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# Grapevine age: Impact on physiology and berry and wine quality

Khalil Bou Nader

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**Grapevine Age:  
Impact on Physiology and Berry and Wine Quality**

Presented by  
Khalil Bou Nader, MSc.

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Members of the jury:

Ms. Annette REINEKE	Professor, Hochschule Geisenheim University	President
Ms. Astrid FORNECK	Professor, Universität für Bodenkultur Wien	Reviewer
Mr. Laurent TORREGROSA	Professor, Montpellier SupAgro	Reviewer
Mr. Vivian ZUFFEREY	Doctor, Institut de recherche Agroscope	Reviewer
Mr. Manfred STOLL	Professor, Hochschule Geisenheim University	Thesis supervisor
Mr. Eric GOMÈS	Professor, Université de Bordeaux	Thesis supervisor



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*“The first hundred years are the hardest.”*

*- Proverb -*

# Abstract

Vine age and its relation to the quality of the wine are topics of recurring interest, both scientific and economic. Consumers and actors in the wine sector seem to agree on the ability of old vines to produce wines of superior character. Despite ongoing research, the validity of this point of view remains debated and questions about the mechanisms through which old vines would end up with superior quality wines remain numerous. To try to answer them, the impact vine age on physiology, tolerance to water stress, and berry and wine quality were studied in an experimental vineyard planted with *Vitis vinifera* L. cv. of identical genetic material (Riesling Gm 239 grafted on 5C Teleki) but planted in different years.

In 2014 and 2015, the vines planted in 2012 had not yet reached their full potential and had a significantly lower vegetative productivity and yield than the vines planted in 1995 and 1971. Moreover, the vines planted in 2012 were not subjected to the same grass treatment as older vines during this period to prevent excessive competition during establishment. The lower capacity of these vines and the absence of cover crop led to greater exposure of clusters to light and greater nitrogen accumulation, which resulted in a higher concentration of amino acids, monoterpenes, norisoprenoids, and flavonols in 2014 and 2015. In the following years (2016 and 2017), the yield and pruning weight of these vines, as well as their berry composition, were comparable to those of the older vines. The parameters of technological maturity ( $^{\circ}$  Brix, total acidity and must pH) were not significantly affected by vine age.

Vines planted in 1995 and 1971 showed similar physiological characteristics throughout the study with the exception of a higher incidence of esca syndrome in the older group. This disease was responsible for the decline in the total yield of vines planted in 1971, but individual yield per vine was equivalent for both groups.

Sensory and chemical analyzes were conducted in 2017 on wines from previous vintages. The wines of the youngest vines were associated with aromas of ripe fruit and the kerosene aroma that is typical of Riesling. These wines were also identified by higher concentrations of potential monoterpenes and norisoprenoids and volatile sulfur compounds in 2014 and 2015 only. The sensory and chemical profiles of wines from vineyards planted in 1995 and 1971 were dependent on the vintage but not on the age of the vines. The wine profiles produced in 2016 were overlapping for the three age groups.

The works described in this thesis manuscript are unique, particularly because the vineyard in which they were conducted was designed specifically to study the effect of the age of the vine under comparable environmental conditions. Once the youngest vines reached their fruiting potential and were conducted in the same way as the older vines, their productivity, the composition of their berries and the quality of the wines they produce converged with those of the two other groups. More interestingly, vines aged 19 and 43 years behaved similarly throughout the study and resulted in wines comparable in terms of sensory analysis, which goes against the idea that the older vines produce wines of a different profile.

Previous studies have shown that the productivity of the vines, whatever their age, could be explained by the wood reserves and the size of the trunk. To have a better idea of differences linked to reserves, the structure-from-motion with multi-view stereo-photogrammetry (SfM-MVS) method was tested to measure trunk thickness and volume. The technique, which allows the creation of scaled, georeferenced 3D models based on photographs, was able to produce accurate models of field-grown grapevine trunks.

**Keywords:** old vine, water deficit, berry composition, wine quality, sensory analysis.

## Zusammenfassung

Das Rebalter und seine Beziehung zur Weinqualität sind Themen von wiederkehrendem Interesse, sowohl wissenschaftlich als auch wirtschaftlich. Vielfach wird behauptet, dass alte Reben Weine mit besonderem Charakter produzieren können. Trotz vielfältiger Forschung bleibt diese Sichtweise jedoch nicht eindeutig belegt, und es gibt immer noch mehr offene Fragen als erklärende Antworten. Deshalb wurde auf einer Rebfläche der Hochschule Geisenheim University hierzu über viele Jahre eine einzigartige Versuchsfläche aufgebaut. Dort ist es möglich, Untersuchungen innerhalb einer Rebfläche (Geisenheimer Fuchsberg) für eine Rebsorte (Riesling) gleichen Klons (Gm 239), gleicher Unterlage (5C Teleki) und auf einheitlichem Standraum ( $2,8 \text{ m}^2$ ) an Reben der drei Pflanzjahre 1971 („alt“), 1995 („alternd“) und 2012 („jung“) durchzuführen. Über einen Zeitraum von vier Vegetationsperioden wurden verschiedene Fragen bearbeitet.

In den Versuchsjahren 2014 und 2015 hatten die im Jahr 2012 gepflanzten Reben noch nicht ihr volles Ertragspotenzial erreicht und zeigten eine deutlich geringere vegetative Produktivität als die in den Jahren 1995 und 1971 gepflanzten Reben. In diesen Anfangsjahren unterschied sich die



Bodenbewirtschaftung zwischen den drei Versuchsgliedern durch offene Bodenbewirtschaftung oder eine Dauerbegrünung. Die geringere Wüchsigkeit der jungen Reben und die höhere Mineralisationsrate der offenen Böden führten zu einer stärkeren Exposition der Trauben und einer stärkeren Anreicherung von hefeverwertbarem Stickstoff, Aminosäuren, Monoterpenen, Norisoprenoiden und Flavonolen in den Jahren 2014 und 2015. In den folgenden Jahren (2016 und 2017) waren Ertrag und Schnittholzgewicht der jungen Reben sowie deren Beerenzusammensetzung mit denen der älteren Rebstöcke vergleichbar. Die Parameter der technologischen Reife ( $^{\circ}$  Brix, Gesamtsäure- und pH-Wert) wurden durch das Alter der Rebe nicht wesentlich beeinflusst.

Die in den Jahren 1995 und 1971 gepflanzten Reben zeigten in der gesamten Studie ähnliche physiologische Merkmale mit Ausnahme eines häufigeren Auftretens von Esca-Symptomen bei den älteren Reben. Diese Krankheit war für den Rückgang des Gesamtertrags der im Jahr 1971 gepflanzten Rebstöcke verantwortlich, wobei hervorzuheben ist, dass der Einzelstockertrag aller drei Versuchsglieder dann auch gleich war.

Im Jahr 2017 wurden sensorische und chemische Analysen der Weine aus früheren Jahrgängen durchgeführt. Die Geschmacksattribute der Weine der jungen Reben wurden mit Aromen von reifen Früchten und dem für Riesling typischen Kerosinaroma in Verbindung gebracht. In diesen Weinen wurden auch in den Jahren 2014 und 2015 höhere Konzentrationen potenzieller Monoterpene und Norisoprenoide sowie flüchtiger Schwefelverbindungen festgestellt. Die sensorischen Profile der Weine aller Versuchsjahre und des Rebalters waren stärker vom Weinjahrgang selbst als vom Alter der Reben geprägt. Sobald die jungen Reben das Ertragspotential erreicht hatten und auf dieselbe Weise wie die älteren Reben bewirtschaftet wurden, stimmten ihre Produktivität, die Zusammensetzung ihrer Beeren und die Qualität der Weine mit denen der beiden anderen Versuchsglieder überein. Interessanterweise traten zwischen den 1971 und 1995 gepflanzten Reben bei physiologischen Messungen und sensorischen Untersuchungen keine Unterschiede auf.

Frühere Studien haben einen Zusammenhang zwischen der Produktivität der Reben und der Reservestoffe im Holz gezeigt. Hierzu wurde im Rahmen der eigenen Untersuchungen mittels der „structure-from-motion with multi-view stereo-photogrammetry“ (SfM-MVS) das Stammvolumen untersucht und erstmals ein 3-D-Modell des Rebstammes publiziert.

**Schlagworte:** alte Reben, Wasserstress, Traubeninhaltsstoffe, Weinqualität, sensorische Prüfung.

# Résumé

L'âge de la vigne et sa relation avec la qualité du vin sont des sujets d'intérêt récurrents, tant scientifiques qu'économiques. Les consommateurs et acteurs de la filière vitivinicole semblent s'accorder à propos de la capacité des vieilles vignes à produire des vins de caractère supérieur. Malgré les recherches en cours, la validité de ce point de vue reste débattue et les questions concernant les mécanismes à travers lesquels de vieilles vignes aboutiraient à des vins qualité supérieure restent nombreuses. Pour tenter d'y répondre, l'impact de l'âge des vignes sur la physiologie, la tolérance au stress hydrique, ainsi que la qualité des baies et du vin ont été étudiés dans un vignoble expérimental constitué de plants de *Vitis vinifera* L. cv. de matériel génétique identique (Riesling de clone Gm 239 greffé sur 5C Teleki) mais aux dates de plantation différentes.

En 2014 et 2015, les vignes plantées en 2012 n'avaient pas encore atteint leur plein potentiel et avaient une productivité végétative et un rendement significativement inférieurs à ceux des vignes plantées en 1995 et 1971. Par ailleurs, les vignes plantées en 2012 n'ont pas été soumises au même traitement d'enherbement que les vignes plus âgées pendant cette période afin de prévenir une compétition excessive pendant leur établissement. La capacité inférieure de ces vignes et l'absence d'enherbement ont mené à une plus grande exposition des grappes à la lumière et une plus grande accumulation d'azote, ce qui s'est traduit par une plus grande concentration en acides aminés, monoterpènes, norisoprénoides, et flavonols en 2014 et 2015. Les années suivantes (2016 et 2017), le rendement et le poids des bois de taille de ces vignes, ainsi que la composition des baies, étaient comparables à ceux des vignes plus âgées. Les paramètres de maturité technologique (°Brix, l'acidité totale et le pH de moûts) n'ont pas été significativement affectés par l'âge des vignes.

Les vignes plantées en 1995 et 1971 ont présenté des caractéristiques physiologiques similaires tout au long de l'étude à l'exception d'une plus grande incidence du syndrome de l'esca chez le groupe le plus âgé. Cette maladie a été responsable de la baisse du rendement à la parcelle des vignes plantées en 1971, les rendements individuels à l'échelle du cep restant équivalents pour les deux groupes.

Des analyses sensorielles et chimiques ont été réalisées en 2017 sur des vins de millésimes précédents. Les vins des plus jeunes vignes ont été associés à des arômes de fruits mûrs et de l'arôme de pétrole typique du Riesling. Ces vins ont aussi été identifiés par de plus hautes concentrations de monoterpènes et norisoprénoides potentiels et de composés soufrés volatils, en

2014 et 2015 uniquement. Les profils sensoriels et chimiques de vins issus des vignes plantées en 1995 et 1971 étaient dépendants du millésime mais pas de l'âge des vignes. Les profils des vins produits en 2016 étaient superposables pour les trois groupes d'âge.

