 | vanillin |
| | 5 | zingerone |
| | 6 | methyl zingerate |
| | 7 | unknown 198 |
| | 8 | guayacol-propanol |
| Terpenes | 1 | linalol oxide (LOF Trans) |
| | 2 | HO-trienol |
| | 3 | trans-pyran linalool oxide trans (LOP Trans) |
| | 4 | trans-pyran linalool oxide (LOP Cis) |
| | 5 | nerol |

| | | |
|--|----|--|
| | 6 | geraniol |
| | 7 | 3,7-dimethyl-1,5-octadien-3,7-diol |
| | 8 | 8-hydroxydihydrolinalool (H8LIN) |
| | 9 | 2,6-dimethylocto-2,7-dien-1,6-diol (diol-3,8, E8LIN) |
| | 10 | Z-8-hydroxylinalol (Z8LIN) |
| | 11 | geranic acid |
| | 12 | p-menth-1-ene-7, 8 diol |

2.8 Statistical analysis

It was performed using the software package INFOSTAT® (University of Cordoba, Argentina). LSD Fisher test for $p < 0.05$ was used to evaluate the existence of significant differences by one-way analysis of variance (ANOVA). INFOSTAT® was also used for Principal components analyzes (PCA).

3. Chapter III: Results

3.1 Influence of the genotype on the accumulation of glycosylated aroma precursors (GAP) concentration

The objective of this section is to analyze the influence of the cultivar in the biosynthesis of glycosylated aroma precursors (GAP) and evaluate how the aromatic molecules groups (alcohols, C13-norisoprenoids, phenols, terpenes and total precursors) change or not. To have an overview of a big number of genotypes, the glycosylated aroma precursors concentration of 8 cultivars was analyzed: Viognier, Chardonnay, Syrah, Grenache, Cabernet-Sauvignon, G5, Marselan and Muscat à petit grain. All of them were evaluated in 2015, except for Muscat which was evaluated in 2016.

Results are shown first as a general overview, where all the cultivars are displayed together, and then analyzed at individual level.

The concentration of glycosylated aroma precursors expressed in $\mu\text{g/L}$ of berry volume by genotype and differentiated by groups of molecules (alcohols, C13-norisoprenoids, phenols, terpenes and total precursors) is shown in Figure III.1. A detailed list of analyzed molecules can be seen in Chapter II, Materials and methods. The data showed in the figure are from control plants, which were sampled when grapes have reached the maximum volume of the berry (V_{max}). V_{max} corresponds to the moment when the plant stops sugar unloading in the berries, also accepted as the physiological maturity. V_{max} was determined by a method based in the Archimedes theory (Lang and Thorpe, 1989) and is described in Chapter II.

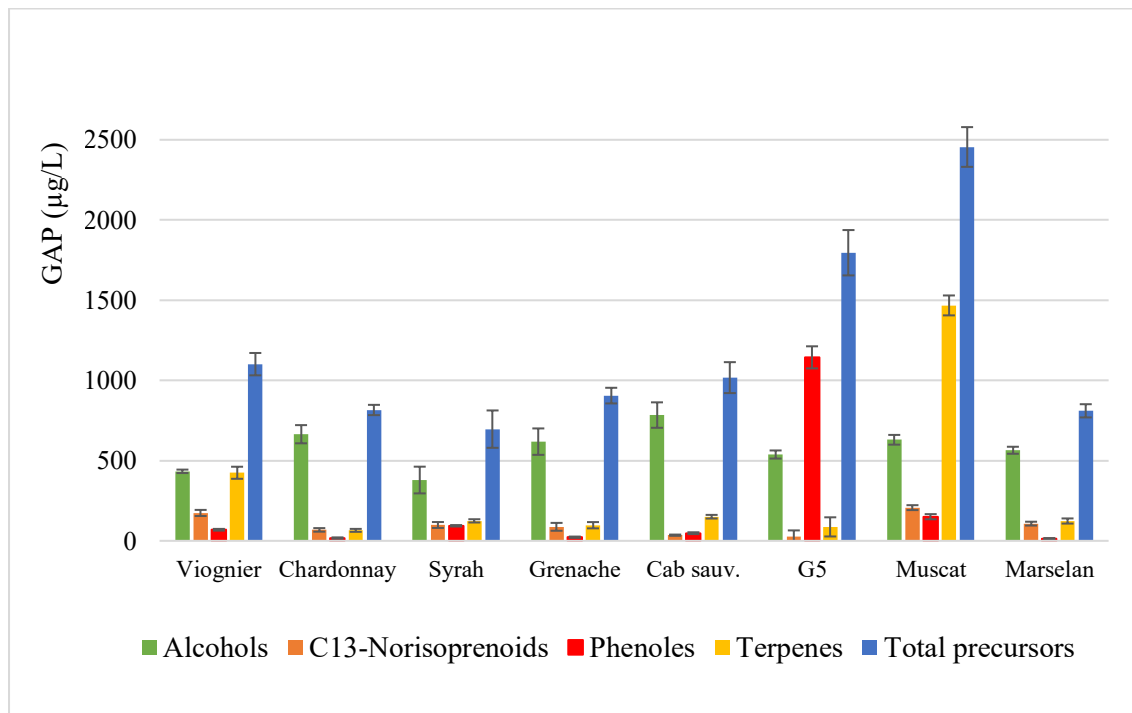


Figure III.1.1: Concentration of GAP ($\mu\text{g/L}$) in control plants berries (2015, except for Muscat, 2016), differentiated by groups of molecules and sampled at physiological maturity.

Values of total aroma precursors in decreasing order are summarized in table III.1.1.

Table III.1.1: Concentration of GAP in berries (2015), sampled at physiological maturity.

| Cultivar | Total GAP ($\mu\text{g/L}$) |
|------------|-------------------------------|
| Muscat | 2455 |
| G5 | 1795 |
| Viognier | 1101 |
| Cab. Sauv. | 1017 |
| Chardonnay | 936 |
| Grenache | 905 |
| Marselan | 811 |
| Syrah | 696 |

Muscat and G5 have the highest concentration of total GAP, while Syrah and Marselan shows the lowest ones. Regarding the groups of molecules, Muscat and G5 shows great concentration of terpenes (linalol oxide, HO-trienol, trans-pyran linalool oxide trans, trans-pyran linalool oxide, nerol, geraniol, 3,7-dimethyl-1,5-octadien-3,7-diol, 8-hydroxydihydrolinalool, 2,6-dimethylocto-2,7-dien-1,6-diol, Z-8-hydroxylinalol, geranic acid, p-menth-1-ene-7, 8 diol, etc.). Instead, Chardonnay, Syrah, Grenache, Cabernet-Sauvignon and Marselan show great amounts of glycosylated alcohols (hexanol, 3-hexen-1-ol cis, 2-hexen-1-ol trans, 1-octen-3-ol, 2-phenylethanol, benzylic alcohol, etc.). Instead, Chardonnay, Syrah, Grenache, Cabernet-Sauvignon and Marselan show great amounts of glycosylated alcohols (hexanol, 3-hexen-1-ol cis, 2-hexen-1-ol trans, 1-octen-3-ol, 2-phenylethanol, benzylic alcohol, etc.). In Viognier, alcohols and terpenes are the most important groups of molecules and are present in similar proportions. Phenols (eugenol, phenol, vanillin, zingerone, methyl zingerate, unknown 198, guayacol-propanol, etc.) and C-13 norisoprenoids (3,4-dihydro-3-oxo-actinidol, 3-hydroxy-B-damascenone, 3-hydroxy-7,8-dihydro-b-ionone, 3-hydroxy-b-ionone, 3-oxo-7,8-dihydro-a-ionol, 3-oxo-Ar-etroAionol, 3-hydroxy-7,8-dihydro- β -ionol, 3-oxo-a-ionol, 3-oxo-A-retroionol, 3-hydroxy-7,8-dihydro- β -ionol, etc.), on the contrary are in very low proportions in all the cultivars, except for G5, where phenols represents the main group of GAP.

A *principal component analyzes* (ACP) (Figure III.1.2) shows the Muscat is separated from the other cultivars due to its high contents of terpenes. The G5, which derives from Muscat de Hambourg, is also in the same direction, but far from the Muscat and closer to Viognier. Grenache and Cabernet-Sauvignon are on the opposite side of Muscat, due to their low concentration of aroma precursors. Interestingly, Marselan, a variety created by INRA from the hybridization of the Cabernet-Sauvignon and Grenache N varieties, Cabernet-Sauvignon

placed between both of them. In general, the group of white varieties is located to the right of the figure (richest in GAP) and the red varieties to the left.

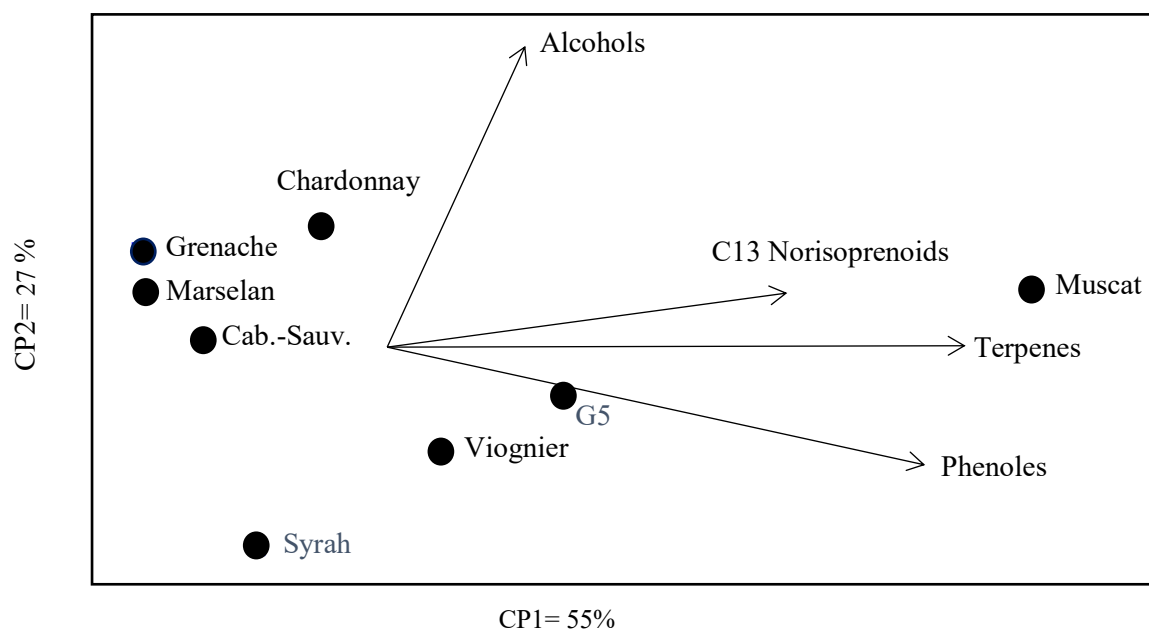


Figure III.1.2: ACP of the GAP concentration ($\mu\text{g/L}$) in control plants berries (2015) by molecule group. Berries were sampled at physiological maturity.

GAP in a berry basis:

From the bibliographic review analyses, it was concluded that most of the research in GAP expresses values in concentration ($\mu\text{g/L}$ of fruit). When result data is shown as concentration, it is not clear if differences are due to the biosynthesis of the GAP molecules or due to a concentration of them. Indeed, if the sampling is after physiological maturity, which is the more frequent situation in previous reports, the concentration is dependent on the level of fruit shriveling. The volume of the berry is a parameter that can change when plants are manipulated with agronomical practices like bunch thinning or irrigation. Then the

concentration of the solutes in the berry can change. Nevertheless, concentration values are useful for winemakers which think in final concentration in the wines.

Therefore, in this research, results are shown when possible in both, in a concentration basis ($\mu\text{g/L}$) and in a per berry basis ($\mu\text{g/berry}$), to avoid confusing metabolism process with a matter of concentration.

Aroma precursors but analyzed in a per berry basis are shown in Figure III.1.3 and Table III.1.2. Results show the same trends and order than those expressed in concentration μL . But when comparing the maximum values of each cultivar, G5 has highest GAP values ($\mu\text{g/berry}$) than Muscat. This is due to the highest volume of the G5 berry.

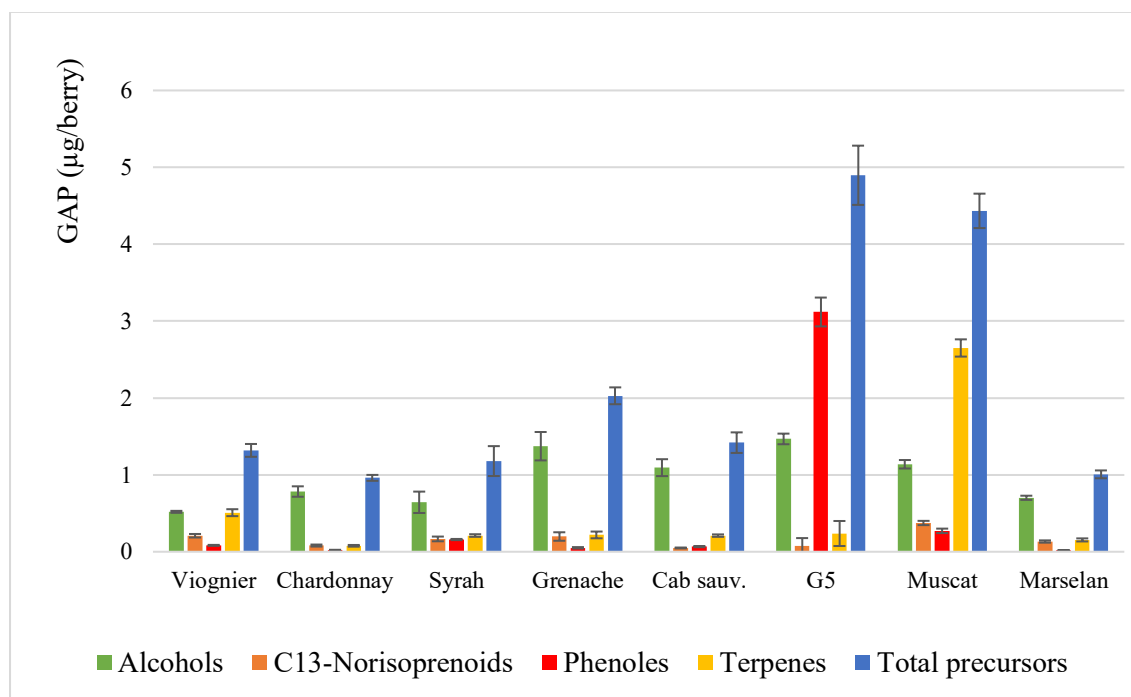


Figure III.1.3: Content of GAP ($\mu\text{g/berry}$) in control plants berries (2015), differentiated by groups of molecules and sampled at physiological maturity.

Table III.1.2: Concentration of GAP in berries (2015), sampled at physiological maturity, expressed in $\mu\text{g}/\text{berry}$ and $\mu\text{g}/\text{L}$.

| Cultivar | GAP ($\mu\text{g}/\text{berry}$) | Cultivar | Total GAP ($\mu\text{g}/\text{L}$) |
|------------|------------------------------------|------------|--------------------------------------|
| G5 | 4,90 | Muscat | 2455 |
| Muscat | 4,35 | G5 | 1795 |
| Viognier | 2,03 | Viognier | 1101 |
| Cab. Sauv. | 1,42 | Cab. Sauv. | 1017 |
| Chardonnay | 1,32 | Chardonnay | 936 |
| Grenache | 1,26 | Grenache | 905 |
| Marselan | 1,18 | Marselan | 811 |
| Syrah | 1,01 | Syrah | 696 |

The ACP is very similar to the previous one expressed in $\mu\text{g}/\text{L}$, but now Grenache is separated from the other red varieties, with higher GAP contents per berry, mainly alcohols, most likely due to the larger size of their berries.

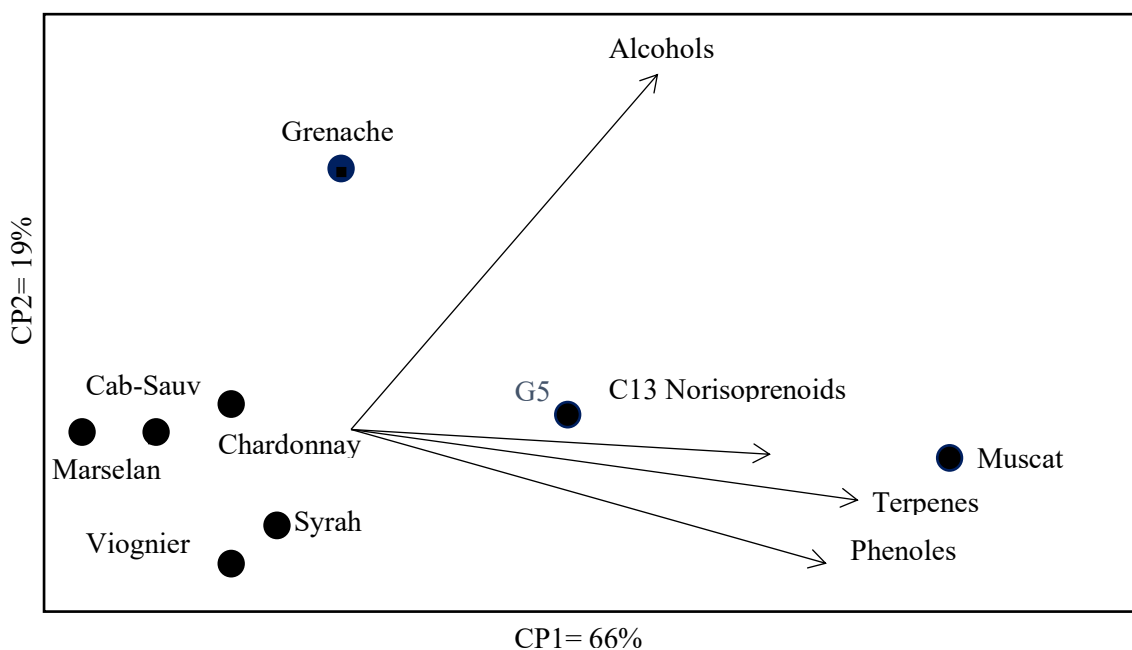


Figure III.1.4: ACP of the GAP content ($\mu\text{g}/\text{berry}$) in control plants berries (2015) by molecule group. Berries were sampled at physiological maturity.