Les travaux décrits dans ce manuscrit de thèse sont uniques, du fait notamment que le vignoble dans lequel ils ont été conduits a été conçu spécifiquement pour étudier l'effet de l'âge de la vigne dans des conditions environnementales comparables. Une fois que les vignes les plus jeunes ont atteint leur potentiel fructifère et ont été conduites de la même manière que les vignes plus âgées, leur productivité, la composition de leurs baies et la qualité des vins qu'elles produisent ont convergé avec celles des deux autres groupes. Plus intéressant encore, des vignes âgées de 19 et 43 ans se sont comportées de la même façon tout au long de l'étude et ont abouti à des vins comparables en termes d'analyses sensorielles, ce qui va à l'encontre de l'idée reçue qui veut que les vignes les plus âgées produisent des vins de qualité différente.

Des travaux précédents ont démontré que la productivité des vignes, quel que soit leur âge, pouvait être expliquée par les réserves de bois et par la taille du tronc. Pour avoir une meilleure idée des différences liées aux réserves, la technique dite « structure-from-motion with multi-view stereo-photogrammetry » (SfM-MVS) a été testée pour mesurer l'épaisseur des troncs et leur volume. Cette technique qui permet la création de modèles tridimensionnels géo-référencés et à l'échelle a pu générer des modèles précis de tronc de vignes plantées en champ.

**Mots-clés :** vieille vigne, déficit hydrique, composition de la baie, qualité du vin, analyse sensorielle.

# Table of contents

Declaration of authorship.....	3
Acknowledgements .....	4
Abstract .....	6
Zusammenfassung.....	7
Résumé.....	9
Table of contents.....	11
List of tables.....	14
List of figures .....	16
List of appendices .....	19
List of abbreviations.....	21
Chapter 1. General Introduction.....	24
1. Wine quality and grapevine age.....	26
1.1. The dimensions of wine quality .....	26
1.2. A bottle of old vines.....	27
1.3. Grapevine age and wine quality in the scientific literature.....	29
2. Grapevine vigor and balance .....	32
2.1. Factors influencing grapevine vigor.....	32
2.2. Consequences of excessive vigor.....	38
2.3. Achieving grapevine balance .....	41
3. Grapevine water relations .....	45
3.1. Cellular and physiological water transport mechanisms.....	45
3.2. Grapevine responses to water deficit .....	46
4. Berry development and composition .....	48
4.1. Berry structure.....	48
4.2. Berry development.....	49
5. The Riesling cultivar.....	51
5.1. Varietal aromas of Riesling.....	52
6. Objectives of the thesis .....	55
Chapter 2. Impact of grapevine age on water status and productivity .....	56
1. Abstract.....	57
2. Introduction.....	57
3. Materials and methods .....	59

3.1.	Weather data.....	59
3.2.	Experimental design and plant material.....	59
3.3.	Gas exchange measurements.....	61
3.4.	Vine capacity and balance.....	61
3.5.	Berry technological maturity and $\delta^{13}\text{C}$ .....	62
3.6.	Experiment on water deficit.....	63
3.7.	Statistical analysis.....	63
4.	Results.....	64
4.1.	Weather data.....	64
4.2.	Vine selection and soil management.....	65
4.3.	Gas exchange measurements.....	68
4.4.	Vine capacity and balance.....	69
4.5.	Berry technological maturity and $\delta^{13}\text{C}$ .....	74
4.6.	Experiment on water deficit.....	74
5.	Discussion.....	78
6.	Conclusion.....	82
7.	Acknowledgments.....	83
8.	Funding.....	83
Chapter 3. Impact of grapevine age on berry structure and secondary metabolites.....		84
1.	Introduction.....	85
2.	Materials and methods.....	86
2.1.	Experimental design and plant material.....	86
2.2.	Berry sampling.....	87
2.3.	Organic and amino acids.....	88
2.4.	Potentially volatile monoterpenes and $\text{C}_{13}$ -norisoprenoids.....	89
2.5.	Flavonol content in berry skin.....	90
2.6.	Statistical analysis.....	91
3.	Results and discussion.....	91
3.1.	Berry composition.....	91
3.2.	Secondary metabolites and light interception.....	97
4.	Conclusion.....	98
Chapter 4. Impact of grapevine age on wine composition and sensory attributes.....		100
1.	Introduction.....	101
2.	Materials and methods.....	102

2.1.	Experimental design and plant material .....	102
2.2.	Winemaking .....	103
2.3.	Sensory analysis .....	103
2.4.	Wine composition .....	105
2.5.	Statistical analysis .....	108
3.	Results and discussion .....	109
3.1.	Sensory analysis .....	109
3.2.	Chemical analysis.....	112
3.3.	MFA with chemical and sensory data .....	113
3.4.	PCA of chemical data for older wines .....	116
4.	Conclusion .....	117
Chapter 5. Evaluating grapevine trunk size with a handheld camera by 3D modeling .....		118
1.	Abstract .....	119
2.	Introduction.....	119
3.	Materials and methods .....	121
3.1.	Plant material and field-measured trunk diameter and circumference.....	121
3.2.	Image acquisition for trunk reconstruction .....	122
3.3.	3D model reconstruction by SfM-MVS .....	123
3.4.	SfM-MVS measurements and model validation .....	124
3.5.	Estimation of trunk volume and crown contribution .....	124
3.6.	Statistical analysis .....	125
4.	Results and discussion .....	126
4.1.	SfM-MVS measurements and model validation .....	126
4.2.	Exclusion of the grapevine crown.....	128
5.	Conclusion .....	129
Chapter 6. General discussion.....		130
Appendices .....		137
References .....		158

# List of tables

Table 1.1. Overview of the current literature related to grapevine age. The grapevine age column refers to the youngest and oldest grapevines in their respective study (adapted from Grigg et al., 2017). .....	31
Table 1.2. Optimal ranges for various grapevine balance indices aggregated from various studies (adapted from Dry et al., 2004). .....	42
Table 1.3. Top five countries by Riesling vineyard area in 2015 (data sourced from OIV, 2018b, 2017). .....	52
Table 2.1. Growing degree-day (GDD) and precipitation data for Geisenheim Fuchsberg, Germany, from April 1st to October 31st for the growing seasons 2014-2017. The yearly average represents the period of 1981-2010. .....	64
Table 2.2. Percentages among vines planted in 1971 and 1995 of healthy vines, vines with esca symptoms, and missing vines as visually assessed at the end of each growing season between 2015 and 2017. .....	70
Table 2.3. Vine balance parameters for three age groups and four replicates over the growing seasons 2014-2017 (n = 4). Fruit yield (which includes rotten berries) and pruning weight refer to values recorded in the field, while adjusted fruit yield and pruning weight take into account the number of missing vines per row (means ± sd). .....	73
Table 2.4. Technological maturity parameters and dry mass carbon isotope discrimination ( $\delta^{13}\text{C}$ ) for the three grapevine age groups (n = 4) over the growing seasons 2014 to 2017 (means ± sd). $\delta^{13}\text{C}$ was only measured in 2016 and 2017. .....	74
Table 3.1. Maturity parameters and amino acid concentrations for the three grapevine age groups (n = 4) over the growing seasons 2015 to 2017. .....	93
Table 3.2. Free and potentially volatile monoterpenes and norisoprenoids, and flavonol glycosides for the three grapevine age groups (n = 4) from 2015 to 2017. .....	94
Table 4.1. Composition of aroma reference standards prepared using aroma standards by soaking in propanediol (Pd). .....	105
Table 4.2. Taste reference standards with low and high concentrations. .....	105
Table 4.3. Average ratings per attribute in the 2017 tasting for wines from the three grapevine age groups (vintages 2014 to 2016). The means and standard deviations were calculated from three replications with 12 panelists. .....	111

Table 4.4. List of the compounds analyzed in wine. ....	113
Table 5.1. Overview of the three grapevine ages used in the 2017 study on trunk size.....	121
Table 5.2. Field-measured and modeled metrics for 5-year-old, 22-year-old, and 46-year-old grapevines (presented as mean $\pm$ standard deviation) and the root mean square error (RMSE) and bias of modelled data. ....	127
Table 5.3. Comparison between total trunk volume and trunk volume 40 cm above the grafting point and the volume fraction formed by the grapevine crown (data presented as mean $\pm$ standard deviation).....	128



# List of figures

Figure 1.1. In the center, the 'Old Vine' of Maribor, Slovenia (Bogdan Zelnik, <a href="http://www.maribor-pohorje.si">www.maribor-pohorje.si</a> . Accessed October 2018). .....	27
Figure 1.2. 'Vegetative' cycle during which shade stimulates vegetative growth at the expense of yield and quality. (b) 'Balanced' cycle where light favors shoot fruitfulness, leading to balance between shoot and fruit growth (adapted from Smart and Robinson, 1991).....	39
Figure 1.3. Schematic model of steady water flow through a plant, from soil to atmosphere. Water uptake at the roots, long-distance transport in the xylem and stomatal resistances are represented as resistors of relative resistance placed in series (Reproduced from Steudle 2000b).....	47
Figure 1.4. Schematic representation of the grape berry at maturity (reproduced from Coombe, 1987). .....	49
Figure 1.5. Diagram showing the double sigmoid growth curve of berry development, including the timing of accumulation of metabolites and indications of xylem and phloem inflow rates (reproduced from Kennedy, 2002).....	50
Figure 2.1. Double Guyot vine selection process in 2014 with trunk diameter and fruitful bud count for all vines and for the 24 selected vines per age group. ....	65
Figure 2.2. Fruitful bud count of selected double Guyot vines from 2014 to 2017 (n = 24).....	66
Figure 2.3. Soil nitrate content measured at horizons 0-30 cm and 30-60 cm over the growing seasons 2014-2017 for double Guyot rows planted in 1971, 1995, and 2012 (means $\pm$ sd).....	67
Figure 2.4. Soil water content measured at horizons 0-30 cm and 30-60 cm over the growing seasons 2014-2017 for double Guyot rows planted in 1971, 1995, and 2012 (means $\pm$ sd).....	67
Figure 2.5. Chlorophyll fluorescence ratio under green excitation (SFR_G) measured at regular time intervals on selected double Guyot vines from flowering to harvest over the growing seasons 2014-2017 (means $\pm$ sd). ....	68
Figure 2.6. Leaf assimilation ( $A_N$ ), stomatal conductance ( $g_s$ ), and transpiration (E) measured periodically on double Guyot vines over the growing seasons 2016 and 2017 with n = 24 (means $\pm$ sd).....	69
Figure 2.7. PCA biplot of point quadrat analysis, cluster architecture and grapevine balance components for 2014-2016. The point labels represent the growing season followed by the grapevine age group ('O' for old grapevines planted in 1971; 'M' for 'mature' grapevines planted in 1995; 'Y' for 'young' grapevines planted in 2012). There are four replicates per growing season per age group. They are represented by the numbers 1 to 4 next to the letter. ....	72

Figure 2.8. Soil water content for the water deficit experiment on cordon vines in 2016 and 2017 at flowering, pea-sized berries, and two weeks after veraison (means  $\pm$  sd) at horizons 0-30 cm and 30-60 cm. .... 76