Table III.1.3: Fresh fruit weight by plant (g) (2015), sampled at physiological maturity.

| | Fresh fruit weight/plant (g) |
|-------------------|-------------------------------------|
| G5 | 5000 |
| Cab. Sauv. | 3933 |
| Grenache | 3241 |
| Viognier | 3204 |
| Marselan | 2860 |
| Syrah | 2694 |
| Muscat | 2212 |
| Chardonnay | 1500 |

Table III.1.4: GAP by plant (2015), sampled at physiological maturity.

| Cultivar | Total aroma precursors (µg/plant) |
|-------------------|--|
| G5 | 8302 |
| Muscat | 4985 |
| Cab. Sauv. | 3637 |
| Viognier | 2929 |
| Grenache | 2765 |
| Marselan | 2028 |
| Syrah | 1712 |
| Chardonnay | 1448 |

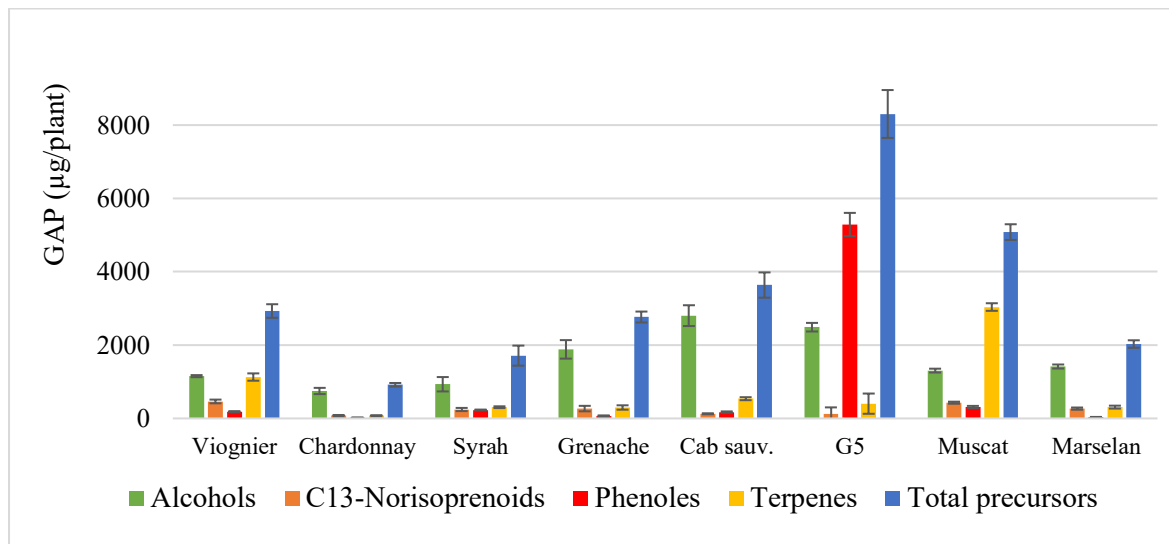


Figure III.1.6: Content of GAP ($\mu\text{g/plant}$) in control plants berries (2015), differentiated by groups of molecules and sampled at physiological maturity.

The ACP (Figure III.1.7) shows that G5 is closer now to Muscat, which has a higher individual content of terpenes. The high fresh fruit weight per plant of the G5 compensates the high terpenes content per berry of the Muscat.

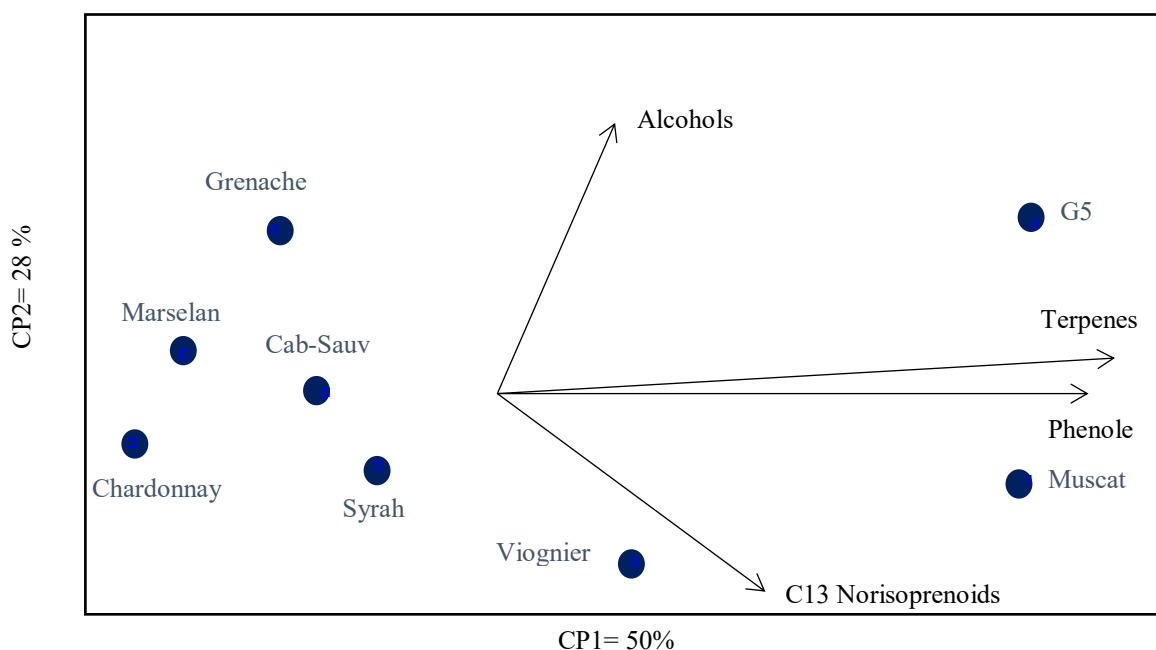


Figure III.1.7: ACP of the GAP content by plant ($\mu\text{g/plant}$) in control plants berries (2015) by molecule group. Berries were sampled at physiological maturity.

When expressed in terms of total aroma precursors per hectare (assuming a constant number of plant/ha = 4000), results show clearly that G5 is the variety with higher contents.

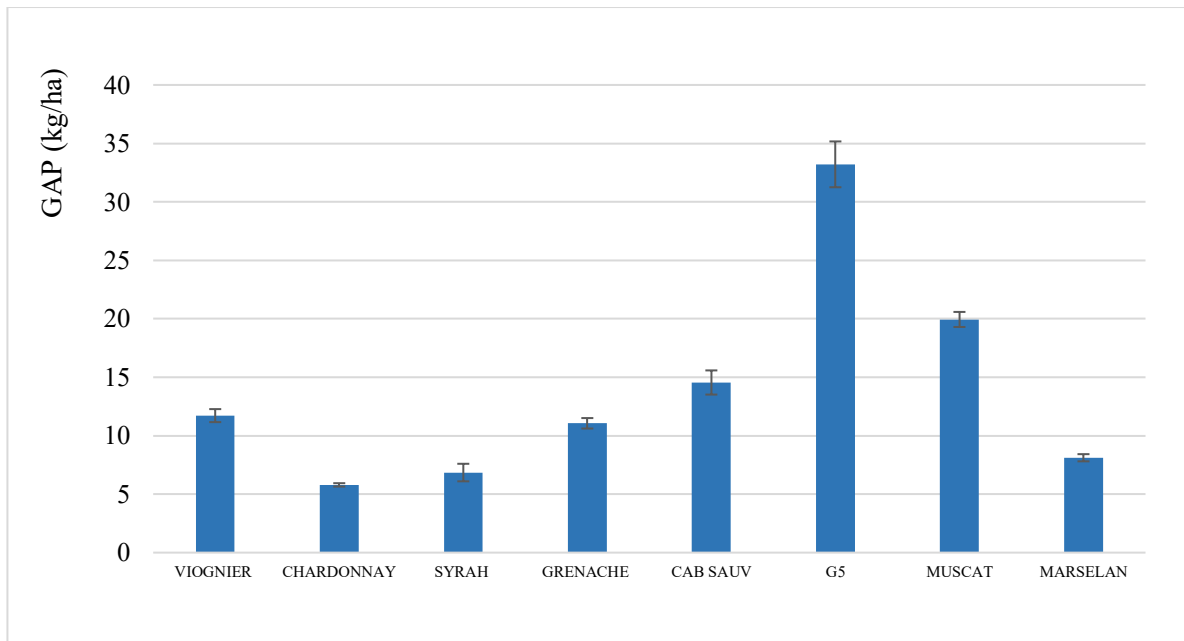


Figure III.1.8: Content of GAP in control plants berries (2015), expressed in kg/hectare, and sampled at physiological maturity.

3.2 Influence of the year on fresh fruit weight and the concentration in GAP

The scope of this point is to analyze the effect of the year on GAP and the relationship with fresh fruit weight. Results show the concentration ($\mu\text{g/L}$) of GAP and the fresh fruit weight (FFW) (kg/plant) along two or three years in Muscat, Cabernet-Sauvignon, Syrah and Chardonnay (Figure III.2.1). There is not a tendency regarding years. Syrah shows in 2015 and 2016 the greatest FFW (kg/plant). In 2017 FFW is decreased in 23%. Cabernet-Sauvignon shows an extraordinary FFW in 2015, 86 % bigger than in 2016. This difference is not seen in the other three cultivars.

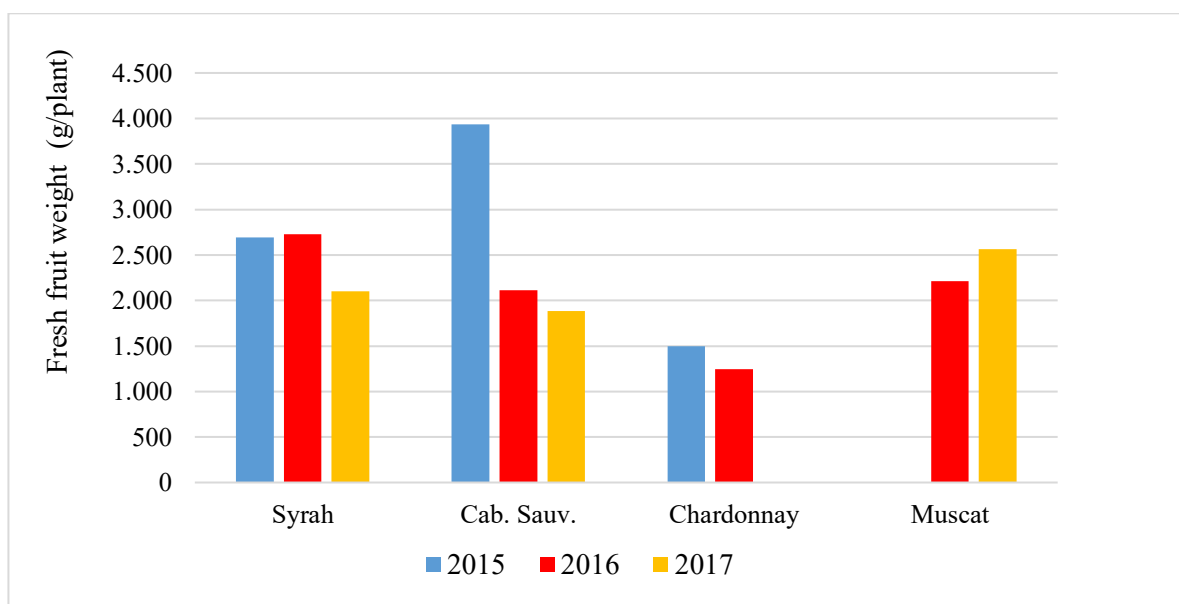


Figure III.2.1: Fresh fruit weight (g/plant) in 2015, 2016 and 2017; sampled at physiological maturity.

When GAP ($\mu\text{g/L}$ or $\mu\text{g/berry}$) are analyzed across the three years, there is not clear tendency either. Although the difference of the year is not evident in the GAP concentration ($\mu\text{g/L}$) (Figure III.2.2) or content ($\mu\text{g/berry}$) (Figure III.2.3), the fact that fruit fresh weight/plant are different between years, this impacts directly on GAP/plant (Figure III.2.4).

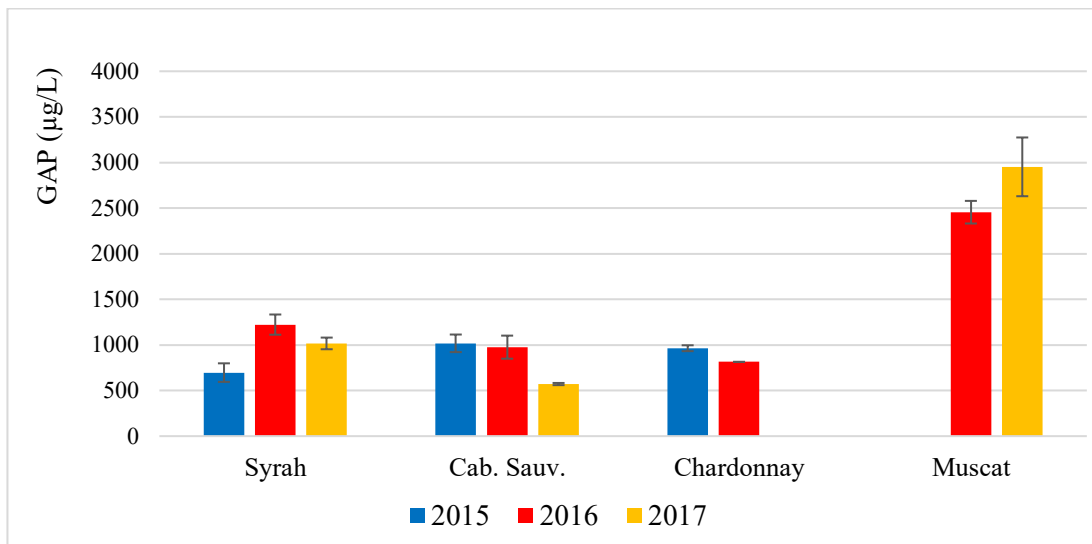


Figure III.2.2: GAP concentration ($\mu\text{g/L}$) in 2015, 2016 and 2017, sampled at physiological maturity.

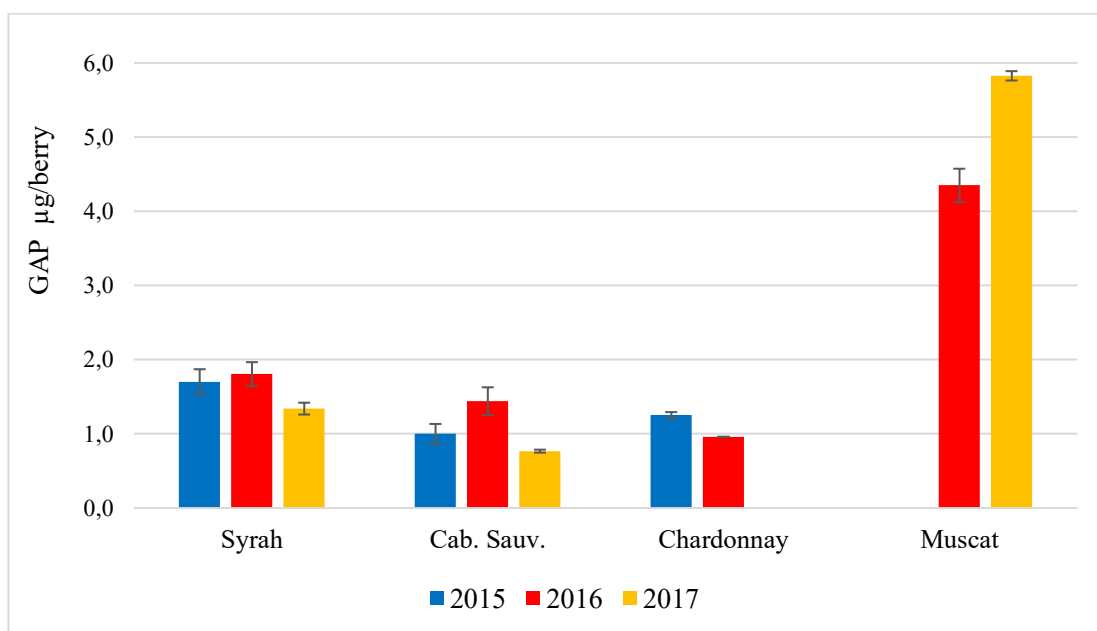


Figure III.2.3: GAP content ($\mu\text{g/berry}$) in 2015, 2016 and 2017, sampled at physiological maturity.

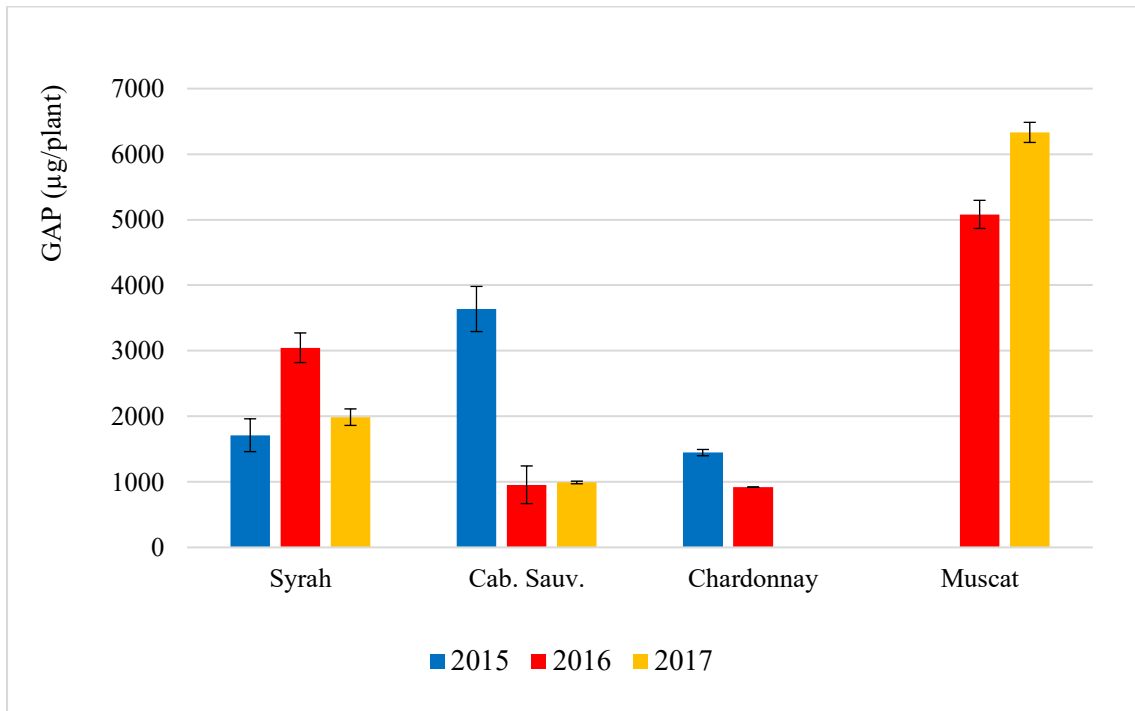


Figure III.2.4: GAP content (µg/plant) in 2015, 2016 and 2017, sampled at physiological maturity.

3.3 Relationship between source/sink balance and GAP biosynthesis.

The scope of this section is to analyze the objective number III, in which we postulate that modifying the source/sink relationship (S/S), also known as leaf/fruit balance, has an impact on the biosynthesis of (GAP). This is particularly relevant because viticulturists often manipulate the S/S by practices like bunch thinning, leaf removal or pruning. In this study, to qualify S/S we calculated the Ravaz index (RI) (Ravaz, 1911). Different ways were performed during the thesis to modulate RI: bunch thinning and/or modifying of the training system (simple Guyot transformed into a double Guyot, increasing the number of buds/plant for a double of fruit charge) (see Chapter II, Materials and methods).

Then this part of the research tries to answer the following questions:

- 1) Is the RI depending on cultivars?
- 2) What is the RI impact on GAP biosynthesis?

1) Is the RI depending on cultivars?

The weight of the winter pruning wood (WP) was not very impacted by the changes in fruit charge (Table III.3.1). WP, which represents the vegetative growth of the year, does not change in the same proportion as fresh fruit weight. For example, when FFW changes 239.05 % (Syrah) and WP only change - 9.57 %.

Table III.3.1: Relationships between fresh fruit weight (FFW), winter pruning wood (WP) and Ravaz index (RI).

| Year Variety | 2015 | | | 2016 | | | 2017 | | |
|-----------------|---------|---------|------|---------|---------|------|---------|---------|-------|
| | FFW | WP | RI | FFW | WP | RI | FFW | WP | RI |
| | g/plant | g/plant | | g/plant | g/plant | | g/plant | g/plant | |
| Viognier | 3.204 | 182 | 17,6 | | | | | | |
| | 1.943 | 225 | 8,6 | | | | | | |
| Chardonnay | 1.500 | 375 | 4,0 | 1245 | 391 | 3,19 | | | |
| | 810 | 419 | 1,9 | 400 | 517 | 0,77 | | | |
| Syrah | 2.694 | 619 | 4,4 | 2726 | 627 | 4,35 | 3994 | 634 | 6,30 |
| | 1.282 | 595 | 2,2 | 752 | 689 | 1,09 | 2102 | 617 | 3,41 |
| | | | 2,5 | | | | 1178 | 701 | 1,68 |
| Grenache | 3.241 | 739 | 4,4 | | | | | | |
| | 2.422 | 767 | 3,2 | | | | | | |
| Cab. Sauv. | 3.933 | 572 | 6,9 | 2113 | 746 | 2,83 | 3083 | 308 | 10,02 |
| | 1.530 | 576 | 2,7 | 953 | 730 | 1,31 | 1887 | 460 | 4,11 |
| | | | | | | | 1134 | 462 | 2,46 |
| G5 | 5.000 | 610 | 8,2 | | | | | | |
| | 2.128 | 552 | 3,9 | | | | | | |
| Marselan | 2.860 | 898 | 3,2 | | | | | | |
| | 1.802 | 724 | 2,5 | | | | | | |
| Muscat | | | | 2212 | 379 | 5,84 | 1027 | 363 | 2,83 |
| | | | | 785 | 386 | 2,03 | 698 | 333 | 2,10 |

2) What is the RI impact on GAP biosynthesis?

The effect of RI in GAP concentration ($\mu\text{g/L}$), in Syrah (2015, 2016, 2017), Cabernet-Sauvignon (2015, 2016, 2017) and Muscat (2016, 2017) is shown in Figure III.3.1. Berries were sampled at physiological maturity. This figure has the aim of a general viewing of the three cultivars in the three years. Each particular situation is analyzed in the following sections for a better interpretation.

When RI changes, the concentration ($\mu\text{g/L}$) of the total glycosylated aroma precursors (GAP) has a different response depending on cultivar. In the case of Muscat, there is a decrease in GAPs concentration when RI increases (or S/S decreased). Syrah, with more dispersed values,

also shows the same tendency. Instead for Cabernet-Sauvignon, there are no changes when RI is modified. These means that there is a genotype-depending response.

The same results are shown in a histogram for an easier interpretation (Figure III.3.2), using discontinues values of (between 0 and 2, between 2 and 4 and between 4 and 6). In Muscat, the concentration of total aroma precursors (expressed in terms of $\mu\text{g/L}$) decreased significantly at higher values of RI.

The results in a per berry basis ($\mu\text{g}/\text{berry}$) (Figure III.3.4) show a tendency similar to that in a concentration basis.

The overload of fruit in Muscat (high RI values) affects negatively the synthesis of GAP. In Cabernet-Sauvignon there is not such effect. In Syrah, results are not so clear in this figure, so it will be analyzed separately.

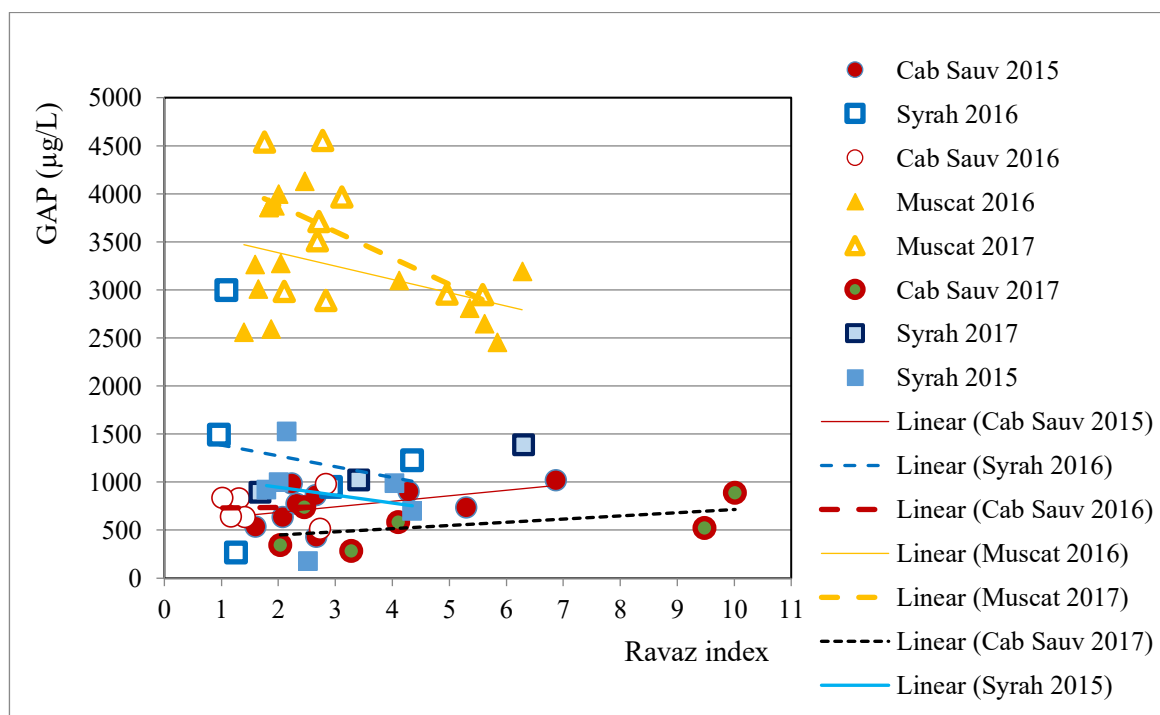


Figure III.3.1: Concentration of GAP ($\mu\text{g/L}$) in Cabernet-Sauvignon, Syrah and Muscat and RI in 2015, 2016 and 2017, sampled at physiological maturity.

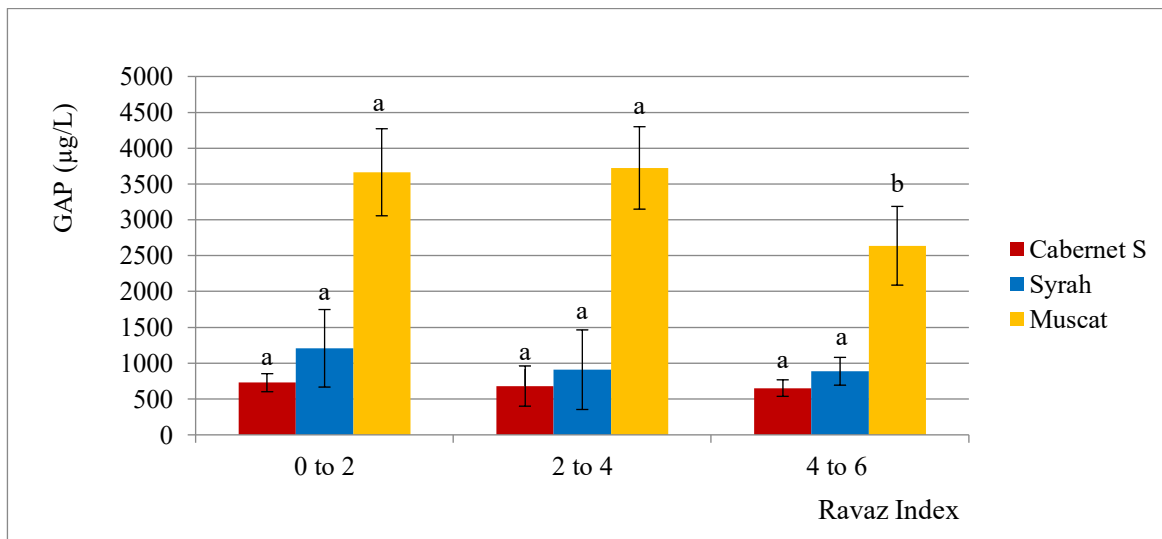


Figure III.3.2: Concentration of GAP ($\mu\text{g/L}$) in Cabernet-Sauvignon, Syrah and Muscat at different levels of RI, all year included, sampled at physiological maturity. For each variety, bars with different letters refers to averages significantly different for $p < 0.05$, analyzed by Fishers Least Significant Difference (LSD) test.

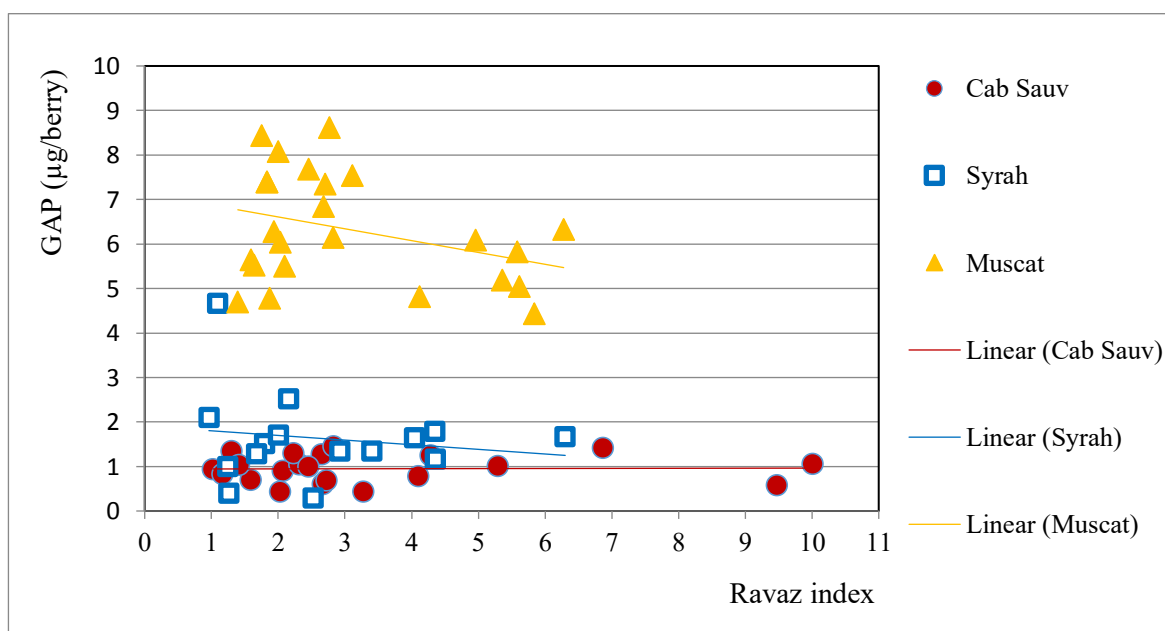


Figure III.3.3: Content of GAP ($\mu\text{g/berry}$) in Cabernet-Sauvignon, Syrah and Muscat, at different levels of RI, all year included, sampled at physiological maturity.

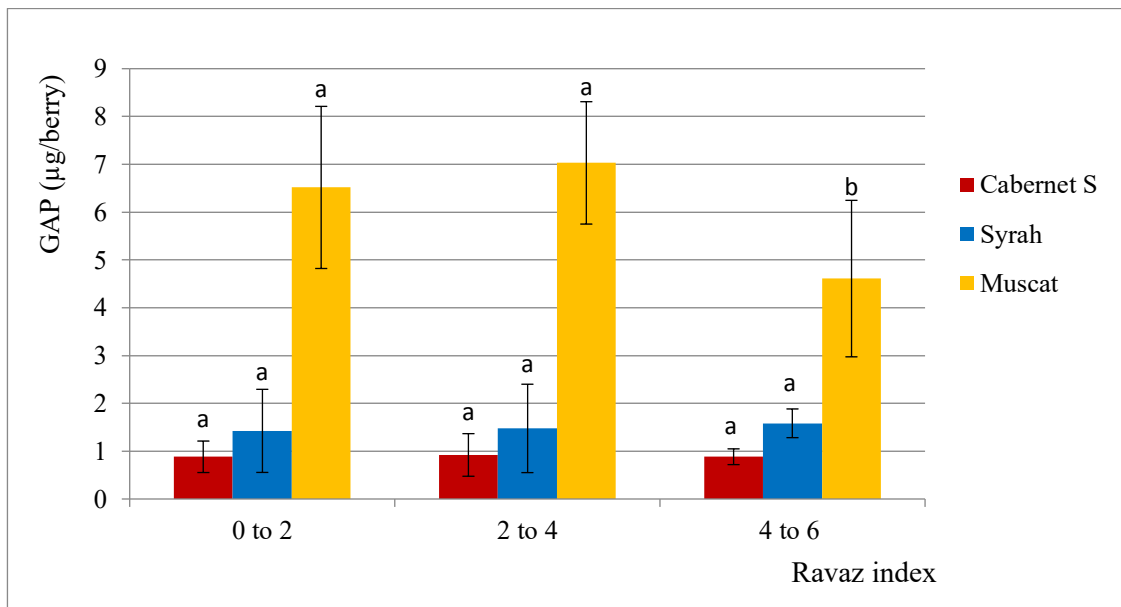


Figure III.3.4: Content of GAP ($\mu\text{g}/\text{berry}$) in Cabernet-Sauvignon Syrah and Muscat at different levels of RI all year included, sampled at physiological maturity. For each variety, bars with different letters refers to averages significantly different for $p < 0.05$, analyzed by Fishers Least Significant Difference (LSD) test.

The amount of GAP by plant ($\mu\text{g}/\text{plant}$) increased when RI increased (Figure III.3.5). This is confirmed for the three cultivars studied, even in the Muscat at high RI values. This is due to the effect of the increment of fresh fruit weight is bigger than the decrease in the biosynthesis of aroma precursors per berry. Results are also shown in an histogramme using discontinues values of RI (Figure III.3.6).