Figure 2.9. Chlorophyll fluorescence ratio under green excitation (SFR\_G) measured at regular time intervals on selected cordon vines for the water deficit experiment from flowering to harvest in 2016 and 2017 (means  $\pm$  sd). .... 76

Figure 2.10. Stomatal conductance ( $g_s$ ) measured periodically on selected cordon vines for the water deficit experiment over the growing seasons of 2016 and 2017 (means  $\pm$  sd). .... 77

Figure 2.11. Predawn water potential ( $\Psi_{PD}$ ) measured on cordon-trained vines for the water deficit experiment over the growing seasons of 2016 and 2017 (means  $\pm$  sd). .... 77

Figure 3.1. PCA score plot of technological parameters and secondary metabolites (amino acids, quercetin glucosides, and free and potentially volatile terpenes and norisoprenoids) from 2015-2017. The point labels represent the growing season followed by the grapevine age group ('O' for 'old' grapevines planted in 1971; 'M' for 'mature' grapevines planted in 1995; 'Y' for 'young' grapevines planted in 2012). There are four replicates per growing season per age group, represented by the numbers 1 to 4 next to the letter. .... 95

Figure 3.2. Loadings plot of technological parameters and secondary metabolites (amino acids, quercetin glucosides, and free and potentially volatile terpenes and norisoprenoids) from 2015-2017. .... 95

Figure 3.3. k-means clustering of the technological parameters and secondary metabolites, with four clusters and 50 iterations. The centroid positions were calculated as to minimize the sum of squares within each cluster. The point labels represent the growing season followed by the grapevine age group ('O' for 'old' grapevines planted in 1971; 'M' for 'mature' grapevines planted in 1995; 'Y' for 'young' grapevines planted in 2012). There are four replicates per growing season per age group. They are represented by the numbers 1 to 4 next to the letter. .... 96

Figure 3.4. PCA biplot of bound and free terpenes and quercetin glucosides in berry must at harvest from 2015 to 2017. The point labels represent the growing season followed by the grapevine age group ('O' for 'old' grapevines planted in 1971; 'M' for 'mature' grapevines planted in 1995; 'Y' for 'young' grapevines planted in 2012). There are four replicates per growing season per age group. They are represented by the numbers 1 to 4 next to the letter. .... 98

Figure 4.1. PCA biplot of the 2017 sensory analysis of grapevines from the three grapevine age groups. The point labels represent the growing season followed by the grapevine age group ('O' for 'old' grapevines planted in 1971; 'M' for 'mature' grapevines planted in 1995; 'Y' for 'young' grapevines planted in 2012). There are four replicates per growing season per age group, represented by the numbers 1 to 4 next to the letter. .... 112

Figure 4.2. MFA Loadings plot of the sensory and chemical and sensory variables analyzed in 2017. .....	115
Figure 4.3. MFA partial score plot with chemical sensory variables analyzed in 2017. The point labels represent the growing season followed by the grapevine age group ('O' for 'old' grapevines planted in 1971; 'M' for 'mature' grapevines planted in 1995; 'Y' for 'young' grapevines planted in 2012). There are four replicates per growing season per age group, represented by the numbers 1 to 4 next to the letter.....	115
Figure 4.4. PCA score plot from the chemical analysis of wines from 2002 to 2016. Some vintages were not available for analysis. The point labels represent the growing season followed by the grapevine age group ('O' for 'old' grapevines planted in 1971; 'M' for 'mature' grapevines planted in 1995; 'Y' for 'young' grapevines planted in 2012). There are four replicates per growing season per age group, represented by the numbers 1 to 4 next to the letter. ....	116
Figure 4.5. PCA loadings plot from the chemical analysis of wines from 2002 to 2016.....	117
Figure 5.1. Example of the experimental setup for taking the pictures (a) with a general view of grapevine with the georeference grid and the point of measurement (white arrow); (b) focus on the point of measurement of trunk circumference and cross-sectional area 10 cm above the grafting point highlighted in the field using a plastic cord; (c) focus on the georeference grid around the base of the trunk. ....	122
Figure 5.2. Images of the SfM-MVS reconstructed grapevine a) with squares representing camera positions when taking photographs; b) showing the intact trunk (left), the trunk cross-section 10 cm above the grafting point with the two orthogonal diameters (middle), and the modeled disc (right).....	123
Figure 5.3. (a) Example of a complex crown on a 46 year old vine; (b) removal of the crown by truncating the trunk volume to 40 cm above the grafting point.....	125
Figure 5.4. Regression analysis of the trunk diameter in the row direction ( $d_{row}$ ) and in the direction perpendicular to the row ( $d_{per}$ ), the trunk cross-sectional area (TCSA), and the trunk circumference with SfM-MVS estimates against field values for grapevines aged 5 (○), 22 (□), and 46 years (△). ....	126

# List of appendices

Appendix 2.1. Images of the plastic covers when retracted and deployed (approximate surface of 20 m <sup>2</sup> ) around the four vines with the “stress” treatment. ....	137
Appendix 2.2. Daily mean temperatures, precipitations, and main phenological stages for the years 2014-2017. The black line represents the global average of daily temperatures from 1981 to 2010. ....	138
Appendix 2.3. LMM results for double Guyot including soil NO <sub>3</sub> <sup>-</sup> and water contents from 0-30 cm and 30-60 cm (n = 4), leaf chlorophyll index, assimilation, stomatal conductance, transpiration (n = 24), and carbon isotope discrimination (n = 4). The data represents three age groups and four replicates over the growing seasons 2014-2017. Three measurements were made for soil NO <sub>3</sub> <sup>-</sup> and water contents per vintage (flowering, veraison, and harvest), while six measures were taken between 400 GDD and harvest for the chlorophyll index depending on weather conditions. Assimilation, stomatal conductance and transpiration measured four times during the season. .	139
Appendix 2.4. Leaf chlorophyll index (SFR_G) for selected vines planted in 1971 (a) and 2012 (b) from 2014 to 2017. The red points represent the vines R#61-15 and R#56b-2, which were found to be positive for GLRaV-1 (grapevine leafroll-associated virus 1) and GFLV (grapevine fan-leaf virus) respectively. ....	140
Appendix 2.5. LMM results for double Guyot vine balance parameters for three age groups and four replicates over the growing seasons 2014-2017 (n = 4). ....	141
Appendix 2.6. LMM results for double Guyot vine balance parameters for vines planted in 1995 and 1971 with four replicates over the growing seasons 2014-2017 (n = 4). ....	143
Appendix 2.7. Eigen values and Eigen vectors from principal component analysis presented in Figure 2.7. ....	144
Appendix 2.8. LMM results for double Guyot berry technological maturity for the three age groups and four replicates over the growing seasons 2014-2017 (n = 4). ....	145
Appendix 2.9. LMM results for the water deficit trial on cordon vines. Soil water content from 0-30 cm and 30-60 cm (n = 4), leaf chlorophyll index (n = 8), stomatal conductance (n = 8), predawn water potential (n = 8), and carbon isotope discrimination (n = 4). The data represents three age groups, two water deficit treatments, and four replicates over the growing seasons 2016-2017. Three measurements were made for soil water content per vintage (flowering, pea size, and two weeks after veraison). Six measures were taken between 400 GDD and harvest for chlorophyll index and stomatal conductance in 2016 and seven measures in 2017, depending on weather conditions. Four measures were taken for predawn water potential during the same period. Carbon isotope discrimination was measured on berry samples at harvest. ....	146

Appendix 3.1. Eigenvalues and eigenvectors from principal component analysis presented in Figure 3.2.....	148
Appendix 3.2. Mean values per cluster from the k-mean clustering in Figure 3.3.....	149
Appendix 3.3. Eigenvalues and eigenvectors from principal component analysis presented in Figure 3.4.....	150
Appendix 4.1. ANOVA P-values from the sensory descriptive analysis in 2017.....	151
Appendix 4.2. Eigenvalues and eigenvectors from principal component analysis presented in Figure 4.1.....	151
Appendix 4.3. Concentrations of free and potentially volatile monoterpenes and norisoprenoids ( $\mu\text{g L}^{-1}$ ) measured in available wines from 2002 to 2016. ....	152
Appendix 4.4. Volatile sulfur compounds ( $\mu\text{g L}^{-1}$ ) measured in available wines from 2002 to 2016. Only H <sub>2</sub> S and DMS were detected in wine.....	153
Appendix 4.5. Chemical compounds by <sup>1</sup> H NMR and CIELab parameters measured in available wines from 2002 to 2016.....	154
Appendix 4.6. Organic acids measured by HPLC ( $\text{g L}^{-1}$ ) in available wines from 2002 to 2016....	155
Appendix 4.7. Eigenvalues and eigenvectors from principal component analysis presented in Figure 4.2.....	156
Appendix 4.8. Eigenvalues and eigenvectors from principal component analysis presented in Figure 4.4.....	157

# List of abbreviations

°Brix	°Brix
3SH	3-sulfanylhexan-1-ol
4MSP	4-methyl-4-sulfanylpentan-2-one
a*	Red/green
ABA	Abscisic acid
A <sub>N</sub>	Leaf assimilation
ArMV	Arabis Mosaic Virus
B	Boron
b*	Yellow/blue
Ca	Calcium
Cl	Chlorine
CS <sub>2</sub>	Carbon disulfide
Cu	Copper
D <sub>2</sub> O	Deuterium oxide
DA	Descriptive analysis
DEDS	Diethyl disulfide
DI	Deficit irrigation
DMAPP	Dimethyl allyl diphosphate
DMDS	Dimethyl disulfide
DMH	Octan-3-ol and 2.6-dimethylhept-5-en-2-ol
DMS	Dimethyl sulfide
DMTS	Dimethyl trisulfide
d <sub>per</sub>	Trunk diameter in the direction perpendicular to the row
d <sub>row</sub>	Trunk diameter in the row direction
E	Transpiration
EDTA	Ethylenediamine tetra acetic acid
EGFV	Ecophysiology and Functional Genomics
EI	Electron impact mode
ELISA	Enzyme-Linked Immunosorbent Assay
EtSAc	Thioaceticacid -S- ethyl ester
EtSH	Ethanethiol
Fe	Iron
GC-MS	Gas chromatography coupled with mass spectrometry
GDC	Geneva double curtain
GDD	Growing degree-day
GFkV	Grapevine Fleck Virus
GFIV	Grapevine Flanleaf Virus
GLRaV-1	Grapevine Leafroll-Associated Virus 1