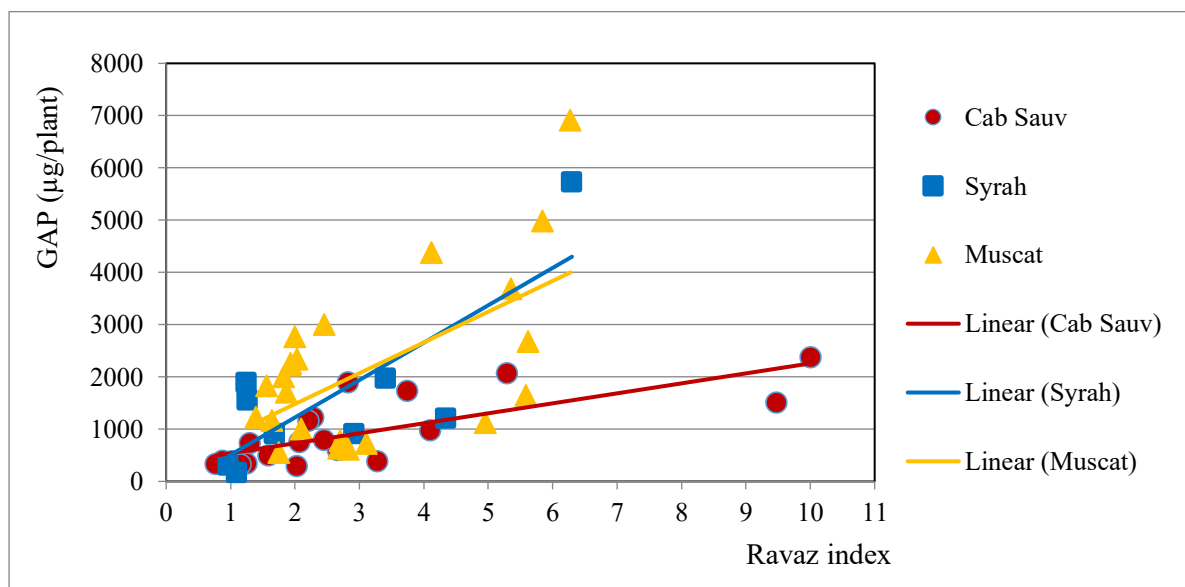


Figure III.3.5: Content of GAP ($\mu\text{g/plant}$) in Cabernet-Sauvignon, Syrah and Muscat at different levels of RI all year included, sampled at physiological maturity.

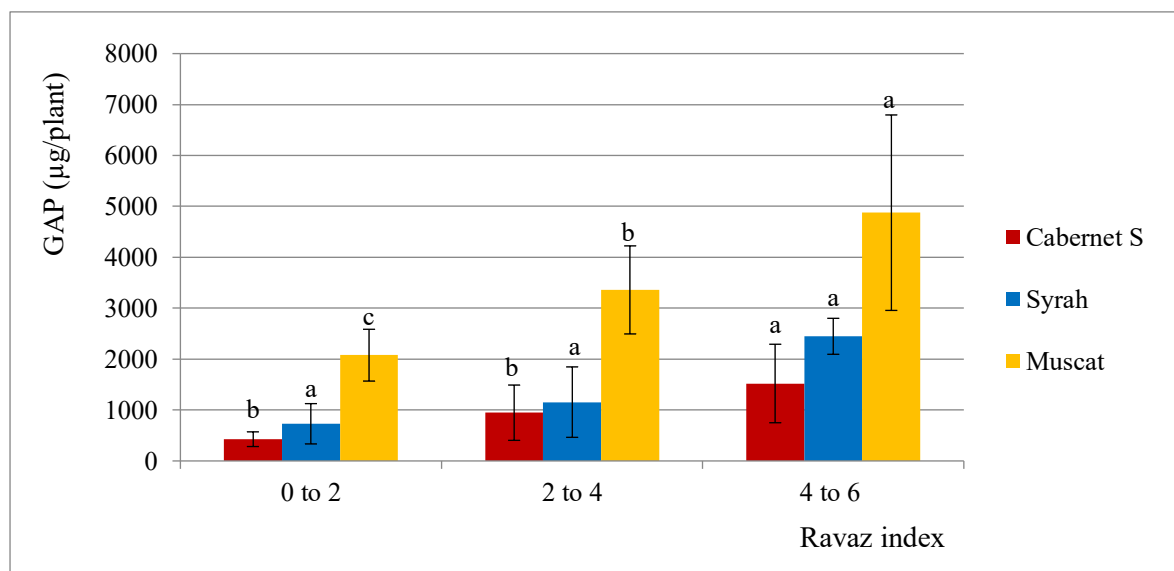


Figure III.3.6: Content of GAP ($\mu\text{g/plant}$) in Cabernet-Sauvignon, Syrah and Muscat at different levels of RI all year included, sampled at physiological maturity. For each variety, bars with different letters refers to averages significantly different for $p < 0.05$, analyzed by Fishers Least Significant Difference (LSD) test.

3.4 Interactions between primary and secondary metabolisms

As presented in Chapter I (Objectives), the scope of this point is to study the interactions between primary and secondary metabolisms in grapes when the source/sink changes. Then, 2015, 2016 and 2017 results are analyzed to identify possible interactions between both metabolisms. Three topics are going to be analyzed:

- Impact of RI on interactions between GAP and sugar content
- Impact of RI on interactions between GAP and anthocyanins
- Does S/S impact the link between primary and secondary metabolisms in the same way in each cultivar?

A comparison between primary (sugar) and secondary (GAP and anthocyanins) metabolism, in relation with the RI is studied in this point. The data used for the dates used for the different comparisons comes from three years of trials and all samples.

Results show that for Syrah and Cabernet-Sauvignon, the concentration (g/L, Figure III.4.1) and content per berry (g/berry, Figure III.4.2) of sugars does not change when RI evolves. Instead, Muscat shows a decrease of sugar concentration when RI increases.

Due to higher yields, sugar content per plant always increases correlatively to the increase in RI for all varieties (Figure III.4.3).

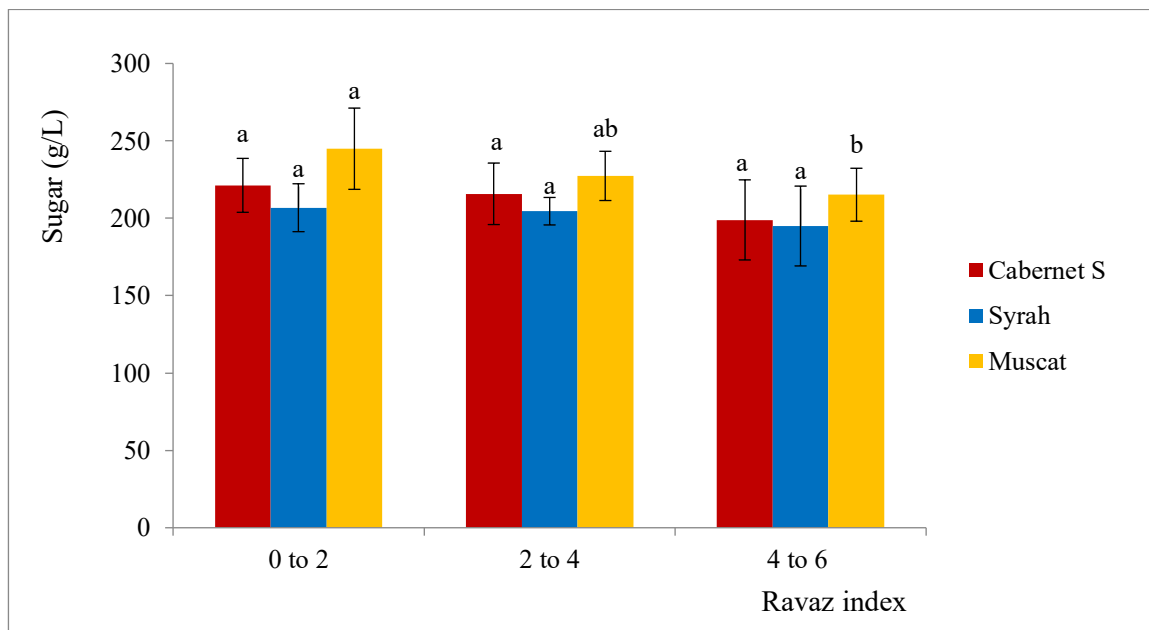


Figure III.4.1: Concentration of sugar (g/L) in Cabernet-Sauvignon, Syrah and Muscat at different levels of RI all year included. For each variety, bars with different letters refers to averages significantly different for $p < 0.05$, analyzed by Fishers Least Significant Difference (LSD) test.

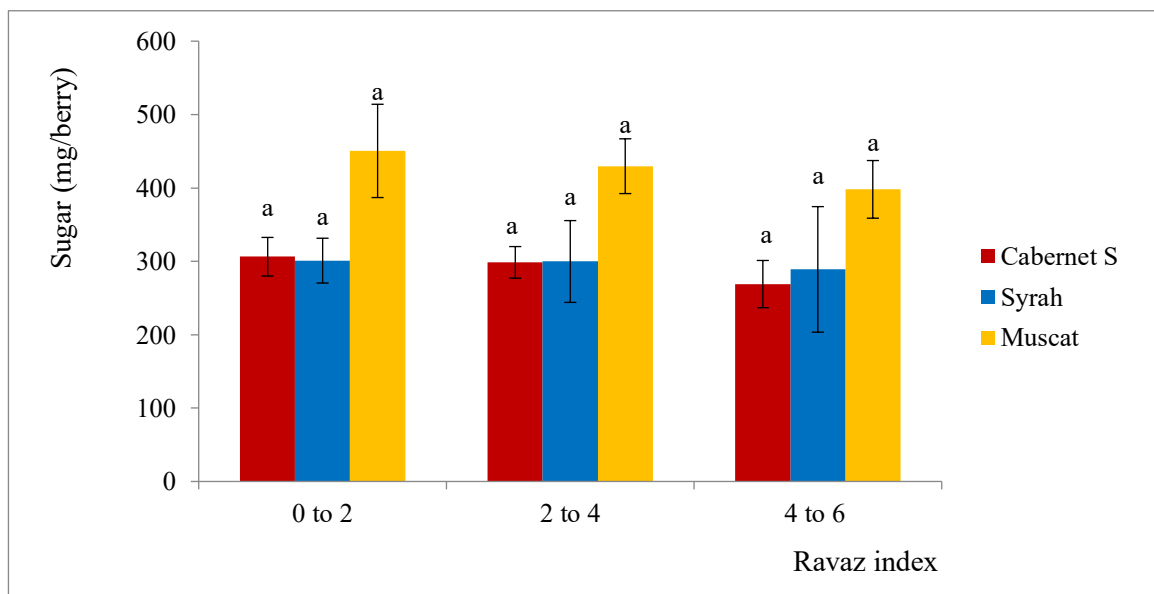


Figure III.4.2: Content of sugar (mg/berry) in Cabernet-Sauvignon, Syrah and Muscat at different levels of RI all year included. For each variety bars with different letters refers to

averages significantly different for $p < 0.05$, analyzed by Fishers Least Significant Difference (LSD) test.

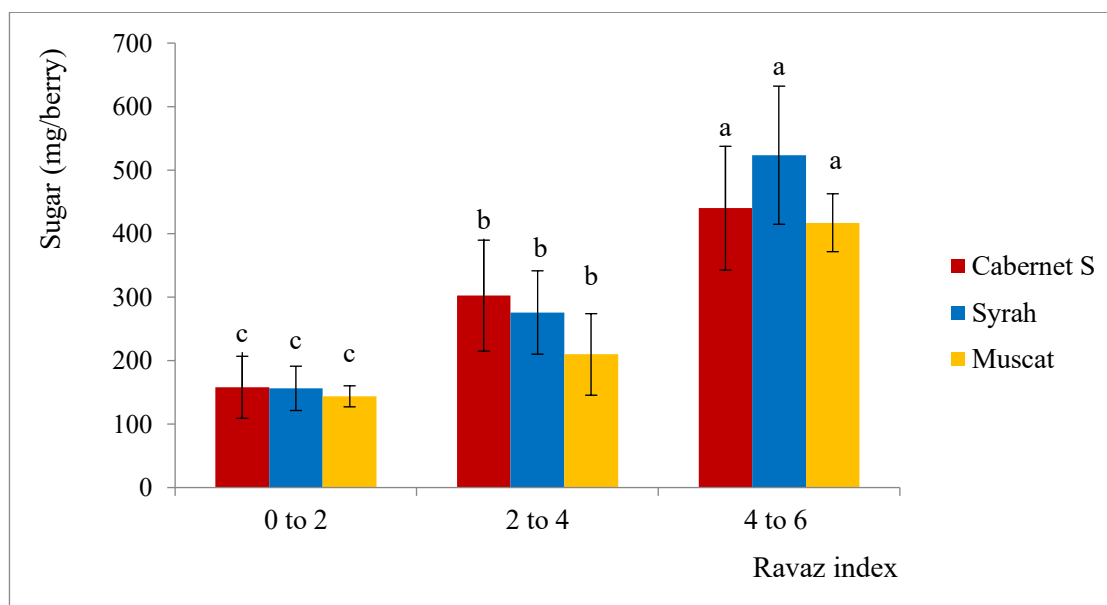


Figure III.4.3: Content of sugar (g/plant) in Cabernet-Sauvignon, Syrah and Muscat at different levels of RI all year included. For each variety bars with different letters refers to averages significantly different for $p < 0.05$, analyzed by Fishers Least Significant Difference (LSD) test.

The impact of decreasing the S/S ratio in anthocyanins concentration (mg/L) and per berry (mg/berry) of red varieties Cabernet-Sauvignon and Syrah is shown in Figures III.4.4 and III.4.5. The general tendency is that anthocyanins decreased when the RI is increased (although there are not significant differences in the conditions of this experimentation).

Anthocyanins per plant in Cabernet-Sauvignon and Syrah (Figure III.4.6) show a clear tendency: when RI increases, anthocyanins/plant increases too (even if error bars are big because the data used comes from 3 years of trials and different moments of sampling).

Muscat is the cultivar with highest values of the ratio between aroma precursors and sugar (Figure III.4.7). White varieties in general have highest values of this ratio, meaning a higher production of GAP by each molecule of sugar produced.

The ratio GAP/sugar does not change in Syrah and Cabernet-Sauvignon when RI changes (Figure III.4.8). Instead, it decreases in Muscat with high RI. This could mean that in a situation of fruit overload, the vines privilege the primary metabolism (sugar production) to the detriment of the aroma precursors. Under the conditions of this experimentation, this is manifest in Muscat, a variety with high production of GAP, and not in the red varieties, naturally less rich in GAP. In red cultivars, anthocyanins have a tendency of decreasing in relation to sugar when RI is increased (although no statistical difference) (Figure III.4.9). Seemingly in red cultivars, anthocyanins are more sensible to the RI changes than aroma precursors.

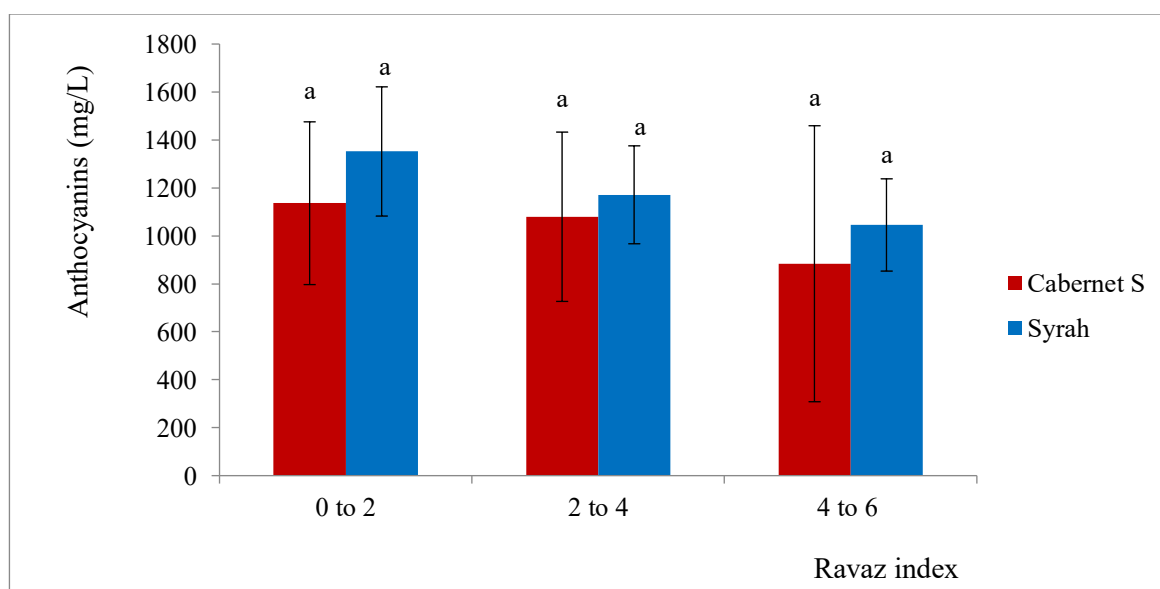


Figure III.4.4: Concentration of anthocyanins (mg/L) in Cabernet-Sauvignon and Syrah at different levels of RI all year included. For each variety, bars with different letters refers to averages significantly different for $p < 0.05$, analyzed by Fishers Least Significant Difference (LSD) test.

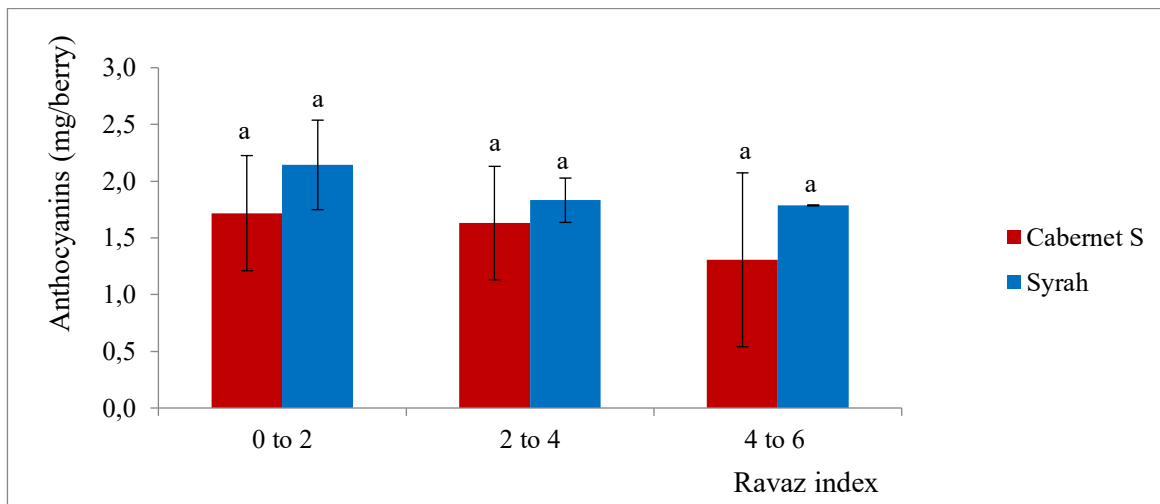


Figure III.4.5: Content of anthocyanins (mg/berry) in Cabernet-Sauvignon and Syrah at different levels of RI all year included. For each variety, bars with different letters refers to averages significantly different for $p < 0.05$, analyzed by Fishers Least Significant Difference (LSD) test.