GLRaV-3	Grapevine Leafroll-Associated Virus 3
GPP	Geranyl phosphate
$g_s$	Stomatal conductance
H <sub>2</sub> S	Hydrogen sulfide
HCA	Hierarchical cluster analysis
HCl	Hydrochloric acid
HGU	Hochschule Geisenheim University
IPP	Isopentenyl pyrophosphate
ISVV	Institute of grapevine and Wine Sciences
K	Potassium
K <sub>2</sub> PO <sub>4</sub>	Dipotassium phosphate
K <sub>2</sub> S <sub>2</sub> O <sub>5</sub>	Potassium metabisulfite
L*	Lightness
LA/Y	Leaf area to fruit yield ratio
LC-MS	Liquid chromatography coupled with mass spectrometry
LLN	Leaf layer number
LMM	Linear mixed-effect models
MeSAc	Thioaceticacid -S- methyl ester
MeSH	Methanethiol (MeSH)
MFA	Multiple factor analysis
Mg	Magnesium
MPa	Megapascal
N	Nitrogen
NH <sub>4</sub> <sup>+</sup>	Ammonium
NMR	Nuclear magnetic resonance
NO <sub>3</sub> <sup>-</sup>	Nitrate
P	Phosphorus
PAR	Photosynthetically active radiation
PC	Principal component
PCA	Principal component analysis
Pd	Propanediol
PG	Percent gaps
PHPD	Pulsed flame photometric detector
PIC	Percentage interior clusters
PIL	Percent interior leaves
PRD	Partial rootzone drying
RDI	Regulated deficit irrigation
REML	Restricted maximum likelihood
rh	Relative humidity
RMSE	Root mean square error
S	Sulfur
S/N	Signal-to-noise ratio

SD	Standard deviation
SfM-MVS	Structure-from-motion with multi-view stereo-photogrammetry
SFR_G	Chlorophyll fluorescence ratio under green excitation
SIM	Selected ion monitoring mode
SO <sub>2</sub>	Sulfur dioxide
SO <sub>3</sub> <sup>2-</sup>	Sulfite ion
SO <sub>4</sub> <sup>2-</sup>	Sulfate ion
SPE	Solid-phase extraction
SW	Sweep width
SWC	Soil water content
SWHC	Soil water holding capacity
TA	Total acidity
TCSA	Trunk cross-sectional area
TDN	1,1,6-trimethyl-1,2-dihydronaphtalene
TSP	3-(trimethylsilyl)propanoic acid sodium salt
TSS	Total soluble solid
UHPLC	Ultra-high performance liquid chromatography
UTCC	Under-trellis cover crop
VPD	Vapor pressure deficit
VSC	Volatile sulfur compounds
VvDXS	Deoxy-D-xylulose synthase
VvTS	<i>Vitis vinifera</i>
WUE	Water use efficiency
Y/P	Yield to pruning weight ratio
YAN	Yeast-assimilable nitrogen
δ <sup>13</sup> C	Dry mass carbon isotope discrimination
Ψ	Water potential
Ψ <sub>M</sub>	Surface tension forces
Ψ <sub>PD</sub>	Predawn water potential
Ψ <sub>soil</sub>	Soil water potential
Ψ <sub>π</sub>	Osmotic potential



# Chapter 1. General Introduction

*“The trick is growing up without growing old.”*

*- Casey Stengel -*

Wine is a unique commodity that has been produced even before history was recorded (Bisson et al., 2002; McGovern, 2013). As noted by Phillips (2000), “it is perhaps the most historically charged and culturally symbolic of the foods and beverages with which we regularly have contact”. The cultivation of the grapevine is an ancestral activity that has shaped the landscape and cultural heritage of numerous regions around the world (Oakes and Price, 2008). Wines produced in these regions are deeply integrated with local cultures and have acquired their own distinctive styles over generations (Duarte Alonso and Northcote, 2009).

Today traditional wine regions still account for a large share of the global wine production. Countries of the European Union led by Spain, France, and Italy represent no less than 43% of the total vineyard area (OIV, 2018a). Nevertheless, the international wine trade has expanded far beyond the Old World. Over the last decades, new markets and consumers have gained familiarity with wine. Wine drinking has also become part of an increasingly globalized lifestyle, which contributes to the spread of the beverage (Lombardi et al., 2016; Smith and Mitry, 2007). In fact, world wine consumption has reached an estimate of 243 million hl in 2017, an increase of 7.5% since the year 2000 (OIV, 2018a). Over the same period, the international vineyard surface has decreased by about 4% due in part to the European Union program (2011/2012 harvest) to regulate viticultural production potential in the EU (OIV, 2018a). The total wine production reached an estimate of 250 million hl in 2017 despite unfavorable climatic conditions in large European wine producing countries, which produced 14.6% less wine than they did in 2016 (OIV, 2018a).

Despite efforts to balance global wine production with global consumption, there has been a recurrent production surplus on the wine market since the early 2000s. This has increased the pressure on wine producers who already have to adapt to an ever-changing consumer landscape. In past generations, these producers along with wine experts held a large influence on the definition of wine quality, and consumers who did not agree were often treated as uncultured (Bisson et al., 2002). Globalization and a better access to wine information have essentially shifted this privilege to wine consumers. As a result, any wine producer who wants to succeed must have a clear understanding of consumer motivations and product quality perceptions (Lockshin and Corsi, 2012).

## 1. Wine quality and grapevine age

### 1.1. The dimensions of wine quality

The notion of product quality has been extensively studied since the 1960s. It has evolved from an abstract idea of excellence to a management framework to guarantee the conformity of a product to specifications (Crosby, 1979). As marketing science gradually acknowledged the impact of consumers, quality was redefined as the degree to which a product can meet consumer expectations (Olshavsky, 1985). This new approach created the concept of “perceived quality” — the gap between what consumers expect from a product, and what they perceive when consuming it (Verdú Jover et al., 2004).

Perceived quality is often seen as the central component of the product experience (Olshavsky, 1985). However, the nature of wine with its high complexity, its strong dependence on the drinker’s level of involvement and culture (Robertson et al., 2018; Sáenz-Navajas et al., 2015), not to mention the “quasi-aesthetic” character of its appreciation (Charters and Pettigrew 2005), makes it particularly difficult to assess its quality.

Several attempts have been made to understand how different consumers behave when evaluating wine. Research suggests that wine perceived quality, similarly to other food products, can be divided into extrinsic and intrinsic dimensions that interact with each other to form a global judgment (Holbrook and Corfman, 1985). Intrinsic attributes are those that directly contribute to the consumption experience like appearance, aroma and taste (Charters and Pettigrew, 2007; Hopfer and Heymann, 2014). Wine experts, who are more familiar with blind tastings, tend to give more weight to intrinsic qualities in their assessment of wine quality. On the other hand, consumer wine evaluation is based on subjective perception that can be altered by extrinsic factors (Sáenz-Navajas et al., 2015). Before purchasing an unfamiliar wine product, consumers attempt to reduce the risk associated with the purchase by relying on a number of cues as proxies for quality. These attributes, such as region of origin (Lockshin and Corsi, 2012), brand (Bruwer et al., 2013), price (Curzi and Pacca, 2015), and packaging (Piqueras-Fiszman and Spence, 2012) extrinsically communicate quality.

In recent years the term “old vines” has emerged as a new external cue that is being increasingly mentioned on wine labels throughout the world.

## 1.2. A bottle of old vines

The grapevine is a perennial plant of remarkable longevity (Grigg et al., 2017; Robinson and Harding, 2015). One of the oldest living and fruiting specimen, a Žametovka grapevine (syn. Blauer Kölner) from Maribor, Slovenia (figure 1.1) was already represented in paintings dating back to 1657. It is believed to be at least 400 years old but still produces an impressive yearly crop of 35 to 55 kilograms (Maribor - Pohorje Tourist Board, 2014; Vršič et al., 2011).



Figure 1.1. In the center, the 'Old Vine' of Maribor, Slovenia (Bogdan Zelnik, [www.maribor-pohorje.si](http://www.maribor-pohorje.si). Accessed October 2018).

Within Europe, grapevines of comparable age are extremely difficult to encounter, due in large part to the phylloxera crisis that occurred in the 1860s. During this decade, the grape phylloxera (*Daktulosphaira vitifoliae*, Fitch) an aphid native to Eastern North America, was introduced in Europe. Phylloxera feeds on grapevine roots and leaves, causing nodosities that prevent water and nutrient uptake and transport. The damage caused by leaf nodosities (gallicoles) is generally limited to a decrease in cane growth, but root nodosities (radicicoles) can cause severe decline or death of the grapevines (Granett et al., 2001). In the course of a few years, phylloxera caused

such damage that a large portion of Europe's vineyard surface was destroyed (Wapshere and Helm, 1987).

Early control methods against phylloxera involved the use of insecticides, but such strategies were abandoned with the realization that roots of American *Vitis* species and hybrids were not severely damaged by the insect's activity (Pouget, 1990). This led to the grafting *Vitis vinifera* cultivars onto resistant American rootstocks and allowed the gradual reconstitution of European vineyards over the following decades (Wapshere and Helm, 1987). Today, approximately 80% of grapevines planted in the world are phylloxera-susceptible cultivars grafted on resistant rootstocks that are hybrids of American *Vitis* species (Whiting, 2004).

Only a few pre-phylloxera vineyards remain where soil conditions are especially unfavorable to the aphid's development, or in regions where natural barriers like deserts prevented its spreading (Ray, 1988; Robinson and Harding, 2015). Two such vineyards, planted with Pinot Noir in the French villages of Ay and Bouzy and owned by the champagne house Bollinger, are still being cultivated. In 1969, the English wine writer Cyril Ray suggested that their fruit be kept aside to produce a separate champagne. The cuvée was named 'Vieilles Vignes Françaises' (old French vines) because the grapevines, in addition to predating the phylloxera era, were trained according to the ancestral layering technique, growing freely and close to the ground, and were planted 'en foule', without any visible uniformity. The Blanc de Noir cuvée quickly rose to become one of the most sought-after products of the champagne house (Ray, 1988).

In more recent years, the term 'old vines' has been placed on various wine labels across the world, but often with a new meaning: it no longer refers to a traditional training system or ungrafted, pre-phylloxera grapevines, but simply to the fact that the wine was produced from grapevines of a certain age. And while the denomination is becoming increasingly common, legislation to regulate its use is still scarce. There is little consensus on the time necessary for a grapevine to be deemed 'old enough' to have any impact on wine quality (Robinson and Harding, 2015). To the author's knowledge, only two wine regions, the Barossa Valley in South Australia and the Napa Valley in the USA, have introduced local charters to keep track of grapevine age and codify the usage of the term 'old vines'. Grapevines need to be at least 35 years old to qualify for the denomination in Barossa Valley, while in Napa Valley the minimal grapevine age has been set to 50 years, although both values do not seem informed by science (Barossa Chapters, 2018; Historic Vineyard Society, 2018).

It is not surprising that old grapevines attract the interest of wine consumers. Their rarity, the fact that they have withstood the test of time and witnessed generations of wine growers, are already commendable in and of themselves. Most important, however, is the common belief that old grapevines produce superior wine since they reduce their yield and achieve a better plant balance.