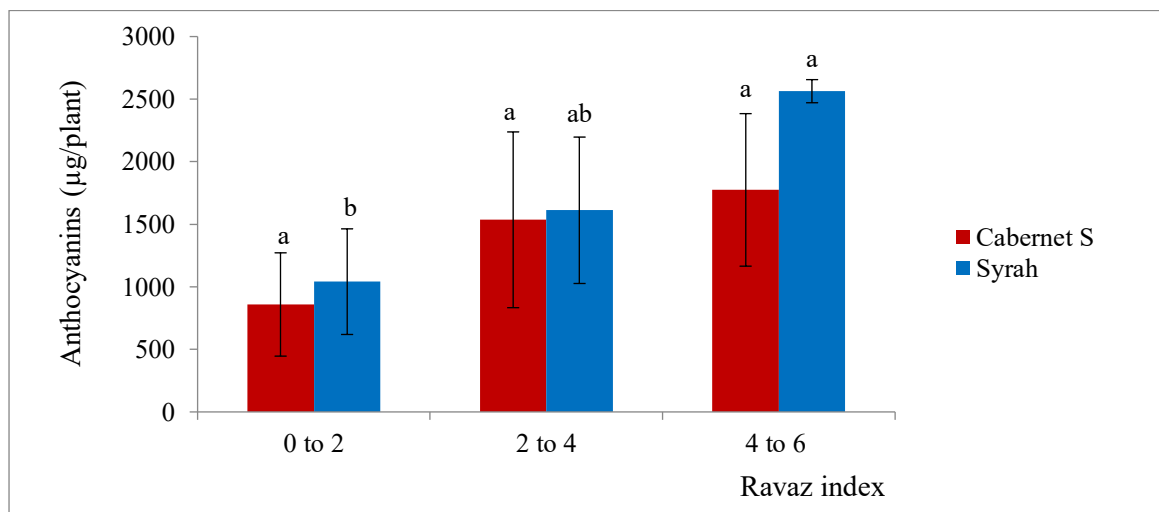


Figure III.4.6: Content of anthocyanins (µg/plant) in Cabernet-Sauvignon and Syrah at different levels of RI all year included. For each variety, bars with different letters refers to averages significantly different for $p < 0.05$, analyzed by Fishers LSD test.

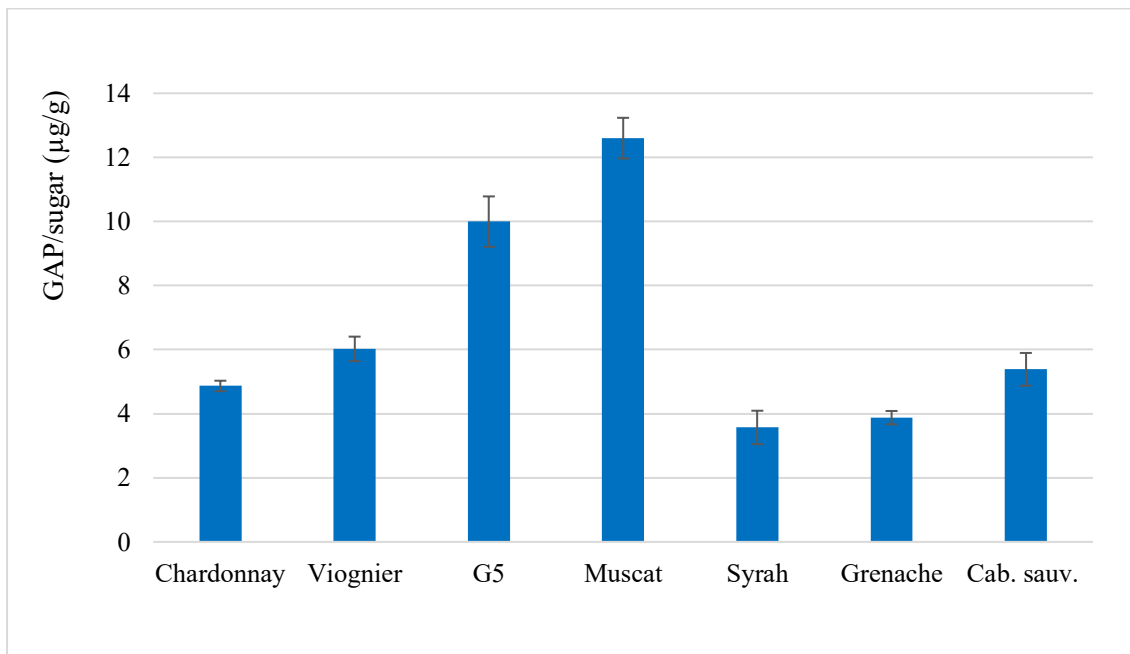


Figure III.4.7: Balance between GAP and sugar (μg of GAP/g of sugar) in 8 cultivars in 2015, in control plants berries, sampled at physiological maturity.

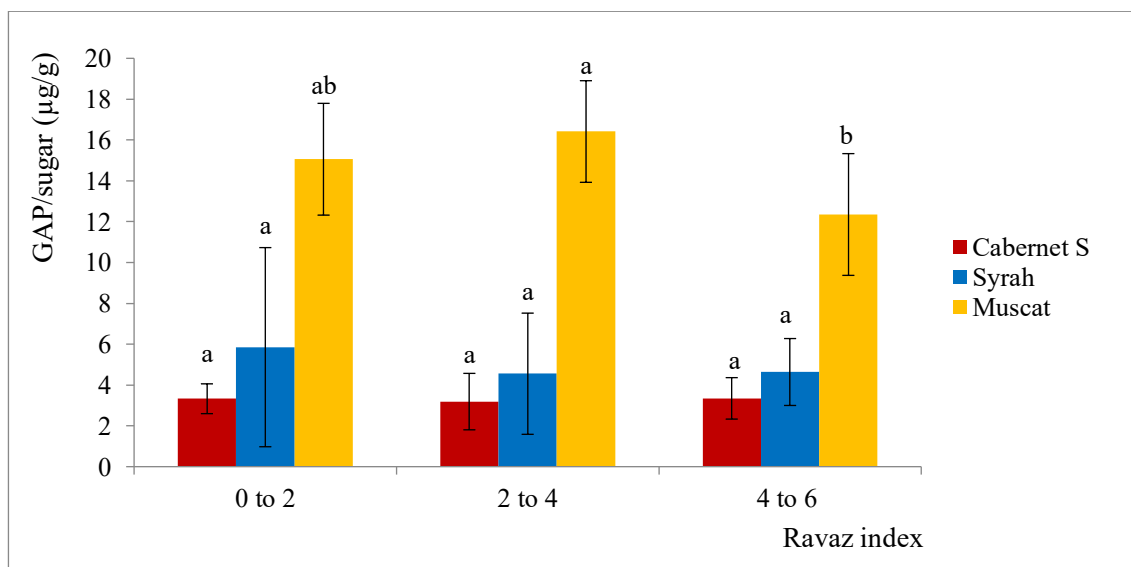


Figure III.4.8: Balance between GAP and sugar (μg of GAP/g of sugar) in Cabernet-Sauvignon Syrah and Muscat varieties at different levels of RI all year included, sampled at physiological maturity. For each variety, bars with different letters refers to averages significantly different for $p < 0.05$, analyzed by Fishers Least Significant Difference (LSD) test.

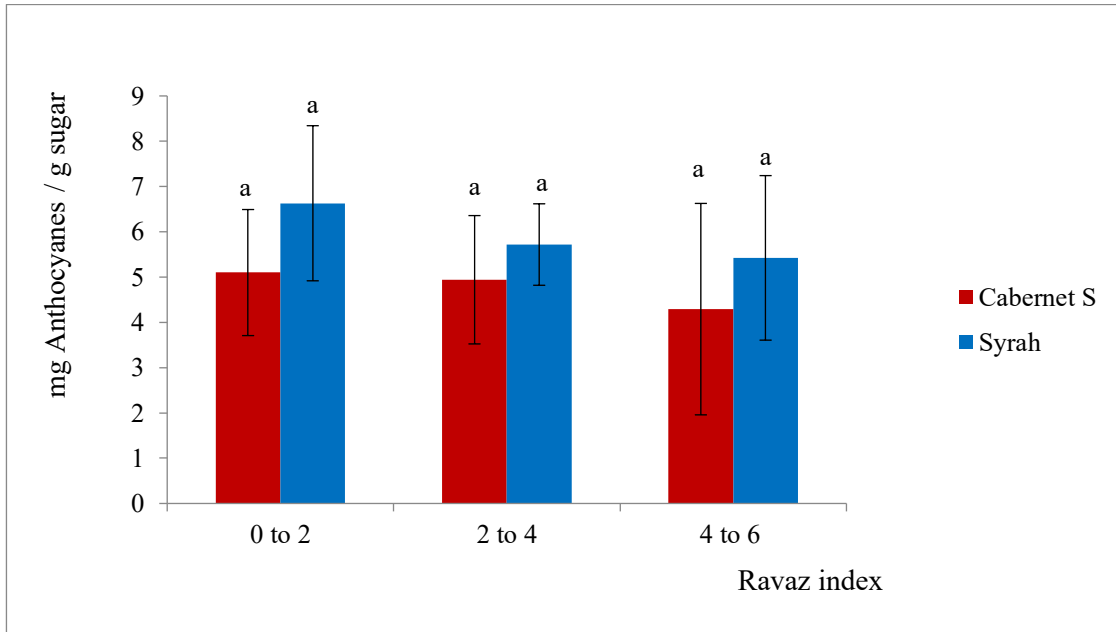


Figure III.4.9: Balance between anthocyanins and sugars (mg of anthocyanin/g of sugar) in Cabernet-Sauvignon and Syrah at different levels of RI, all year included, sampled at physiological maturity. For each variety, bars with different letters refers to averages significantly different for $p < 0.05$, analyzed by Fishers Least Significant Difference (LSD) test.

3.5 Influence of bunch thinning timing on GAP biosynthesis

In this section, the timing of bunch thinning as a practice to modify S/S balance is analyzed. Therefore, trials consisted in thinning the grape bunch in two periods of the growing cycle. First, 2-3 weeks before onset of ripening, when green berries grow at high rate before veraison (pea size) (rapid growth stage - RGS), and the second one at the onset of ripening (OOR). Bunch thinning levels were as 50% for 2015 and 2017, and 70% for 2016.

Syrah showed an increment of GAP ($\mu\text{g}/\text{berry}$) when the balance S/S was modified early, at RSG in 2015 (Figure III.5.1) and 2016 (Figure III.5.2). In 2017, no differences were found (data not shown).

Cabernet-Sauvignon showed no difference in the content of GAP ($\mu\text{g}/\text{berry}$) when the balance S/S was modified early (RGS) or later (OOR) (Figures III.3.5.3 and III.5.4).

In Muscat, the bunch thinning performed late, at OOR, increased the content of GAP /berry in 2016 (Figure III.5.5). No differences were found in 2017 (Figure III.5.6).

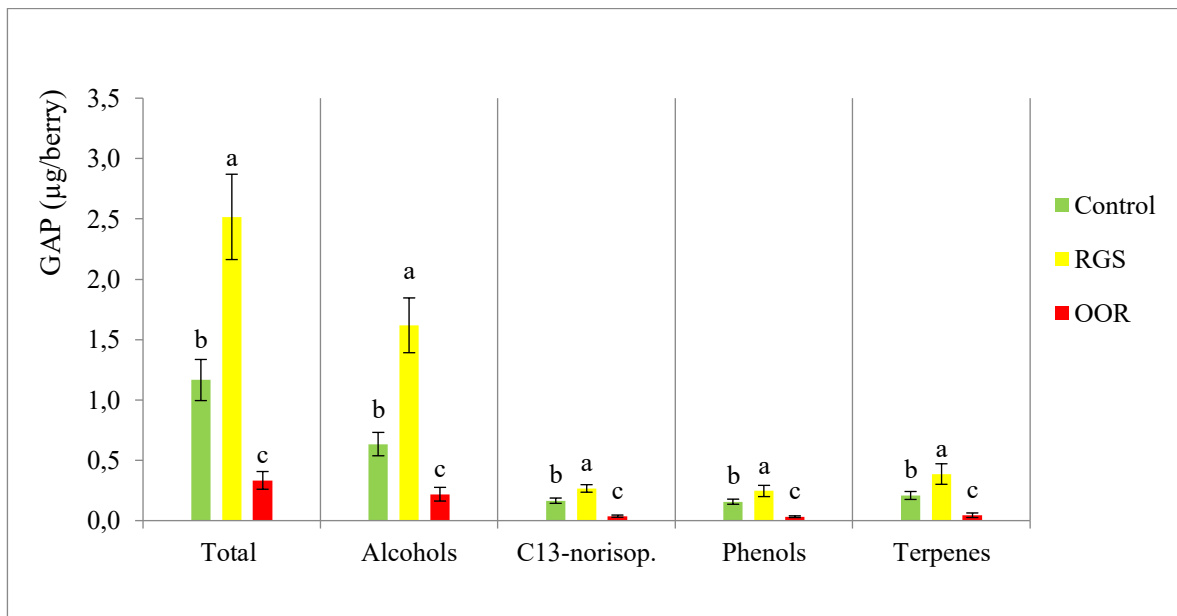


Figure III.5.1: Content of GAP (µg/berry), at physiological maturity, after 50 % of bunch thinning at rapid growth stage (RGS) and at onset of ripening (OOR). Bars with different letters refers to averages significantly different for $p < 0.05$.

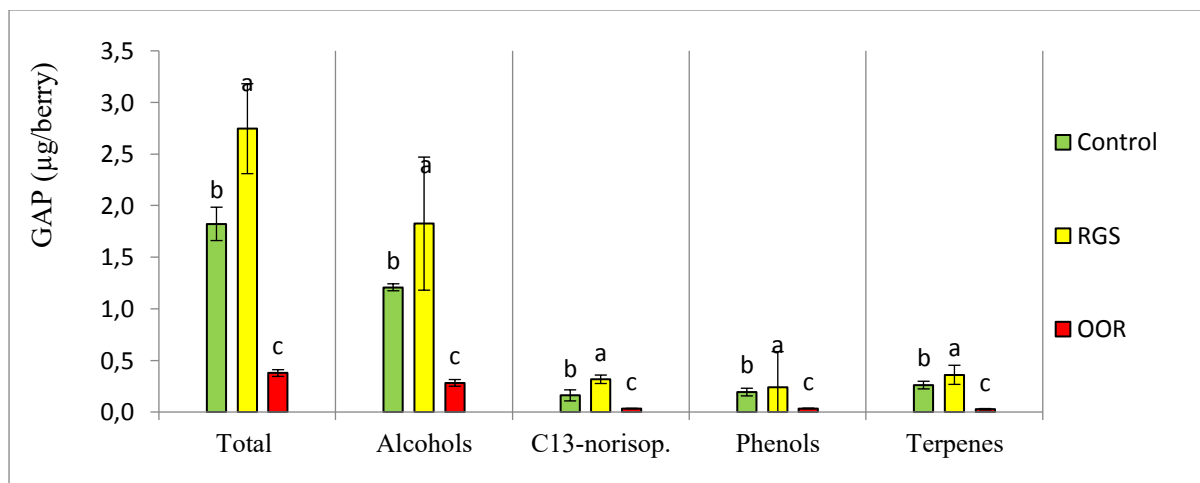


Figure III.5.2: Content of GAP (µg/berry) in berries of Syrah 2016, sampled at one moment of the ripening period after 70 % of bunch thinning at rapid growth stage (RGS) and at onset of ripening (OOR). Bars with different letters refers to averages significantly different for $p < 0.05$.

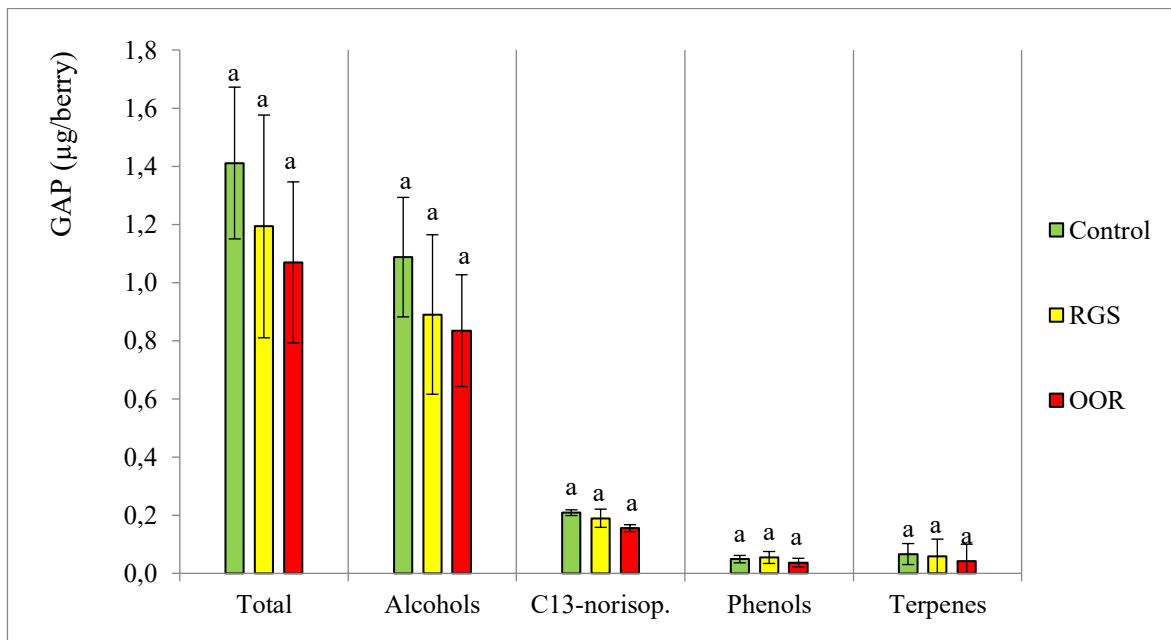


Figure III.5.3: Content of GAP in Cabernet-Sauvignon 2015 berries (µg/berry) at physiological maturity (04/09/2015), after 50 % of bunch thinning at rapid growth stage (RGS) and at onset of ripening (OOR).