### 1.3. Grapevine age and wine quality in the scientific literature

It has long been suggested that grapevines produce wines of increasing quality as they grow older, which as a concept finds no parallel in other crops. Nevertheless, the idea has been perpetuated by popular media and even features in trade journals, books, and peer-reviewed publications (Goode, 2005; Heymann and Noble, 1987; Howell, 2001; Koblet and Perret, 1982; Robinson and Harding, 2015; Smart, 1993). Although the literature supporting the claim that wine quality increases with vine age is still scarce, a growing body of research on the subject has developed over the last 15 years, as summarized in table 1.1. Some studies have focused on berry metabolites and wine sensory attributes. In two studies on several cultivars, old grapevine berries had a higher total acidity (TA) and a lower pH than young grapevines. Old grapevine wines were also slightly better rated in sensory analysis, with more differences for some red cultivars (Reynolds et al., 2008; Zufferey and Maigre, 2008). Heymann and Noble (1987) compared Californian Cabernet Sauvignon wines from different properties whose grapevine ages ranged from 5 to 20 years, and found a positive correlation between grapevine age and berry aroma intensity and a negative correlation between grapevine age and vegetal aromas. In a Chinese experiment that studied the aromatic profile of the autochthonous Beihong cultivar, wines from 12-year-old grapevines were reportedly more concentrated in total volatile compounds and had higher odor activity values than 3 and 6-year-old grapevines (Du et al., 2012). Other research has investigated the influence of grapevine age on its vegetative and reproductive performance. Zufferey and Maigre (2007) studied six cultivars between 5 and 34 years old in Switzerland and recorded higher net photosynthesis, predawn water potential, and pruning weight for older grapevines of all cultivars. In an experiment that included grapevines aged 6 to 168 years old distributed across five vineyards, Grigg et al. (2017) found that the older grapevines even produced higher fruit yields compared to young grapevines.

In what may seem as contradictory to the previously described research, two trials in Australia (Considine, 2004) and Tunisia (Ezzili, 1992) reported old grapevines to be equally or less vigorous than young grapevines. These apparent discrepancies may arise from several factors.

The most obvious is the diversity of climatic regions in which the studies were conducted. In addition, the grapevine ages that were considered ‘young’ and ‘old’ vary widely among studies (table 1.1). This is due in part to the difficulty in finding adequate plant material and implementing field experiments on grapevine age with comparable conditions. In fact, the clonal material of very old vineyards may be challenging to acquire decades later, unless the grapevines are own-rooted and propagated directly in the field. For grafted grapevines, the nature of the scion and rootstock combination can also influence grapevine longevity (Bauerle et al., 2008; Keller et al., 2012; Lider et al., 1978), and this adds another layer of complexity to such comparisons. Finally, the effects related to grapevine age can be difficult to separate from seasonal and site variability (Grigg et al., 2017; Reynolds et al., 2008; Zufferey and Maigre, 2008).

Over their lifetime grapevines are constantly adapting their canopy size, wood reserves and rooting depth (Tyminski, 2013; Williams et al., 1990). This adaptation can have an important influence on grapevine productivity and on grape and wine quality (Dry et al., 2004; Reynolds and Wardle, 1989; Smart and Robinson, 1991). In order to better understand the implications of grapevine age on physiology, the next section of this chapter reviews the concepts of grapevine vigor and grapevine balance and the factors that impact them. Another section is dedicated to water relations in grapevines, as previous research has suggested that young grapevines were more sensitive to drought than older grapevines (Zufferey and Maigre, 2007). The last two sections focus on berry development and on varietal aromas, in particular for the white cultivar Riesling (*Vitis vinifera* L.).

Table 1.1. Overview of the current literature related to grapevine age. The grapevine age column refers to the youngest and oldest grapevines in their respective study (adapted from Grigg et al., 2017).

Measures	Vine age (years)		Location	Cultivars	Findings	References
- Fruitset kinetics	13	50	Tunisia (El Khanguet)	Alicante, Grenache Noir	Older grapevines had lower vigor and reduced fruit set.	Ezzili (1992)
- Vegetative - Fruit	6	50	Australia (Western Australia)	Zante Currant	Older grapevines had lower vigor and berry number per bunch. Grapevine age was not related to total yield, bunch number or berry volume.	Considine (2004)
- Vegetative - Fruit - Wine	5	34	Switzerland (Wädenswil)	Chasselas, Pinot Blanc, Arvine, Gamay, Syrah, Humagne Rouge	Older grapevines had higher TA, YAN and pruning mass. Age had no impact on sugar concentration. Wines of old grapevines were more preferred early and after 4 years of aging.	Zufferey and Maigre (2008, 2007)
- Vegetative - Fruit - Wine	4	14	Canada (Ontario)	Cabernet-Sauvignon, Cabernet Franc, Pinot Noir and Pinot Meunier	Old grapevines had higher yield, bunch number, bunch mass and berry mass and lower TSS in one season only. Age had little impact in second season. Wine pH and TA were contrasting in each season, and wines from old grapevines were more vegetal in 2002 but not in 2003.	Reynolds et al. (2008)
- Vegetative	5	18	China (Beijing)	Kyoho	Vine age was correlated with seasonal carbon storage and total dry matter production.	Chiarawipa et al. (2013)
- Vegetative - Fruit	6	168	Australia (South Australia)	Syrah	Older grapevines had a higher yield, which may be due to their increased size. The effects associated with planting site were more important than the effect of grapevine age.	Grigg et al. (2017)
- Wine	5	20	USA (California)	Cabernet-Sauvignon	Vine age was correlated with berry aroma and fruit flavor in finished wines. Wines of old grapevines had higher ratings. Negative correlation between grapevine age and green bean and vegetative flavor in wines. Younger grapevines from cooler areas produced more vegetative wines.	Heymann and Noble (1987)
- Wine	3	12	China (Beijing)	Beihong	As grapevine age increased, the concentration of total volatiles and the odor activity values of the wines increased.	Du et al. (2012)



## 2. Grapevine vigor and balance

Vigor was described by Winkler (1974) as “the quality or condition that is expressed in rapid growth of the parts of the vine”. Shoots with thick stems, large leaves, and numerous secondary (lateral) shoots that grow rapidly even after veraison are considered highly vigorous. On the contrary, shoots that display poor vigor are characteristically short, with small leaves and internodes (Kliewer and Dokoozlian, 2005).

A distinction is often made between vigor and capacity. In the viticultural sense, grapevine capacity is related to the total production of a grapevine rather than its growth rate (Howell, 2001). Although the two parameters may be interchangeable at the shoot level—vigorous shoots tend to have a high capacity for the production of biomass and fruit—these parameters are not always equivalent at the whole grapevine level. A young, non-fruiting grapevine and a mature but severely pruned grapevine may both exhibit high shoot vigor and low capacity. Conversely, a mature, non-pruned grapevine may have a low shoot vigor but a relatively high capacity (Dry and Loveys, 1998).

### 2.1. Factors influencing grapevine vigor

#### 2.1.1. Climatic conditions

##### 2.1.1.1. Radiation and temperature

Sunlight is an important source of energy for green plants, and wavelengths that fall within the visible range of 400-700 nm in particular are necessary for photosynthesis. Consequently, this wavelength range is often referred to as photosynthetically active radiation (PAR). On a sunny day, PAR can be above  $2000 \mu\text{E m}^{-2}\text{s}^{-1}$ , and overcast conditions can reduce this value to less than  $300 \mu\text{E m}^{-2}\text{s}^{-1}$ . Grapevine leaves have a light absorption capacity that is generally saturated between 700 and  $1500 \mu\text{E m}^{-2}\text{s}^{-1}$  (Smart and Robinson, 1991; Yu et al., 2009). Below saturation levels, light is not sufficient for maximum rates of photochemistry (Allen and Ort, 2001), while above these levels leaves are said to be  $\text{CO}_2$  limited because enzymatic reactions regeneration cannot keep pace with the photochemistry triggered by strong radiation (Yu et al., 2009). This phenomenon is directly related to the afternoon depression in photosynthesis observed in plants (Correia et al., 1990). Light absorbed by a single leaf usually represents 85 to 90% of incident light in the PAR range. The rest is either reflected on the leaf surface (6%) or transmitted through leaves (4-9%; Smart, 1985). As a result, leaves located deep inside the canopy (after the third leaf layer) receive negligible amounts of sunlight. And even if they are able to achieve

a positive carbon balance since they also respire less, their overall contribution to photosynthesis is only 10-20% of the contribution of fully exposed leaves (Keller, 2010).

Grapevines are not suited for areas where average air temperature is less than 10° C during the growing season, under which their development is stalled (Gladstones, 2011). Typically, shoot elongation and leaf expansion accelerate with rising temperatures and reach an optimum at around 25-30 °C. The growth rate slows down with further temperature increases, until it stops at approximately 35-40 °C depending on the cultivar (Greer, 2018). Modeling studies have shown that above this threshold carbon fixation becomes limited by stomatal closure, but also by the rate of RuBP carboxylation at the chloroplast level (Greer and Weedon, 2012). In cool climate regions where overcast conditions are frequent, grapevines will have a tendency to invest additional resources in the production of larger, more photosynthetically capable leaves than in warm climate in order to compensate for the lower light intensity (Keller, 2010).

On a daily basis, the canopy temperature changes with fluctuations in radiation and air movement. Exterior leaves and bunches that are heated by the sun and tend to have elevated tissue temperatures, especially under wind-still conditions; berries exposed to bright sunlight on calm days can be warmed up to 15° C above air temperature (Cola et al., 2009). Sun-exposed leaves do not experience such increases in temperature because of the emission of long-wave radiation, heat loss due to air circulation around the leaf, and evaporative cooling caused by transpiration (Jones, 2013).

#### 2.1.1.2. Wind and relative humidity

Wind exerts a mechanical force on canopies that intensifies with shoot length. Grapevines usually respond to this action by developing shorter but thicker shoots, with smaller leaves and lower stomatal density. This common behavior in the plant kingdom is called ‘thigmomorphogenetism’ (Braam, 2005). Strong winds with speeds above 6 m s<sup>-1</sup> can induce visible damage to the grapevine. Wind also decreases the leaf’s boundary layer resistance, which is an advantage in warm climates because it favors evaporative cooling through transpiration (Jones, 2013). Nevertheless, in order to avoid excessive water losses, grapevines partially close their stomata when wind speed becomes greater than 2.5 m s<sup>-1</sup> (Williams et al., 1994). This strategy allows the grapevine to save water but comes at the expense of lower photosynthetic CO<sub>2</sub> assimilation (Davies et al., 1987; Martínez-Lüscher et al., 2015; Stoll et al., 2000). On the other hand, if wind speed is too low, leaves may deplete the surrounding CO<sub>2</sub> in the air, reducing their photosynthetic ability (Vogel, 2009). Wind speeds lower than 0.5 m s<sup>-1</sup>

also lead to more humidity at the center of canopies, which encourages the development of fungal diseases (Reynolds and Vanden Heuvel, 2009).

Leaf transpiration does not have a significant impact on canopy humidity. Instead, canopy humidity mostly depends on two factors: relative humidity (rh) and air temperature. This is due to their effect on the vapor pressure deficit (VPD) between leaves and their surrounding atmosphere. A higher VPD increases the air's capability to extract water vapor and is correlated with evaporation. As a response, leaf stomatal conductance will tend to decrease in an attempt to control water deficit, which will inhibit CO<sub>2</sub> absorption and photosynthesis (Williams and Baeza, 2007). In addition, high water pressure deficit hinders leaf growth by decreasing cell division and expansion rates. For an equal amount of soil water available, grapevines growing in drier climates will have smaller leaves and more open canopies than grapevines growing in humid climate (Keller, 2010).

#### 2.1.2. Soil characteristics

Grapevines are grown in a wide variety of soils. Soil physical properties such as texture and depth, water holding capacity, and mineral and organic matter contents have a strong impact on resource availability and root development, but also shoot growth and berry quality.