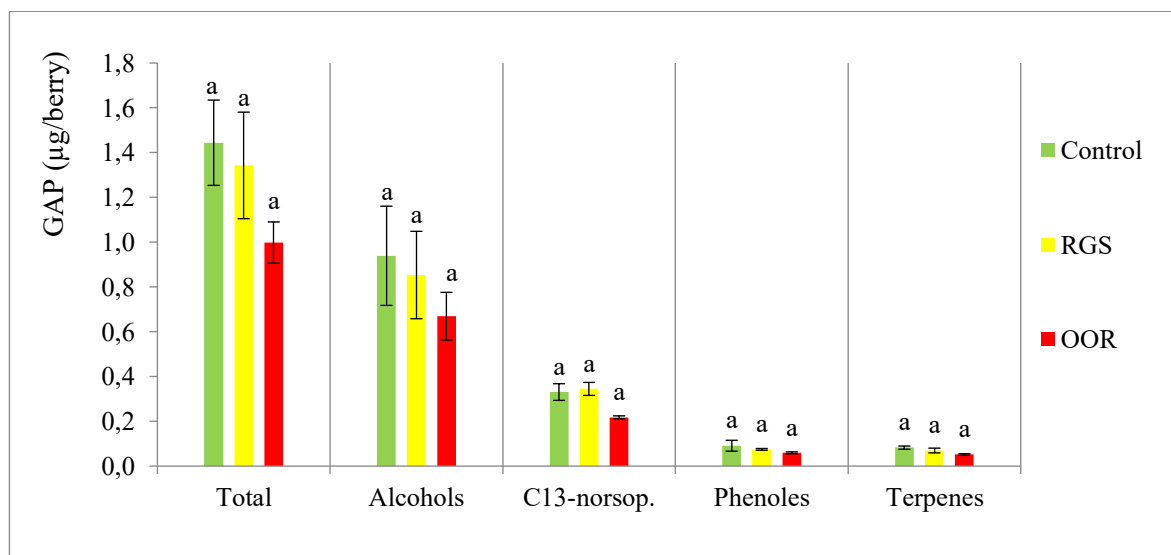


Figure III.5.4: Content of GAP in Cabernet-Sauvignon 2016 berries (µg/berry) at physiological maturity, after 70 % of bunch thinning at rapid growth stage (RGS) and at onset of ripening (OOR). Bars with different letters refers to averages significantly different for $p < 0.05$.

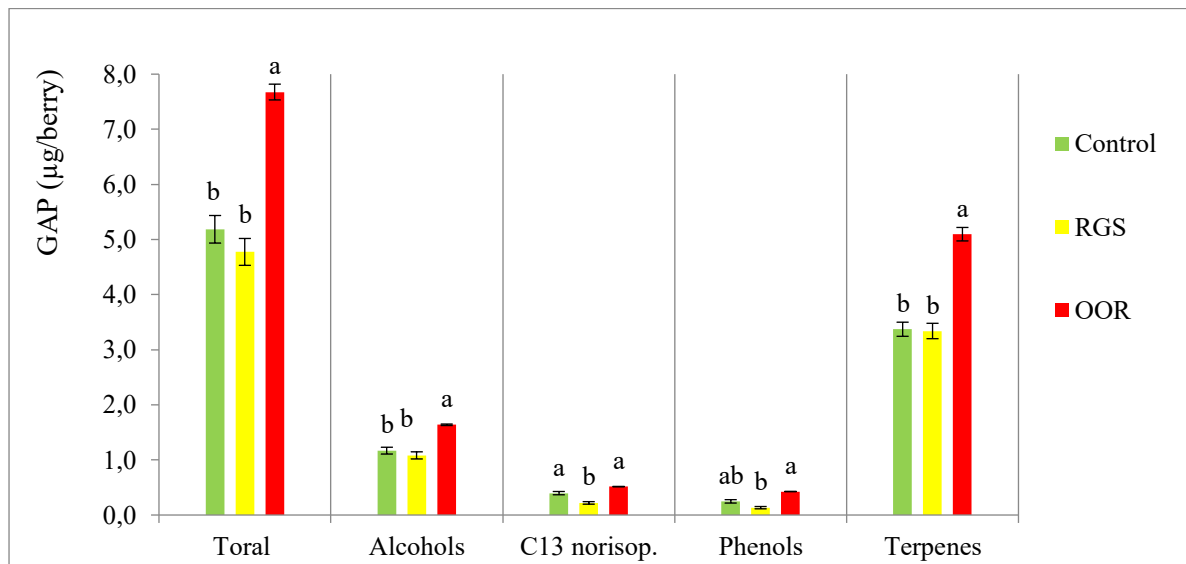


Figure III.5.5: Content of GAP in Muscat 2016 berries (µg/berry) at physiological maturity, after 70 % of bunch thinning at rapid growth stage (RGS) and at onset of ripening (OOR). Bars with different letters refers to averages significantly different for $p < 0.05$.

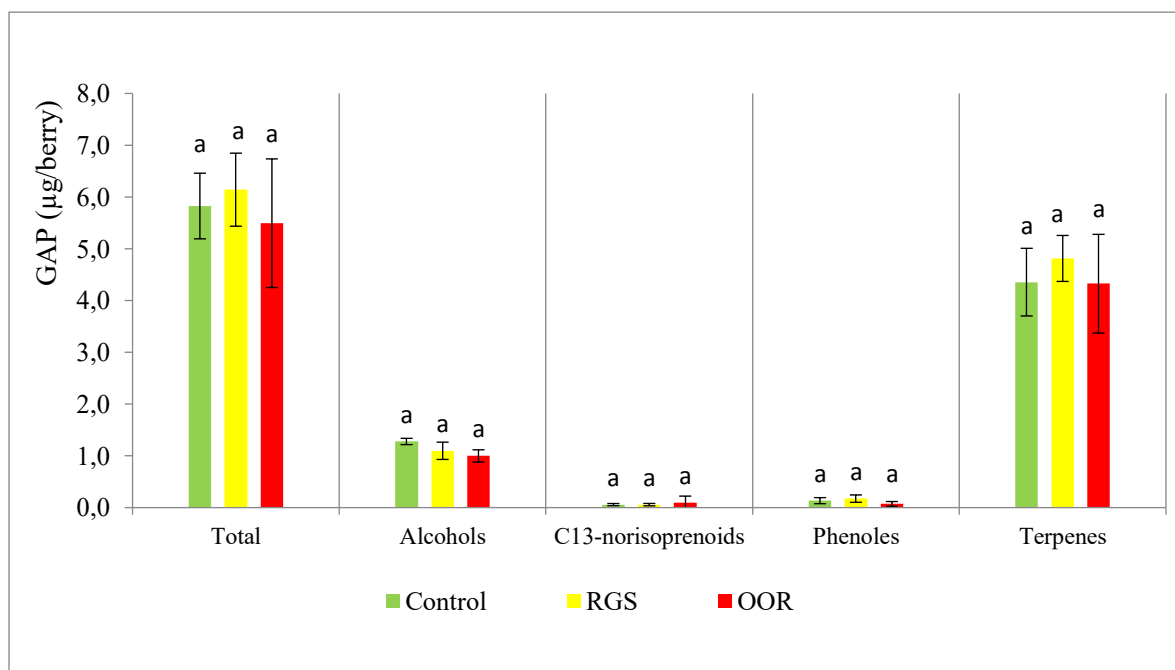


Figure III.3.5.6: Content of GAP in Muscat 2017 berries (µg/berry) at physiological maturity, after 50 % of bunch thinning at rapid growth stage (RGS) and at onset of ripening (OOR). Bars with different letters refers to averages significantly different for $p < 0.05$.

3.6. Variations for the loss in sugar and the gain in GAP when source/sink is modulated

The aim of the topic is to analyze how much sugar has to be lost (by increasing S/S) at plant level to obtain an increase in GAP concentration in the fruit. The cultivars chosen for this analyzes are those with positive response to the modification of the S/S balance (Muscat and Syrah).

The date of sampling is similar to the technological maturity date, used in commercial vineyards, one to three weeks after physiological maturity. The moment of bunch thinning chosen is RGS for Syrah. For Muscat, the period of bunch thinning chosen was OOR (which resulted to be more effective than thinning in RGS).

The level of FFW expressed in g, for each RI and the difference (lost) in fruit when RI is reduced is shown in Table III.6.1. Then the GAP concentration ($\mu\text{g/L}$) is shown together with the difference (gain or loss) after reducing RI.

In most situations, the concentration of GAP/L is increased when RI decreased. But when data is analyzed at plant level, results show that there are negative results for all varieties (except for Syrah 2015, where a little gain is produced). This means that the gain in GAP concentration do not compensate the GAP plant lost by reducing FFW. Then all the productive system is losing GAP when RI is reduced.

Table III.6.1: Balance sugar/GAP when RI is modified

| Cultivar | Year | RI | FFW/plant | FFW difference | GAP (µg/l) | GAP difference | GAP /plant | GAP difference |
|-----------------|-------------|-----------|------------------|-----------------------|-------------------|-----------------------|-------------------|-----------------------|
| | | | g/plant | g/plant | µg/L | µg/L | µg/pl | µg/plant |
| Syrah | 2015 | 4,35 | 2.694 | | 696 | | 1.711 | |
| | | 2,15 | 1.282 | -1412 | 1.519 | 822 | 1.763 | 52 |
| Syrah | 2016 | 2,92 | 1.832 | | 948 | | 1.567 | |
| | | 0,96 | 663 | -1.169 | 1.492 | 544 | 616 | -951 |
| Syrah | 2017 | 4,23 | 2.679 | | 871 | | 2.150 | |
| | | 2,08 | 1.281 | -1.398 | 1.209 | 338 | 945 | -1.206 |
| | | 1,01 | 706 | -1.973 | 803 | -68 | 523 | -1.627 |
| Muscat | 2016 | 5,36 | 2.028 | | 2.811 | | 5.441 | |
| | | 2,46 | 806 | -1.222 | 4.127 | 1.316 | 3.157 | -2.284 |
| Muscat | 2017 | 4,96 | 2.274 | | 2.956 | | 6.332 | |
| | | 3,11 | 1.035 | -1.239 | 3.962 | 1.006 | 3.857 | -2.475 |

3.7 The economic balance between reducing yield (kg/ha) and increasing the potential of grape quality

A simulation was made to calculate the impact on the final yield (kg of fruit per hectare), when S/S is reduced to increase quality.

As in section 3.6, the date of sampling is similar to the technological maturity date, used in commercial vineyards, approximately one to three weeks after physiological maturity. The moment of bunch thinning chosen is RGS, except in Muscat where the period of bunch thinning chosen was OOR (which resulted to be more effective than thinning in RGS).

Table III.7.1 shows the level of yield (expressed in kg/ha), and the difference (lost) in fruit when RI is reduced for Muscat and Syrah. Then the GAP concentration ($\mu\text{g/L}$) percentual difference is shown.

Results shows that Syrah has got interesting increments of GAP/L, with percentual increments of 118.1%, 57.3 % and 38.9 % in 2015, 2016 and 2017 respectively. Muscat also shows increments of 46.8 % and 34% of GAP concentration in 2016 and 2017 respectively.

The goal would be to evaluate, if the mentioned increments on GAP concentration, impacts on wine quality and prize and if this extra income compensate the great loss of fruit (between 50 and 60 % of yield). This estimation exceeds the purpose of the present research.

Table III.7.1: Yield /GAP balance

| | Year | Yield/ha | Yield difference | GAP ($\mu\text{g/L}$) difference |
|---------------|-------------|-----------------|-------------------------|--|
| | | kg/ha | kg/ha | % |
| Syrah | 2015 | 10.774 | | |
| | | 5.128 | -5.646 | 118 |
| Syrah | 2016 | 7.327 | | |
| | | 2.651 | -4.676 | 57 |
| Syrah | 2017 | 10.714 | | |
| | | 5.123 | -5.591 | 39 |
| | | 2.824 | -7.890 | -8 |
| Muscat | 2016 | 8.113 | | |
| | | 3.226 | -4.887 | 47 |
| Muscat | 2017 | 9.095 | | |
| | | 4.139 | -4.956 | 34 |

4. Chapter IV: Discussion

Results presented in this Thesis only includes glycosylated aroma precursors; instead methoxypyrazines, volatile thiols and free volatiles aroma molecules were not considered. Then, we do not take into account all the aromas which account for the sensory attributes of grapes and wines.

Results from 2015 showed that there is a great difference in GAP ($\mu\text{g/L}$) values between varieties, as noted in Figure III.1.1, being Muscat and G5 the varieties with higher concentration of GAP. Instead Syrah and Marselan shows the lowest ones. The aromatic composition is different between varieties. Muscat and G5 are the varieties with the greatest aromatic potential, the first one due to its rich profile in terpenes and the second one due to an important concentration in phenols. This is confirmed even when values are expressed as $\mu\text{g/berry}$, $\mu\text{g /plant}$ or as kg of GAP/hectare . This is coincident with literature (Song et al., 2012; Friedel et al.2016; Schwab et al.2015; Hjelmeland, 2014) nevertheless in this research, values were obtained in one experiment with identical agronomical and analytical condition (with exception of soil).

Regarding the groups of molecules, Muscat shows great concentration of terpenes (linalol oxide, HO-trienol, trans-pyran linalool oxide trans, trans-pyran linalool oxide, nerol, geraniol, 3,7-dimethyl-1,5-octadien-3,7-diol, 8-hydroxydihydrolinalool, 2,6-dimethylocto-2,7-dien-1,6-diol, Z-8-hydroxylinalol, geranic acid, p-menth-1-ene-7, 8 diol, etc.), giving the typical aromatic flavor to grapes, as described in literature (Mateo and Jiménez, 2000; Darriet and Thibon, 2012; Robinson et al. 2014). Instead, Chardonnay, Syrah, Grenache, Cabernet-Sauvignon and Marselan show great amounts of glycosylated alcohols (hexanol, 3-hexen-1-ol cis, 2-hexen-1-ol trans, 1-octen-3-ol, 2-phenylethanol, benzylic alcohol, etc.). In Viognier,

alcohols and terpenes are the most important groups of molecules and are present in similar proportions. Phenols (eugenol, phenol, vanillin, zingerone, methyl zingerate, unknown 198, guayacol-propanol, etc.) and C-13 norisoprenoids (3,4-dihydro-3-oxo-actinidol, 3-hydroxy-B-damascenone, 3-hydroxy-7,8-dihydro-b-ionone, 3-hydroxy-b-ionone, 3-oxo-7,8-dihydro-a-ionol, 3-oxo-Ar-etroAionol, 3-hydroxy-7,8-dihydro-b-ionol, 3-oxo-a-ionol, 3-oxo-A-retroionol, 3-hydroxy-7,8-dihydro-B-ionol, etc.), contrarily, are in very low proportions in all the cultivars, except for G5. The participation of C-13 norisoprenoids in grape aroma is greater than alcohols, due to the low perception threshold of these molecules (Winterhalter and Rouseff, 2002). C-13 norisoprenoids are among the most flavor compounds in wines and contribute to floral and fruity attributes (Winterhalter and Schreier, 1994).

The ACP (Figure III.1.2) shows the Muscat is isolated from the other cultivars because of its high contents of terpenes. The G5, which derives from Muscat de Hambourg, is also in the same way, but away from the Muscat and closer to Viognier. Grenache and Cabernet-Sauvignon are on the opposite side of Muscat, due to their low concentration of aroma precursors. Interestingly, Marselan, a variety created by INRA from the hybridization of the Cabernet-Sauvignon and Grenache N varieties, it is placed between both of them. In general, the group of white varieties is located to the right of the figure (richest in GAP) and the red varieties to the left.

GAP analyzes expressed as GAP/berry are shown in Figure III.1.3 and Table III.1.2. Results show the same tendency and order than those expressed in concentration $\mu\text{g/L}$. But when comparing the maximum values of each cultivar, G5 has highest GAP values ($\mu\text{g/berry}$) than Muscat. This may be because the highest volume of the G5 berry.

In Figure III.1.4, the ACP of the GAP content ($\mu\text{g/berry}$) is shown. The ACP is similar to the previous one expressed in $\mu\text{g/L}$, but now Grenache is away from the other red varieties, with

higher GAP contents per berry, mainly alcohols. This could be because of the larger size of the Grenache's berries.

Considering all the molecules analyzed (Figure III.1.5), Muscat shows a great correspondence with approximately 12 molecules of terpenes and phenols. Viognier also shows great correspondence with 7 molecules. Instead, Chardonnay, Syrah, Grenache, G5 and Cabernet-Sauvignon shares the same group of molecules.

When GAP are expressed in $\mu\text{g/plant}$ (Tables III.1.3 and III.1.4 and Figure III.1.6), the plant fresh fruit weight becomes a crucial factor. G5 shows the great content of GAP/plant, due to both, the high values of the fruit/plant and the high Content of GAP in berries. The ACP (Figure III.1.7) shows the G5 close to Muscat (which has a bigger individual content of terpenes). The high fresh fruit weight per plant of the G5 compensates the high terpenes content per berry of the Muscat. In Figure III.1.7, results are expressed in GAP/per hectare (assuming a constant number of plant/ha = 4000). Results shows that G5 is the variety with highest content of GAP/ha.

In point 3.2, the impact of the year on GAP and the relationship with fresh fruit weight (FFW) was analyzed along three years. Results showed that there is a big effect of the climatic and agronomical conditions in each period on FFW (g/plant) (Figure III.2.1). Syrah showed in 2015 and 2016 the greatest FFW (g/plant) and in 2017 FFW is decreased. For Cabernet-Sauvignon results showed an a very high fruit production in 2015. But this was not noticed in the rest of the cultivars suited. This difference may be due to the combination of climate and soil conditions of the Cabernet-Sauvignon vineyard in the previous years to the present research, resulting in a bigger fertility of the vines. Even more, the biology of grapevine is complex, as fertility is influenced principally by the agro-climatic conditions of two seasons (Keller, 2010).

The effect of the “year” on the GAP can be seen in figures II.2.2 and II.2.3. As in FFW, the effect of the year did not show a clear effect or tendency. But when analyzing results at plant level, the impact of the increment in FFW/plant is very high and has a big effect on GAP/plant.

These results demonstrate that it is not possible to find a trend in a three-year experiment, and a longer set of years should be needed to understand the influence of the year effect in the GAP concentration. This topic becomes relevant in the context of a changing climate (Torregrosa et al., 2017), in order to preview the potential chances in GAP concentration, which can impact wine aromatic quality.

In the experiments performed during 2015, 2016 and 2017 (point 3.3), the effect of modifying the S/S balance on GAP and primary metabolism was studied. The first question postulated was: is the RI depending on cultivars?

The weight of the winter pruning wood (WP) was not very affected by the changes in fruit charge (Table III.3.1). WP (vegetative growth of the year), does not change too much when FFW is modified. Results showed that in Syrah, the FFW increased 239.05 % while WP was decreased - 9.57 %. These values of Ravaz (4 to 10) are among the recommended in literature, depending on authors (Reynolds 2018; Zhuang 2014; Kliewer and Dokoozlian 2005; Steyn 2016). In Zhuang’s trial, changes of 57,1% in fresh fruit weight level corresponded to 7,14 % in WP in 2011 and changes of 63,15 % in fresh fruit weight level corresponded to 0 % in WP in 2012. Then, when the fruit charge is modified, there is little or no consequence in the vegetative growth, meaning that there are minimal or no changes in the partition of carbohydrates for these cultivars. Theoretically, there should be an extra amount of available carbohydrates when S/S ratio increased, and this could result in an increment of the secondary metabolites biosynthesis (considering that all the GAP derives from carbohydrates, as shown in Chapter I, Review’s figure N°1).

The second question in this section was: what is the RI impact on GAP biosynthesis?

Results from Figures III.3.1 and III.3.2, showed that when RI changes, GAP concentration was impacted differently depending on cultivar. Muscat showed a decrease of GAP concentration when RI was increased, and Syrah shows the same tendency. Instead for Cabernet-Sauvignon, there are no changes when RI is modified. This means that the response depends on the genotype. When results are expressed in $\mu\text{g}/\text{berry}$ (Figure III.3.3 and II.3.3.4), the results have the same tendency as when expressed in $\mu\text{g}/\text{L}$. This could mean that there is not only a matter of concentration and dilution of the GAP: there could be a difference at biosynthesis level. But, in all the varieties, when GAP was expressed in terms of $\mu\text{g}/\text{plant}$ (Figure III.3.3 and III 3.4), an increment of RI resulted in an increment of GAP ($\mu\text{g}/\text{plant}$), due to the big difference in fresh fruit weight (g/plant). Kok et al. 2011 also found an increment in GAP when S/S was modified by bunch thinning in Sauvignon blanc, an aromatic cultivar. Kock's results are then similar to those obtained in Muscat in the present research. But, results from Kok's research are expressed in concentration only ($\mu\text{g}/\text{L}$); then it is not possible to distinguish if there is an effect of concentration and/or dilution of the GAP or to an increment in the biosynthesis of the GAP. Reynolds et al. 2007, also reported increments of GAP concentration after bunch thinning in Chardonnay.

In point 3.4, the interactions between primary and secondary metabolism were studied. Results showed that when RI is modified, in Syrah and Cabernet-Sauvignon, the concentration and content of sugar do not change (Figure III.4.1 and Figure III.4.2). On the other hand, Muscat sugar concentration decreased when RI increased. And as expected, the GAP content per plant increases correlatively to the increase in RI for all varieties (Figure III.4.3).

The content and concentration of anthocyanins were also studied in red cultivars. When RI was modified, the values of anthocyanins decreased when RI increased (Figures III.4.4 and

III.4.5). This is coincident with Zhuang (2014) research, where the effect of bunch thinning leads to a higher concentration of anthocyanins in Cabernet franc. Anthocyanins per plant in Cabernet-Sauvignon and Syrah (Figure III.4.6) increased when RI increased, as a result of the increment on fruit per plant.

Figure III.4.7 shows that Muscat has highest values of the ratio between GAP and sugar. Results also shows that white varieties in general have highest values of this ratio than red cultivars, meaning a higher production of GAP by each molecule of sugar produced.

The ratio GAP/sugar does not change in Syrah and Cabernet-Sauvignon when RI is increased (Figure III.4.8). Instead, it decreases in Muscat with high RI. This could mean that in a situation of fruit overload, the vines privilege the primary metabolism (sugar production) to the detriment of the aroma precursors. Under the conditions of this experiment, this is manifest in Muscat, a variety with high production of GAP, and not in the red varieties, naturally less rich in GAP.

In conclusion, the modification of the S/S balance did not have an impact on the sugar concentration in Cabernet-Sauvignon and Syrah. Instead, in Muscat, the sugar concentration was decreased when RI was increased. When results are expressed in mg of sugar/plant there is always an increment of sugar/plant when RI is increased.

The analyzes of the Content of GAP produced by each gram of sugar produced (ratio GAP/sugar), showed that Muscat and G5 has got the highest values between the 8 cultivars. Is there a relationship between high GPA concentrations in white cultivars with the absence of anthocyanins? Do white varieties have more resources available to produce aroma precursors without the need to produce anthocyanins? These questions cannot be answered in

the present research with current information. Further experiments are needed in order to respond to those questions.

In Chapter 3.5, results showed that the timing of bunch thinning for increased S/S ratio impacted on GAP ($\mu\text{g}/\text{berry}$) biosynthesis in Muscat and Syrah. Muscat GAP content ($\mu\text{g}/\text{berry}$) was increased when bunch thinning was performed in onset of ripening (OOR). Similar results were obtained regarding the moment of bunch thinning by Kok et al. 2011 in Sauvignon.

Instead, in Syrah, GAP were increased when bunch thinning was performed in an earlier stage (rapid growth stage-RGS) (Figures III.5.1 to III.5.6). This could be explained based on the different timing on biosynthesis in each GAP group. Alcohols, C13-norisoprenoids and phenols (the predominant GAP molecules in Syrah) are biosynthesized early, in RGS (Mendez-Pintos et al., 2009; Mathiew et al. 2005). Then, the bunch thinning performed in RGS could have impacted in the biosynthesis of these molecules. On the other hand, terpenes which are the most important GAP molecules in Muscat, are biosynthesized during the veraison (OOR) (Schwab et al., 2015), and the bunch thinning performed at this moment could impact them. Cabernet-Sauvignon, less sensitive to S/S balance changes, did not show responses to the timing of bunch thinning.

The effect of changes in S/S ratio on GAP concentration was analyzed from a production perspective (Table III.6.1). For this experiment, berries were sampled at technical maturity, showing different results from berries sampled at physiological maturity. Results showed that generally GAP concentration ($\mu\text{g}/\text{L}$) increased when S/S balance increased (or RI decreased), but when analyzed at plant level, results showed that GAP (mg/plant) tend to decrease when RI decreased, indicating that there is not enough compensation of the lost fruit.

When analyzing the production of GAP at vineyard level, results show that there are increments of GAP (Table III.7.1). But to obtain increments of GAP between 25 and 100% (approximately), depending on cultivar, yield should be reduced in approximately 50 or 60%. An increment of GAP concentration of the grape juice before winemaking can potentially (as they are aroma precursors) increase wine quality. Is there an economic compensation of losing approximately one half of the fruit production to potentially enhance the aromatic quality of wine? This question cannot be answered in the current research. Further experiments are needed in this area.

5. Chapter V: Conclusions and perspectives

The aromatic composition of grapes showed a great difference between the 8 cultivars analysed. This variability was put into evidence when the molecules of each cultivar were analyzed. Results also showed that the total amount of GAP fluctuated between cultivars, being Muscat and G5 the cultivars with more GAP/berry. Even more, Muscat and G5 showed the higher values of GAP/sugar ratio. White cultivars in general, showed higher values of GAP/sugar than red cultivars. Results were also analyzed regarding the year effect, and the agroclimatic conditions that each year effect had on yield and GAP concentration.

The responses to the source/sink (S/S) balance modification were also genotype-dependent. The concentration of GAP was not impacted when S/S balance was modified in Cabernet-Sauvignon. Instead, Muscat and Syrah showed increments of GAP/berry at physiological maturity when the S/S balance was increased. The time of the modification of the S/S balance impacted in both cultivars. Muscat showed a positive response to changes in S/S balance at onset of ripening and Syrah at rapid growth stage. It would have been critical to evaluate this fact from a molecular point of view and then analyze the response of modifying S/S balance in gene transcription and metabolic products. This was not analyzed as it was not the aim of the present study.

Then, agronomical practices which modify S/S balance, such as bunch thinning, pruning or leaves removal should be evaluated for each cultivar and agro-climatic situation, analyzing their impact on berries' GAP concentration. Many of the mentioned practices are often used in an empirical way without supporting research.

From a viticultural perspective, the increase of S/S ratio resulted in increments of GAP concentration, when berries were sampled at technical maturity. But increasing the GAP

concentration with agronomical practices such as bunch thinning has a very high cost in terms of productivity of the system due to the significant decrease in yields. It is essential for viticulture, that future research could evaluate how does the increment of GAP in grape juice, impacts wine aroma molecules after winemaking and the final product value. It would be critical to decide the convenience of creasing S/S ratio, or what is the same, to loss fruit yield. It is important to evaluate and compare this practice against other ways of enhancing the aroma profile in grapes, as using precision irrigation of vineyards, minimizing the impact on yields. New experiments could compare both effects on aroma precursors and stablish which is the best practice for each agro-climatic situation.

New research will be required to analyze the effect of climate change on aroma molecules. In the present research, it was demonstrated that a three-years experiment is not enough and longer set of years are needed to analyze and predict the effect of climate on aroma precursors and on the effect on the final product. As these studies could take a longer period to obtain reliable data, it would be possible to analyze hypothetical scenarios using simulation programs, which are useful in other areas of agronomy.

More research is still needed to completely understand the relationship between aroma precursors and primary metabolism. In this way, would be important to increase the research of new hybrids resistant to fungal diseases, which produce less sugar. This becomes particularly important in a climatic changing scenario where temperatures are increasing, resulting in higher levels of sugar content in berries and higher alcohol content in the resulting wines.

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7. Annex 1: INRA UR Pech Rouge laboratory

| | | |
|---|--|-----------------------------------|
|  | Mode opératoire | Réf. : MO-LAB-16 Version : 5 |
| | Préparation des échantillons de contrôle maturité | Date : 16/02/2015 Page 145/156 |

| | Rédacteur | Vérificateur | Approbateur |
|------------|----------------|----------------|----------------|
| Nom : | VEYRET Mélanie | SAURIN Nicolas | AGUERA Evelyne |
| Fonction : | TR | Animateur AQR | Animateur AQR |

1. Objet et domaine d'application

Ce document décrit les opérations de préparation et de traitement du raisin dans le cadre du contrôle maturité.

2. Documents de référence

I-LAB-02 Utilisation du Dyostem

MO-LAB-60 V1 pH AT par Oenotrateur CRISON

I-LAB-32 V2 Diluteur spectro thermo

I-LAB-33 V2 Spectro Thermofisher

3. Liste de diffusion et si nécessaire niveau de confidentialité

4. Hygiène et sécurité

- Blouse
- Gant et hotte aspirante pour la préparation de la solution d'extraction

5. Principe de la méthode

6. Matériels nécessaires

- Plateaux compteurs de baies
- Analyseur de baies - Dyostem
- Bacs plastiques
- Fouloir de laboratoire
- Cônes en plastique
- Spatule
- Tubes à centrifuger de 45mL
- Balance
- Centrifugeuse - Ependorf
- Réfractomètre
- Pipettes jetables compte-gouttes
- Titrateur automatique de l'acidité et du pH - CRISON
- Flacons de macération 250mL
- Table d'agitation
- Tubes en plastique adaptés au spectrophotomètre
- Spectrophotomètre - Thermofisher

7. Réactifs (chimiques et biologiques)

- Solution d'extraction : 8.5 litre d'eau osmosée / 1.5 litre d'éthanol à 96% / 8.5 ml d'HCl 37%

8. Contraintes de la méthode

Le dosage des anthocyanes nécessite 1h de temps de macération.

9. Contenu du mode opératoire

9.1 Analyse classique de maturité (Sucres / Acidité Totale / pH)

- Prélever, compter 200 baies de raisin à l'aide des plateaux compteurs de baies (100 baies par plateau)



Plateaux compteurs de baies

- Si besoin, effectuer l'analyse Dyostem : cf I-LAB-02 Dyostem



Dyostem

- Peser les 200 baies comptées
- Positionner un bac en plastique adapté sous le petit fouloir de laboratoire
- Fouler l'ensemble des 200 baies, récupérer un maximum de jus, pellicule, pulpe... à l'aide d'un cône de pipette en plastique.



Foulage des baies

- Prélever dans un tube à centrifuger 40 ml de jus et centrifuger l'échantillon 5000 tr / 5min / 20°C. ! Penser à équilibrer par pesée les tubes !
- Pour fouler l'échantillon suivant, ne pas laver le fouloir, l'essuyer au maximum avec un papier essuie main.
- Analyser sur jus foulé :
 - Sucres : mesurer le ° Brix à l'aide d'un réfractomètre : positionner une goutte de jus dans le réfractomètre à l'aide d'une pipette compte-goutte jetable et lire la valeur.
- Entre chaque échantillon, rincer le réfractomètre à l'eau osmosée et l'essuyer.
- Se reporter à l'abaque en Annexe 1 pour obtenir la quantité de sucres en g/l ainsi que le TAP (Taux d'Alcool Probable en % v/v) correspondant.



Réfractomètre

Attention : avant véraison, ne pas faire le °Brix et congeler 4mL de jus pour l'analyse enzymatique (Glucose-Fructose) des sucres.

- Acidité Totale et pH :

cf MO-LAB-60 CRISON Analyse réalisée par le personnel du laboratoire

9.2 Autres paramètres d'analyse de maturité (Azote/Anthocyanes et IPT)

➤ Azote

L'analyse de l'azote sur les baies de raisin est effectuée par méthode enzymatique sur l'analyseur séquentiel du laboratoire d'analyses œnologiques. Cette analyse est réalisée par Mélanie VEYRET sur des séries d'échantillons, il faut donc stocker par congélation les échantillons.

Sur jus foulé et centrifugé :

- Prélever (dans un tube à centrifuger de 5 mL) 4mL de jus
- Congeler l'échantillon dans le tiroir du congélateur dans le laboratoire d'analyses œnologiques.

! Penser à remplir la fiche de suivi des échantillons congelés située sur le congélateur !

➤ **Anthocyanes / IPT**

- Prélever, compter 200 baies de raisin à l'aide des plateaux compteurs de baies
- Peser les 200 baies comptées
- Positionner un bac en plastique adapté sous le petit fouloir de laboratoire
- Fouler l'ensemble des 200 baies
- Broyer l'ensemble des baies avec le mixer pendant 2 minutes, vitesse maximale
- Prélever alors 50g de broyat dans un flacon de 250 ml
- Ajouter 100ml de solution d'extraction

Préparation de la solution : 8.5 litre d'eau osmosée / 1.5 litre d'éthanol à 96% / 8.5 ml d'HCl 37%

! Penser aux équipements de sécurité gants, blouse et hotte !