##### 2.1.2.1. Texture and depth

Soil texture is defined by the relative amounts of clay, silt, and sand particles that compose it. The size of particles influences their total surface area and the size of the pores and channels in the soil (Brady and Weil, 2002). Soil texture influences soil water holding capacity (SWHC), and thus the availability of water for uptake by the roots (Lovisolo et al., 2016). In one study, a higher percentage of sand was positively correlated with the total soluble solid (TSS) and polyphenol concentration (De Santis et al., 2017). Other work has found that soil texture did impact wine quality, but that its effects were mitigated by climatic factors such as growing season temperatures and precipitations (Reynolds et al., 2013; van Leeuwen et al., 2004).

According to several studies, soil depth until the water table has a much higher impact on rooting depth distribution, along with the presence of high bulk density elements that cannot be penetrated by roots such as stones or pebbles (Saayman and Van Huyssteen, 1983; Smart et al., 2006; van Leeuwen et al., 2004; Williams and Smith, 1991).

### 2.1.2.2. Water availability

The principal limitation to plant growth and yield formation is inadequate water supply (Kramer and Boyer, 1995; Serra et al., 2014). Under natural conditions, water is provided by rainfall and snow and is temporarily stored in the soil until it evaporates. Plant water availability is influenced by the amount, but also by the timing of precipitation events. SWHC varies with soil depth, texture, and organic matter content (Rowe, 1993; White et al., 2007). Loamy soils are able to hold up to six times more water than coarse sand, but this water may not be available to grapevines because of elevated surface tension forces ( $\Psi_M$ ) (Hsiao and Xu, 2000). In fact, water contained in pores smaller than 0.2  $\mu\text{m}$  would necessitate a suction of around 1.5 MPa, which cannot be achieved by grapevines. This pressure level represents the lower limit of grapevine available water, or ‘permanent wilting point’ of the grapevine (Watt et al., 2006). A coarse soil on the other hand dries much faster than loam. This can cause important decreases in soil water potential ( $\Psi_{\text{soil}}$ ) making water withdrawal more difficult (Jackson et al., 2008; Sperry et al., 1998).

Excessive soil water can also be detrimental to grapevine root development. In case of waterlogging, which is sometimes the result of flooding events or excessive irrigation, soil moisture can increase way above field capacity. Water will then drive gases, including oxygen ( $\text{O}_2$ ) out of soil pores. As a result, roots cannot respire properly and can suffer from hypoxia ( $\text{O}_2$  deficiency) or even anoxia (complete  $\text{O}_2$  absence) if conditions are prolonged (Keller, 2010).

### 2.1.2.3. Organic matter and minerals

The minerals present in the soil are derived from organic matter but also from the degradation of the mother rock. They are classified as: (1) macro-elements that form the basis of grapevine nutrition such as nitrogen (N), phosphorus (P), potassium (K), and calcium (Ca), and (2) micro-elements like sulfur (S), magnesium (Mg), iron (Fe), boron (B), and copper (Cu).

Organic matter (humus) is an essential source of nutrition for plants, as its degradation provides a large fraction of nitrogen and other minerals necessary for growth. Nitrogen mineralization, the degradation of nitrogen from organic matter to nitrate ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ) by soil microorganisms, is the main process by which plants can access soil nitrogen (Hart et al., 1994). Plants may also use amino acids and other organic forms of nitrogen directly obtained from the depolymerization of larger nitrogen-containing molecules, though to a lesser extent (Schimel and Bennett, 2004). Photosynthesis requires high quantities of nitrogen in order to convert light into energy. Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is the enzyme

responsible for CO<sub>2</sub> fixation by leaves and can account for up to 40% of the leaf's organic nitrogen (Evans, 1989). Harvest also exports a large quantity of nitrogen outside of the vineyard (Schreiner et al., 2006). Overall it is estimated that grapevines require between 30 and 80 kg ha<sup>-1</sup> y<sup>-1</sup> of nitrogen depending on their productivity, and this quantity needs to be replenished yearly while accounting for potential leaching (Delas, 2010).

The majority of grapevine nutrients are acquired in their ionic form by roots. Soluble nutrients mostly diffuse towards roots as part of soil water, therefore their availability is also a function of water availability in the soil. As earlier, a loamy soil will usually store more nutrient ions than a sandy soil. Soil pH is another parameter that influences nutrient availability as it will determine the fraction of minerals present in ionic form (Keller, 2010).

### 2.1.3. Viticultural management practices

#### 2.1.3.1. Scion and rootstock combination

Potential shoot vigor differs among genotypes. For instance, Muscat of Alexandria is considered as having low potential shoot vigor compared to Syrah, which is often referred to as a vigorous cultivar. The two cultivars planted on the same site would have to be trained differently (e.g. by varying the number of nodes at pruning) to deliver similar results in shoot growth (Dry and Loveys, 1998).

In addition to shoot vigor, genotypes behave differently when subjected to water deficit, specifically with their control of stomatal conductance (Chaves et al., 2010; Gaudillère et al., 2002; Lovisolo et al., 2010). Those that demonstrate a better sensitivity to water deficits like Grenache have been classified as isohydric ('drought-avoiding' or 'pessimistic'), while the others like Syrah were regarded as anisohydric ('optimistic') with their response (Schultz, 2003).

Grapevine rootstock are primarily used to provide susceptible *Vitis vinifera* cultivars with resistance to soil-borne infestation by phylloxera and nematodes. Secondly, rootstocks are also bred for their tolerance to adverse soil characteristics such as drought, waterlogging, high or low pH, and salinity, as well as their ability to influence scion vigor and fruit ripening (Lovisolo et al., 2016).

However, while some rootstocks tend to confer a lesser degree of vigor to the scion than others in a humid environment (Hatch et al., 2011), the variation in grapevine growth, yield formation and fruit ripening and composition is dominated in dry climate conditions by factors such as

water deficit and spatial disparity across the vineyard site. Rootstock effects also depend on interactions with their grafting partner (Keller et al., 2012; Tandonnet et al., 2010).

#### 2.1.3.2. Planting density

Planting density varies considerably among world wine regions. It is not uncommon to find vineyards with planting densities of 10,000 grapevines per hectare in Bordeaux and Champagne, while some vineyards in Spain are planted with only a few hundred grapevines per hectare due to water scarcity and heat intensity (Gladstones, 2011).

Vine spacing and arrangement has a relatively significant effect on yield. Increasing grapevine distance allows grapevines roots to explore larger soil volumes, and usually results in larger leaf area and yield per grapevine. However depending on row length this may ultimately lead to lower yields per hectare (Bernizzoni et al., 2009; Turkington et al., 1980; Winkler, 1969).

Also counterintuitively, high density planting does not necessarily decrease shoot growth and yield. Narrower grapevine spacings may lead to smaller root systems per grapevine, but these root systems will have a higher root density, allowing grapevines to better utilize soil reserves (Archer and Strauss, 1985). In high potential soils, closely spaced grapevines must be pruned to a restricted number of nodes to prevent shoots from adjacent grapevines to overlap on the trellis, which typically increases shoot vigor and canopy shading (Smart and Robinson, 1991).

#### 2.1.3.3. Training system

The amount and distribution of leaf area in a canopy as well as its interaction with above-ground climate are determining factors for canopy microclimate and grapevine vigor (Deloire, 2012; Jackson and Lombard, 1993; Smart, 1985). Training systems, which allow the physical manipulation of a plant's form (Reynolds and Vanden Heuvel, 2009) are therefore of central importance. Training was already a widespread viticultural practice in the ancient vineyards of the Rome (Winkler, 1974) and today a myriad of training systems, some still indigenous to their geographical region of origin, can be found throughout the world.

Regardless of their complexity, training systems can be brought down to four basic combinations. The grapevine trunk can be either trained to form a 'head' or a 'cordon' (horizontal extension of the trunk), and the resulting structure can either support 'spurs' (two-node bearing units) or 'canes' (longer bearing units) that will give rise to new shoots in the following season. Training systems therefore include two central components: (1) the amount of perennial wood, represented by the height of the trunk and the presence/absence of cordon,

and (2) the pruning method, which can be either spur or cane pruning, although it may sometimes incorporate both (Reynolds and Vanden Heuvel, 2009). The choice of training system at the time of planting must take into account vineyard potential and grapevine spacing (Bernizzoni et al., 2009). A suitable system should allow maximal light interception without overexposing clusters to sunlight, and it should optimize the leaf to fruit ratio to optimize both berry quality while avoiding shoot overlap between grapevines on the same row (Reynolds and Vanden Heuvel, 2009). The amount of perennial wood must also be adjusted to allow the reestablishment of growth around budburst and to reduce the risk of winter injury in aboveground organs (Holzapfel et al., 2010).

#### 2.1.4. Biotic stresses

Biotic stresses such as those incurred by fungi and herbivorous insects can affect grapevine growth by reducing photosynthetic capacity and leaf area (Moriondo et al., 2005). Powdery mildew and downy mildew, caused respectively by *Uncinula necator* and *Plasmopara viticola*, are common cryptogamic diseases that affect virtually all *Vitis vinifera* cultivars (Doster and Schnathorst, 1985).

Grapevine viruses may also have a detrimental impact on vigor (Credi and Babini, 1997). They are often transmitted by vectors like dagger nematodes or mealy bugs and can only be countered by preventative approaches, the most cost-effective being the use of resistant cultivars and rootstocks when available. Even so, such plant material may only slow down the infection, as no complete resistance has been observed in *Vitis vinifera* hybrids yet (Oliver and Fuchs, 2011).

In addition to cryptogamic and viral diseases, trunk diseases can have an important impact on grapevine productivity. Such is the case of the esca disease, which is caused by complexes of xylem-dwelling fungi (Bertsch et al., 2013; Mugnai et al., 1999). Common wood symptoms for esca include discoloration with black necrosis in sectors of the xylem, while visible symptoms include ‘tiger-striped’ leaves and spotting of the berry skin, also known as ‘black measles’. Esca may lead to apoplexy, with leaf fall and berry shriveling on shoots, and ultimately to grapevine death (Bertsch et al., 2013).

#### 2.2. Consequences of excessive vigor

Lateral shoot number and mean length per lateral shoot (Smart and Coombe 1983) as well as the percentage of leaf area made up by laterals (Myers et al., 2008) increase with the vigor of

the primary shoot. This excessive growth may lead to a dense canopy of several leaf layers, which is detrimental for yield formation and berry quality buildup.

### 2.2.1. Reduced yield and grape quality

As noted by Smart and Robinson (1991), high vigor vineyards, especially in the case of mature grapevines trained to restrictive training systems, tend to undergo a vegetative growing cycle that favors shoot growth over fruit production. These grapevines have a high number of leaf layers, resulting in denser canopies with low light interception at their center. An important consequence of overly dense canopies is reduced yield due to low fruit initiation in the buds, development of primary bunch necrosis, and poor fruit set (Collins and Rawnsley, 2005). In response to the lower yield, vegetative growth is further encouraged and the shade problems increase, so the vicious cycle of vegetative growth is perpetuated (figure 1.2). In the first year of production, vigorous varieties on high vigor potential soils and simple trellising systems will often produce high yields, but due to the vegetative growth cycle, yield is likely to decline in future seasons (Smart and Robinson, 1991).

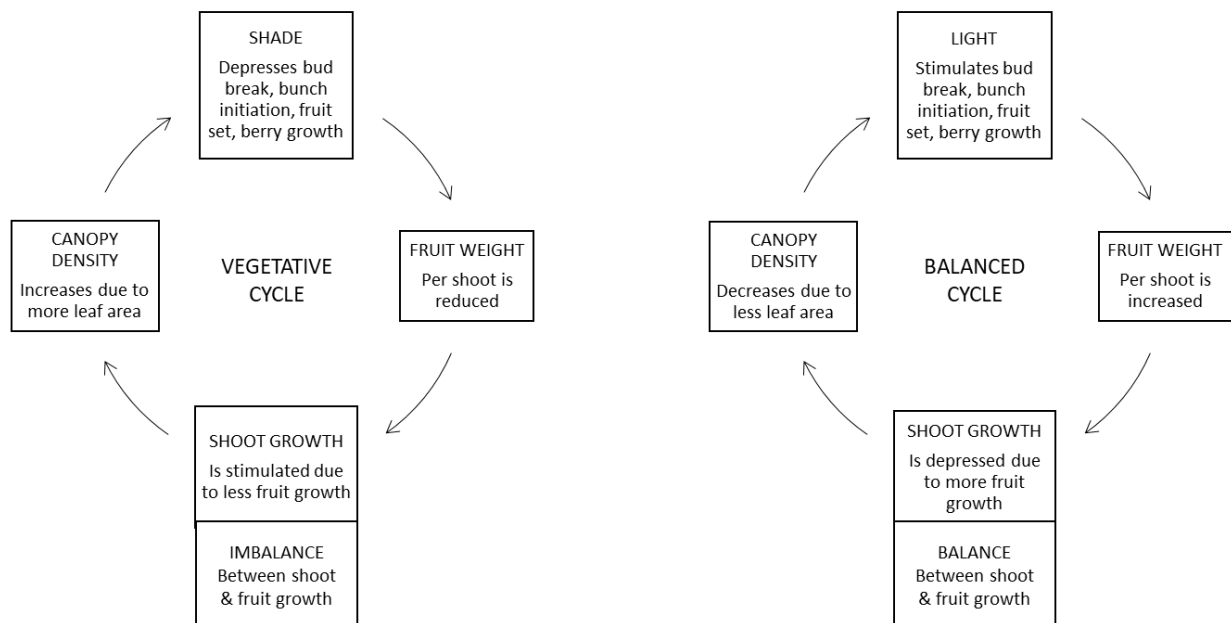


Figure 1.2. 'Vegetative' cycle during which shade stimulates vegetative growth at the expense of yield and quality. (b) 'Balanced' cycle where light favors shoot fruitfulness, leading to balance between shoot and fruit growth (adapted from Smart and Robinson, 1991).

In addition to lower yields, grapes grown in a shaded environment have lower sugar and tartaric acid accumulation, but increased concentrations of malic acid and potassium, and higher pH



values compared to non-shaded grapes (Smart, 1985). Substantial changes in secondary metabolites involved in wine flavors and aromas are also noted, including polyphenols and anthocyanins (Downey et al., 2006), skin flavonols (Diago et al., 2012), monoterpenes (Skinkis et al., 2010), and norisoprenoids (Lee et al., 2007).

### 2.2.2. Increased cryptogamic disease pressure

A dense canopy usually contributes to the development of grape pathogens (Austin et al., 2011). *Uncinula necator* and *Plasmopara viticola* are able to develop on leaf tissues, but also on green stems, inflorescences, and fruit. Early season infections interfere with fruit set and cause dramatic yield reductions if not controlled (Chellemi and Marois, 1992; Oliva et al., 1999). Fungicide applications are usually necessary until the fruit reaches a certain degree of maturity, although some varieties such as Muscat of Alexandria, Tokaz, Carignane, and Thompson Seedless may be more resistant to infection (Chellemi and Marois, 1992; Gadoury et al., 2003).

*Botrytis cinerea* is another grapevine fungus whose growth is favored by dense canopies and bunch compactness. This pathogen is especially damaging in cool climate regions with frequent rain events late in the growing season, in many cases causing penalties in quality and yield. The first symptoms of infection usually appear by the stage of veraison, and yield losses generally occur because of stalk rot (leading to premature cluster fall) or losses of juice and berry desiccation (Gubler et al., 1987). *Botrytis cinerea* converts glucose and fructose to gluconic acid and glycerol and produces the Laccase enzyme that catalyzes the oxidation of phenolic compounds, causing must browning (Zimdars et al., 2017). *Botrytis cinerea* also consumes high quantities of yeast-assimilable nitrogen (YAN) and secretes polysaccharides such as  $\beta$ -glucans that hinder wine fermentation and clarification due to their viscosity (Dimopoulou et al., 2017).

Several studies have shown that fruit grown in the shade is more prone to powdery/downy mildew and bunch rot infection than fruits that are exposed to sunlight (Bavaresco et al., 2008; Chellemi and Marois, 1992; Zahavi et al., 2001). In a trial on *Vitis vinifera* L. cv. Rielsing in the Niagara region, Canada, (Percival et al., 1994) showed that machine-assisted leaf removal at pea size stage on both sides of the fruiting zone of vigorous grapevines reduced the incidence of bunch rot by as much as 20% and the percentage of clusters with slight symptoms by 17%. In the case of powdery mildew, the phenomenon was explained in terms of plant-pathogen interaction by the synergistic effects of UV-B radiation and the rise in temperature of exposed grapevine tissues, both of which inhibit the development of fungi (Austin et al., 2009; Keller et al., 2003). Other advantages of low canopy density are improved fungicide coverage of clusters

and the possible increase in the volatility of sulfur due to the warming of exposed tissues, which provides further suppression effects on the pathogens (Austin et al., 2011).

### 2.2.3. Higher management costs

Higher disease pressure, yield losses and grape quality reduction combined with vigorous and long-lasting shoot growth all contribute to increase vineyard management costs. More elaborate trellis systems like divided canopies are needed to support foliage weight and reduce canopy shading. Summer pruning becomes necessary to allow machine circulation between rows and make cultural operations like weed control, harvesting and foliage spraying more efficient. However, this creates another problem since lateral shoot growth is favored by the trimming of vigorous shoots, leading to more crowding in the remaining canopy (Poni et al., 2014). For this reason, establishing a vineyard with the adequate parameters of planting density, cultivar/rootstock combination, and management strategies is crucial for the long-term productivity of the vineyard.

### 2.3. Achieving grapevine balance

Grapevine vigor is often regarded as a pejorative term, although neither low nor high shoot vigor are necessarily undesirable as long as the grapevine produces a high yield of fruit at a desirable quality, i.e. as long as it is balanced (Dry and Loveys, 1998). Balance from a viticultural point of view can be expressed as the equilibrium between vegetative growth and crop load. Although this concept has gained extensive attention in recent decades, it was first imagined by Ravaz (1911) in the early 20th century, who devised a fruit to pruning weight ratio as a practical method for winegrowers to evaluate grapevine balance. His concept has evolved over time (Patridge, 1925; Shaulis, 1982) and is now also understood in terms of leaf area needed to ripen a unit of crop weight (Kliewer and Dokoozlian, 2005; Petrie et al., 2000a, 2000b) or leaf area per meter of canopy (Filippetti and Intrieri, 2001; Howell, 2001). Dry et al. (2004) have reviewed grapevine balance indices that are commonly used in contemporary viticulture (table 1.2). The general recommendation for the yield to pruning weight ratio (Y/P) is between 5 and 10, with values above this range leading to insufficient grape maturity. However, the optimal Y/P ratio is highly dependent on planting conditions and genotypes. Low Y/P values are associated with excessive vegetative growth, but studies have shown that values as low as three may be optimal for cultivars with small bunches in cool climate (Kliewer and Dokoozlian, 2005). The leaf area to fruit yield ratio (LA/Y) and other ratios described in table 1.2 are inversely related to Y/P, with higher values indicating excessive vigor.

Table 1.2. Optimal ranges for various grapevine balance indices aggregated from various studies (adapted from Dry et al., 2004).

Balance Index	Abbreviation	Optimal value
fruit weight per grapevine (kg) / pruning weight per grapevine (kg)	Y / P	3 - 12
total leaf area per grapevine (m <sup>2</sup> ) / fruit weight per grapevine (kg)	LA / Y	0.6 - 1.5
pruning weight (kg) / unit of canopy length (m)	P	0.3 - 1.0
total leaf area per grapevine (m <sup>2</sup> ) / surface area per grapevine (m <sup>2</sup> )	TLA / VSA	< 2.5
total leaf area per grapevine (m <sup>2</sup> ) / unit canopy length (m)	-	2 - 5
mean cane weight (g)	-	25 - 45

Several management practices can be employed to achieve grapevine balance. In cool climate, the goal will often be to restrict grapevine vigor to avoid its negative consequences (Schultz, 1995), while it is more important in warm climate to ensure that the leaf area is sufficient to optimally ripen the crop (Palliotti et al., 2014; Schultz, 1997).

### 2.3.1. Leaf and shoot removal

The vegetative growth cycle can be broken by reducing shade in the canopy directly or by reducing grapevine vigor. Management practices such as shoot thinning and leaf removal are cost-effective and flexible methods to achieve more open canopies (Bavaresco et al., 2008; Nicolosi et al., 2012; Pellegrino et al., 2014). The amount of leaf area taken out can be relatively precise and the location from where the leaves/shoots are removed (e.g. in the cluster zone or above the cluster zone, inner leaves or outer leaves) can have more or less impact on vigor and berry quality (Stoll et al., 2013). The main downside of these methods is that they can only serve as temporary or ‘Band-Aid’ treatments to control excessive vigor since their effects only last for a single growing season (Smart and Robinson, 1991). Defoliation around the cluster zone can be performed manually or mechanically between fruit set and ripening in order to expose clusters to sunlight and wind, and to improve spraying effectiveness (Bavaresco et al., 2008; Percival et al., 1994). At veraison, leaf removal operations in very dense canopies resulted in increased sugars, flavonoids, and aromas and lower acidity and botrytis and powdery mildew infections (Austin et al., 2011; Percival et al., 1994; Poni et al., 2004; Stoll et al., 2013). In addition to obtaining a more favorable canopy microclimate, this practice also modifies the source/sink distribution and enhances the activation of light-induced enzymes such as the

phenylalanine ammonia-lyase that participates in the formation of skin phenolic compounds (Dokoozlian and Kliewer, 1995; Haselgrove et al., 2000).

Shoot thinning or ‘desuckering’ is usually performed in spring on water shoots (‘suckers’) that compete with bearing shoots for grapevine resources and cause unfavorable shading (Smart and Robinson, 1991). Shoot thinning can also be performed on bearing shoots, but only if crop load is deemed excessive with regards to leaf area, in which case the practice is commonly called ‘summer pruning’. Depending on the cultivar, research reported reduced crop load and cluster compactness as well as improvements in berry composition as a consequence of shoot thinning, notably in the case of total soluble solids (TSS), total acidity (TA), and total polyphenols (Morris et al., 2004; Reynolds et al., 2005; Sun et al., 2011; Tardaguila et al., 2008). However, shoot thinning early in the season can have negative impacts on canopy microclimate in case of high vigor, as remaining shoots will tend to grow longer and develop more laterals (Reynolds et al., 2005).