- Positionner le flacon sur la table d'agitation et macérer pendant 1heure avec agitation permanente



Flacons contenant 50g de broyat + 100ml de solution d'extraction, disposés ensuite sur la table d'agitation

- Après macération, prélever 10mL de solution dans un tube de 13mL
- Centrifuger l'échantillon 8000 tr / 5min / 20°C! **Penser à équilibrer par pesée les tubes!**
- Transférer délicatement le surnageant dans un tube en plastique de 10mL



Surnageant transféré dans les tubes en plastique

- Analyse sur surnageant : Dosage des anthocyanes (Méthode Puissant Léon) et IPT.
cf I-LAB-33 Spectrophotomètre. Analyse réalisée par le personnel du laboratoire
Dilution de l'échantillon au 20^{ème}, 50^{ème} ou 100^{ème} en diluant avec la solution d'HCl 1M.
Mesure du spectre sur une cuve de 10mm (méthode « cuve 10mm »).

$$\text{ANTHOCYANES (mg/l)} = \text{DO520} \times 22.76 \times \text{facteur de dilution} \times 3$$

$$\text{DO 280 ou IPT} = \text{DO280} \times \text{facteur de dilution} \times 3$$

ANNEXE 1 – Abaque de correspondances des ° Brix

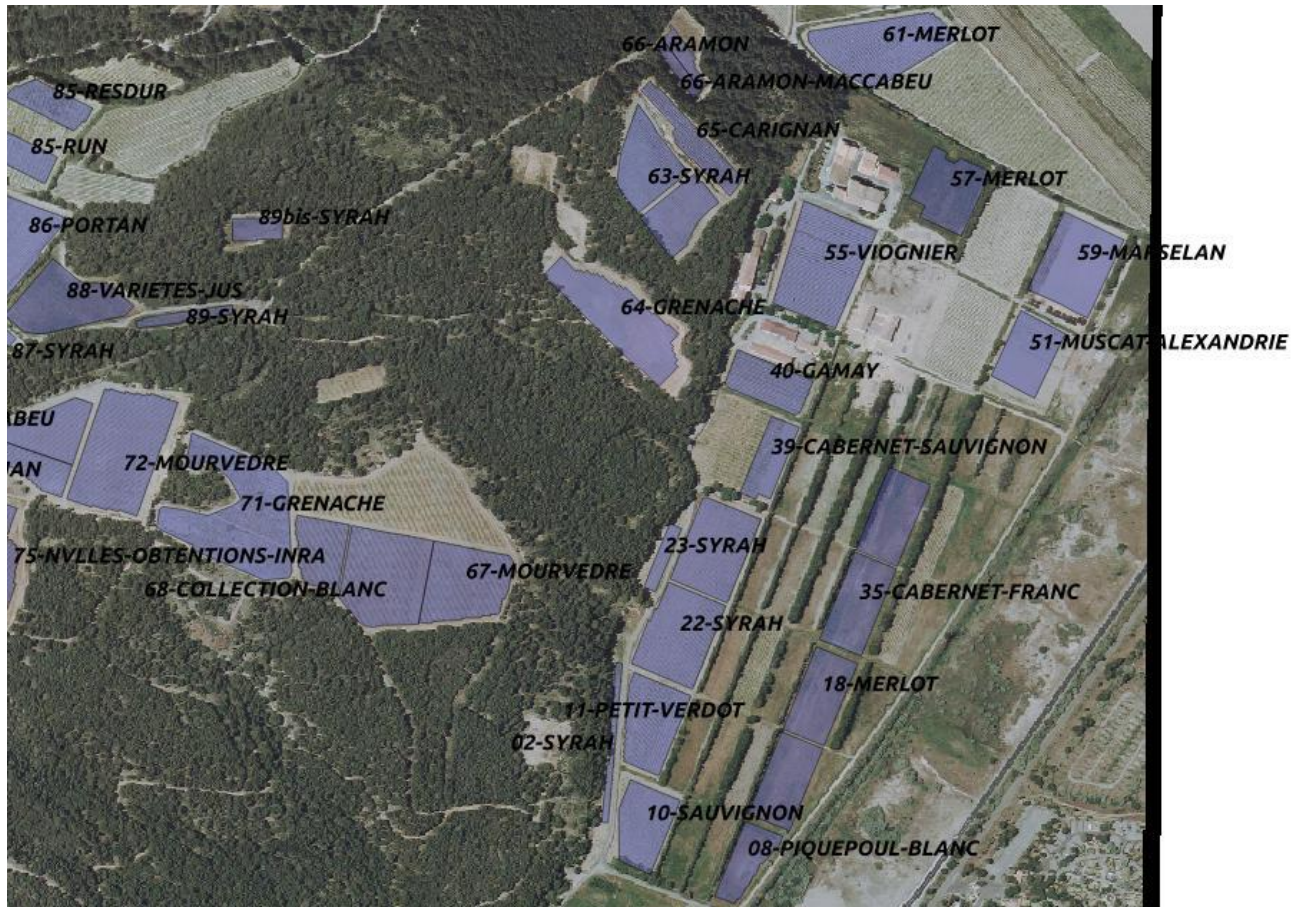
(Le Degré d'alcool probable est calculé sur une base de 16.83g de sucres pour 1 degré.)

| D° BRIX | | Sucres g/l | | Alcool Probable | D° BRIX | | Sucres g/l | | Alcool Probable | D° BRIX | | Sucres g/l | | Alcool Probable | D° BRIX | | Sucres g/l | | Alcool Probable |
|------------|---|---------------|---|--------------------|------------|---|---------------|---|--------------------|------------|---|---------------|---|--------------------|------------|---|---------------|---|--------------------|
| 10.0 | → | 82.3 | → | 4.89 | 15.0 | → | 136.0 | → | 8.08 | 20.0 | → | 191.9 | → | 11.40 | 25.0 | → | 249.7 | → | 14.84 |
| 10.2 | → | 84.5 | → | 5.02 | 15.2 | → | 138.2 | → | 8.21 | 20.2 | → | 194.2 | → | 11.54 | 25.2 | → | 251.7 | → | 14.96 |
| 10.4 | → | 86.6 | → | 5.15 | 15.4 | → | 140.4 | → | 8.34 | 20.4 | → | 196.5 | → | 11.68 | 25.4 | → | 254.4 | → | 15.12 |
| 10.6 | → | 88.6 | → | 5.26 | 15.6 | → | 142.6 | → | 8.47 | 20.6 | → | 198.8 | → | 11.81 | 25.6 | → | 256.4 | → | 15.23 |
| 10.8 | → | 90.8 | → | 5.40 | 15.8 | → | 144.8 | → | 8.60 | 20.8 | → | 201.1 | → | 11.95 | 25.8 | → | 259.1 | → | 15.40 |
| | | | | | | | | | | | | | | | | | | | |
| 11.0 | → | 92.9 | → | 5.52 | 16.0 | → | 147.0 | → | 8.73 | 21.0 | → | 203.3 | → | 12.08 | 26.0 | → | 261.1 | → | 15.51 |
| 11.2 | → | 95.0 | → | 5.64 | 16.2 | → | 149.2 | → | 8.87 | 21.2 | → | 205.7 | → | 12.22 | 26.2 | → | 263.8 | → | 15.67 |
| 11.4 | → | 97.1 | → | 5.77 | 16.4 | → | 151.5 | → | 9.00 | 21.4 | → | 207.9 | → | 12.35 | 26.4 | → | 265.8 | → | 15.79 |
| 11.6 | → | 99.3 | → | 5.90 | 16.6 | → | 153.7 | → | 9.13 | 21.6 | → | 210.2 | → | 12.49 | 26.6 | → | 268.5 | → | 15.95 |
| 11.8 | → | 101.4 | → | 6.02 | 16.8 | → | 155.9 | → | 9.26 | 21.8 | → | 212.5 | → | 12.63 | 26.8 | → | 270.5 | → | 16.07 |
| | | | | | | | | | | | | | | | | | | | |
| 12.0 | → | 103.6 | → | 6.16 | 17.0 | → | 158.1 | → | 9.39 | 22.0 | → | 214.8 | → | 12.76 | 27.0 | → | 273.2 | → | 16.23 |
| 12.2 | → | 105.7 | → | 6.28 | 17.2 | → | 160.4 | → | 9.53 | 22.2 | → | 217.2 | → | 12.91 | 27.2 | → | 275.2 | → | 16.35 |
| 12.4 | → | 107.9 | → | 6.41 | 17.4 | → | 162.6 | → | 9.66 | 22.4 | → | 219.5 | → | 13.04 | 27.4 | → | 277.9 | → | 16.51 |
| 12.6 | → | 110.0 | → | 6.54 | 17.6 | → | 164.8 | → | 9.79 | 22.6 | → | 221.7 | → | 13.17 | 27.6 | → | 279.9 | → | 16.63 |
| 12.8 | → | 112.2 | → | 6.67 | 17.8 | → | 167.0 | → | 9.92 | 22.8 | → | 224.1 | → | 13.32 | 27.8 | → | 282.6 | → | 16.79 |
| | | | | | | | | | | | | | | | | | | | |
| 13.0 | → | 114.3 | → | 6.79 | 18.0 | → | 169.3 | → | 10.06 | 23.0 | → | 226.4 | → | 13.45 | 28.0 | → | 284.6 | → | 16.91 |
| 13.2 | → | 116.5 | → | 6.92 | 18.2 | → | 171.5 | → | 10.19 | 23.2 | → | 228.7 | → | 13.59 | 28.2 | → | 287.3 | → | 17.07 |
| 13.4 | → | 118.6 | → | 7.05 | 18.4 | → | 173.8 | → | 10.33 | 23.4 | → | 231.1 | → | 13.73 | 28.4 | → | 289.3 | → | 17.19 |
| 13.6 | → | 120.8 | → | 7.18 | 18.6 | → | 176.0 | → | 10.46 | 23.6 | → | 233.4 | → | 13.87 | 28.6 | → | 292.0 | → | 17.35 |
| 13.8 | → | 122.9 | → | 7.30 | 18.8 | → | 178.3 | → | 10.59 | 23.8 | → | 235.8 | → | 14.01 | 28.8 | → | 294.0 | → | 17.47 |
| | | | | | | | | | | | | | | | | | | | |
| 14.0 | → | 125.1 | → | 7.43 | 19.0 | → | 180.5 | → | 10.72 | 24.0 | → | 238.2 | → | 14.15 | 29.0 | → | 296.7 | → | 17.63 |
| 14.2 | → | 127.3 | → | 7.56 | 19.2 | → | 182.8 | → | 10.86 | 24.2 | → | 240.3 | → | 14.28 | 29.2 | → | 299.4 | → | 17.79 |
| 14.4 | → | 129.5 | → | 7.69 | 19.4 | → | 185.1 | → | 11.00 | 24.4 | → | 243.0 | → | 14.44 | 29.4 | → | 301.4 | → | 17.91 |
| 14.6 | → | 131.6 | → | 7.82 | 19.6 | → | 187.4 | → | 11.13 | 24.6 | → | 245.0 | → | 14.56 | 29.6 | → | 304.4 | → | 18.09 |
| 14.8 | → | 133.8 | → | 7.95 | 19.8 | → | 189.7 | → | 11.27 | 24.8 | → | 247.7 | → | 14.72 | 29.8 | → | 306.4 | → | 18.21 |
| | | | | | | | | | | | | | | | 30.0 | → | 308.6 | → | 18.34 |

8. Annex 2: Irrigation and training systems

| Variety | Irrigation | Training system |
|--------------------|---------------|--------------------------------|
| Viognier | 60-80 mm/year | Guyot |
| Chardonnay | 30-40 mm/year | Bilateral cordon (spur pruned) |
| Grenache | 30-40 mm/year | Bilateral cordon (spur pruned) |
| Marselan | 30-40 mm/year | Bilateral cordon (spur pruned) |
| Syrah | 30-40 mm/year | Guyot |
| Cabernet-Sauvignon | 0 mm/year | Bilateral cordon (mechanized) |
| G5 (VDQA genotype) | 30-40 mm/year | Bilateral cordon (spur pruned) |
| Muscat | 30-40 mm/year | Bilateral cordon (spur pruned) |

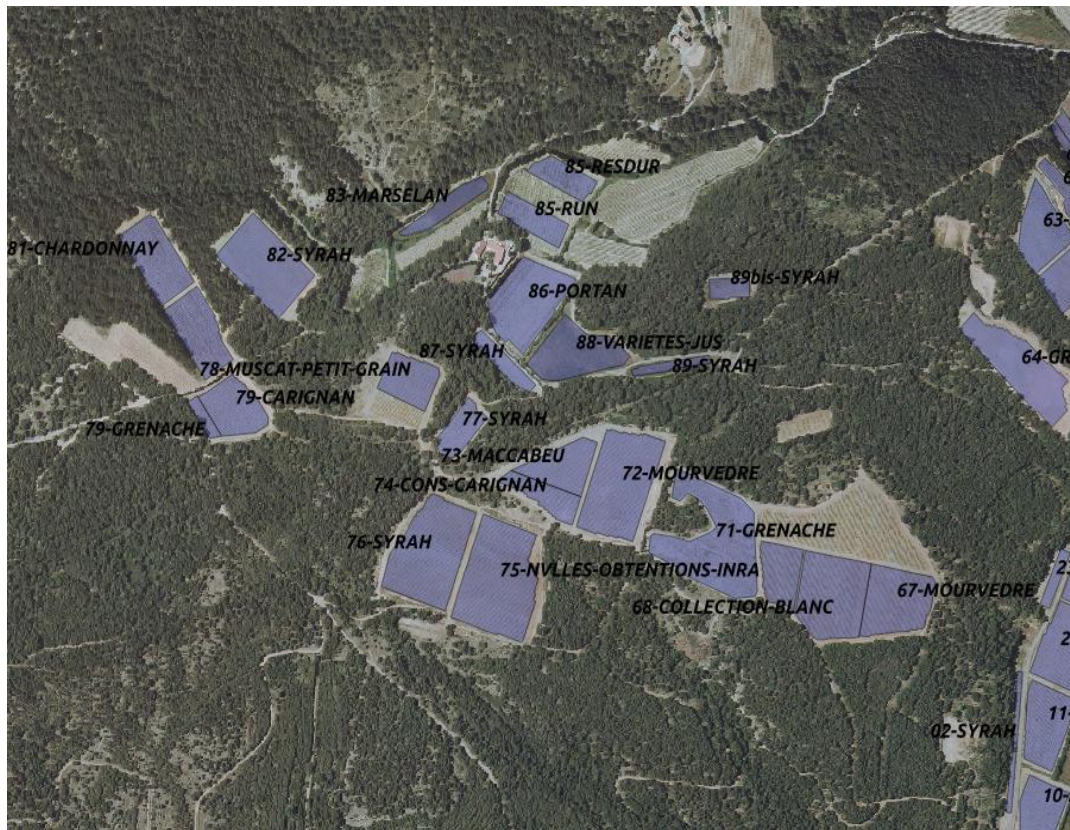
9 Annex 3: Plots distribution at Pech Rouge



Plot number 39: Cabernet-Sauvignon

Plot number 55: Viognier

Plot number 64: Grenache

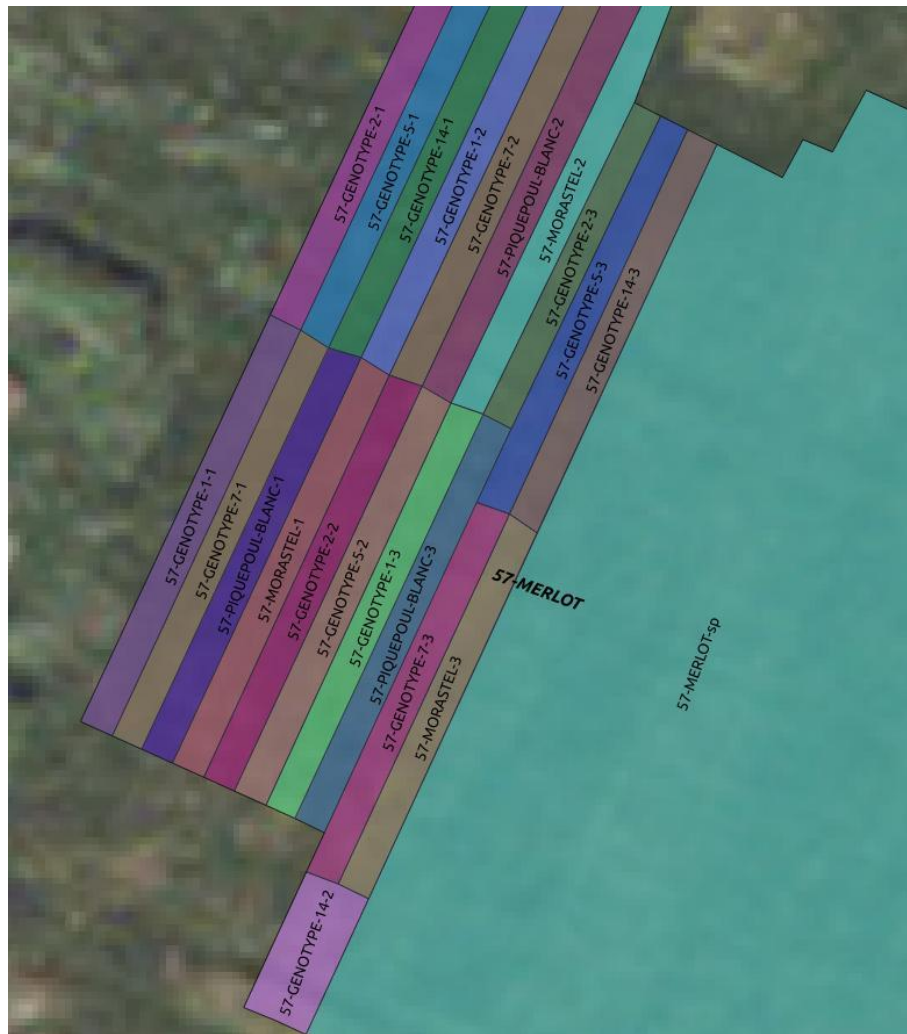


Plot number 75; Marselan

Plot number 78: Muscat à petit grains

Plot number 81: Chardonnay

Plot number 82: Syrah



Plot number 57: genotype G5

10 Annex 4: Berry sorting by density

| Genotype | Density | 140 | 160 | 180 | 200 |
|-----------------------|-------------|-------|-------|-------|-------|
| | | g/L | g/L | g/L | g/L |
| | °Brix range | 18-19 | 20-21 | 22-23 | 24-25 |
| Cabernet Sauvignon I | C | | | 22,65 | |
| | PP | | 21,90 | | |
| | RD | | | 22,50 | |
| Cabernet Sauvignon II | C | | | 22,70 | |
| | PP | | | | 24,30 |
| | RD | | | 23,10 | |
| Syrah I | C | | 20,40 | | |
| | PP | 19,80 | | | |
| | RD | 18,60 | | | |
| Syrah II | C | | 21,20 | | |
| | PP | | 21,00 | | |
| | RD | | 20,70 | | |

Note: values in red correspond to °Brix values of the chosen group