Shoot trimming is another practice aiming to control vigor. It refers to cutting off the shoot apical meristem in the summer to inhibit the growth of primary shoots and is usually performed by machine. Shoot positioning must be done adequately beforehand, and varieties with an upright behavior such as Cabernet Sauvignon are usually easier to manage (Poni et al., 2014). Again, if performed in a high vigor situation, trimming will tend to trigger lateral growth near the cutting position (Smart and Robinson, 1991).

### 2.3.2. Training system adaptation

A long-term alternative to leaf and shoot removal is to modify the training system and pruning level to better counterbalance the effects of climate and vineyard potential.

It is well understood that pruning too severely in winter often leads to fewer but more vigorous shoots (Winkler, 1974). Leaving a sufficient number of nodes in winter should lead to shoots that are still growing after fruit set, but at a slow rate. Combined with a light trim in summer, this method ensures that lateral growth is reduced (Pallioti et al., 2010). Increasing the number of nodes is also the idea behind minimally pruned vineyards, where grapevines are not pruned and shoots remain short and cease their growth around flowering (Poni et al., 2014; Smart and Robinson, 1991). But increasing the number of nodes requires more space on the trellis to prevent shading. This is possible with spur-pruned cordons but more difficult to apply in the case of the Guyot training system.

An alternative is the formation of divided canopies, which can ensure that a sufficient number of nodes are retained without the risk of shoot crowding. By creating two or more canopies from a single curtain, fruiting nodes can be better spread out in space, allowing for higher yield, better light interception and resulting in higher fruitfulness and improved berry composition (Smart, 1985). Notable examples of horizontally divided trellis systems include the Geneva double curtain (GDC) and the lyre, and the vertically divided system in the Scott Henry (Reynolds and Vanden Heuvel, 2009).

### 2.3.3. Root restriction

Intentional root volume restriction by using an artificial barrier that allows drainage has already been used as a means to restrict the vigor of various fruit trees (Ismail and Davies, 1998; Yeh and Chiang, 2001). Although experiments with container-grown grapevines demonstrated that shoot growth was correlated to root volume regardless of the plant's water and nutritional status (Hatch et al., 2011; Yang et al., 2007), root restriction remains difficult to implement (Dry and Loveys, 1998). Root pruning is easier to apply and was proven to be effective at reducing grapevine shoot growth (Giese et al., 2015; McCartney and Ferree, 1999; Smart et al., 2006), but is also not frequently used in commercial viticulture.

In regions where irrigation is beneficial for grapevine cultivation, deficit irrigation (DI), regulated deficit irrigation (RDI) and partial rootzone drying (PRD) can be used to manage vigor (Beis and Patakas, 2015). DI and RDI are based on controlling the water supply to induce a light water stress during the growing season to reduce vegetative growth and improve berry quality (Chaves et al., 2007). In DI, the same irrigation regime is applied throughout the season while in RDI water can be completely restricted during drought-insensitive phenological stages like the end of the ripening period (Lopes et al., 2011). PRD aims to achieve the same benefits by drying approximately half of the root system while the other half is still irrigated, and switching between dry and wet side at regular time intervals (Stoll et al., 2000). The literature suggests that DI, RDI, and PRD, depending on their timing and environmental conditions, are useful techniques to increase grapevine water use efficiency while maintaining crop yield and quality (Beis and Patakas, 2015; Chaves et al., 2010; Lopes et al., 2011).

### 2.3.4. Cover crop

Cover crops have been increasingly implemented in vineyards around the world in the last decade. Whether purposely seeded or consisting of resident species that naturally cover the vineyard floor, they bring about several benefits such as soil protection from erosion and

crusting, improved soil fertility, structure, better water infiltration and water holding capacity. Cover crops were also found to encourage soil biological diversity and the presence of beneficial predators, weed suppression, and provide early firm footing for cultural operations (Guerra and Steenwerth, 2011). Cover crops can also provide intentional competition for water and soil nutrients to help regulate grapevine vigor (Ingels et al., 2005). The ability to choose from a wide assortment of plant species comprising, among others, the *Poaceae* (cereals or grasses) and *Fabaceae* (legumes) families, and the possibility to mow the cover crop anytime during the growing season allows the adaptation of the timing and intensity of the competition to best suit vineyard needs. In a trial by Hatch et al. (2011) on Cabernet Sauvignon in Virginia, creeping red fescue (*Festuca rubra*) was associated to a permanent, mixed sward of tall fescue (*Festuca arundinacea*) and orchard grass (*Dactylis glomerata*) as an under-trellis cover crop (UTCC). The treatment was compared with a weed-free strip under the trellis obtained by herbicide application. Results showed that UTCC was successful at suppressing vegetative growth during the seasons of 2008 and 2009, as measured by rate and seasonal duration of shoot growth, lateral shoot development, dormant pruning weights, and trunk circumference.

While cover crops can be used advantageously to control vigor in some situations, their effects should be closely monitored to protect grapevines from uncontrolled competition that would lead to undesirable reductions in capacity. In a study in Switzerland, the competition exerted by a cover crop of tall fescue was too high for the grapevine, even with a deep soil and abundant water resources (David et al., 2001). In order to prevent such a situation, cover crop species should be selected while taking into account the timing and duration of their water use peak so that it does not coincide with that of the grapevine (Celette et al., 2008).

### 3. Grapevine water relations

#### 3.1. Cellular and physiological water transport mechanisms

Water constitutes a large fraction of a grapevine's fresh weight and is involved in virtually all the processes related to metabolism, including photosynthesis, thermal regulation, and ion assimilation. At the cellular level, water transport occurs radially through apoplastic and cell-to-cell pathways. The apoplastic pathway refers to water movement around protoplasts. The cell-to-cell pathway combines two processes that are experimentally difficult to separate. The first is the flow of water molecules through the cytoplasm and plasmodesmata between cells

(symplastic pathway), while the second is the diffusion of water across the phospholipid bilayer of cell membranes (transcellular pathway) (Steudle, 2000a; Vandeleur et al., 2009).

The cell-to-cell pathways are mostly regulated by aquaporins present in cell membranes. These channel proteins, depending on their number and activity, allow the rapid diffusion of water between cells (Gambetta et al., 2013). Several plasma membrane- and tonoplast-bound aquaporins have been identified and functionally characterized in grapevine (Shelden et al., 2009). In addition to water transport, specialized aquaporins can modify the concentration in cells of specific solutes such as sugars, organic acids like malate, and inorganic ions like potassium ( $K^+$ ) and chlorine ( $Cl^-$ ). Aquaporin 'gates' that are guarded by a negative charge are impermeable to anions, while those that are guarded by a positive charge are impermeable to cations (Maurel et al., 2008). As such, aquaporins are effective means of regulating the osmotic potential ( $\Psi_\pi$ ) of cells.

Water is axially transported inside the grapevine through xylem vessels. The xylem is radially connected to the apoplasts through a cell wall continuum, and is surrounded by parenchyma cells that also contribute to water exchanges (Steudle, 2000a). Xylem water transport is largely passive. In early spring, root water uptake is triggered by the release in the xylem sap of stored reserves in the form of osmotically active ions and organic molecules. The differences in water concentrations create an osmotic gradient throughout the grapevine and between the root surface and the soil. This leads to a positive pressure that pushes water towards higher organs. After leaf expansion, water uptake becomes mainly dependent on the hydraulic pull created by leaf transpiration. By losing water to the atmosphere, the intrinsic leaf water pressure decreases, which creates a pressure gradient that pulls water from lower parts of the grapevine (Keller, 2010).

### 3.2. Grapevine responses to water deficit

Grapevines experience water stress when the transpiration rate at the stomata exceeds the absorption rate of water at the roots. In fact, transpiration consumes 95-98% of all the water acquired by the grapevine, and it is the main driving force behind water movement during the growing season (Keller, 2010). In order to limit water loss, grapevines are able to enact a series of hydraulic resistances (figure 1.3). The main hydraulic resistance occurs at the leaf level with stomatal closure (Steudle, 2000b). When  $\Psi_{soil}$  declines, abscisic acid (ABA) is synthesized in roots and shoots and is translocated through the xylem sap to the guard cells in the leaves to

trigger stomatal closure (Chaves et al., 2010; Stoll et al., 2000). At the same time, ABA promotes auxin-dependent root and root hair growth in order to increase soil volume exploration and the size of the soil-root exchange surface (Lovisol et al., 2016).

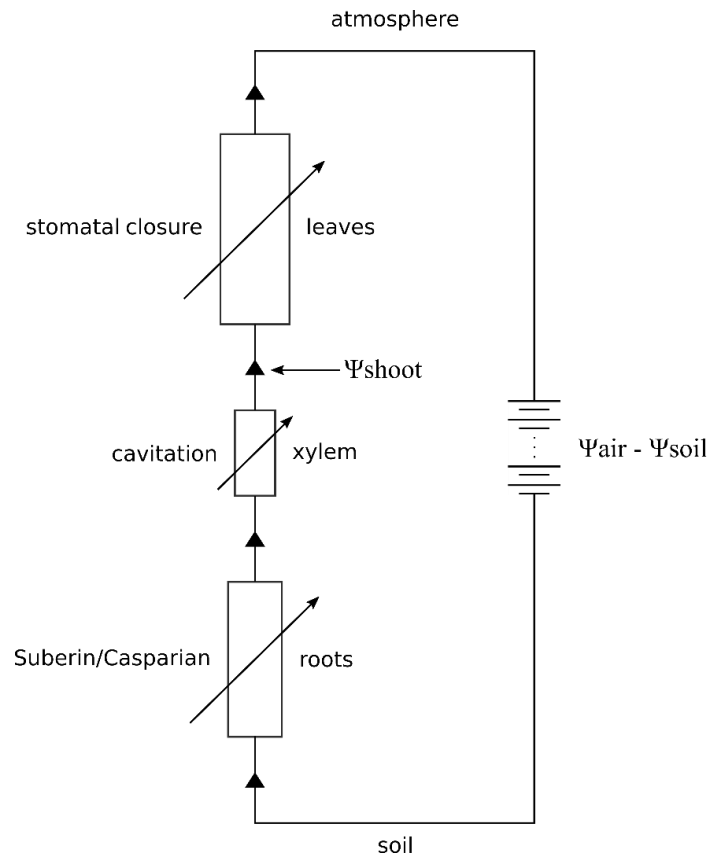


Figure 1.3. Schematic model of steady water flow through a plant, from soil to atmosphere. Water uptake at the roots, long-distance transport in the xylem and stomatal resistances are represented as resistors of relative resistance placed in series (Reproduced from Steudle 2000b).

The hydraulic pull at the leaf stomata leads to a hydraulic pressure difference between the roots and xylem, and this pressure drives water through the roots using both apoplastic and cell-to-cell pathways. In the absence of water stress, the cell-to-cell pathway is mostly used. This pathway has a high hydraulic resistance to limit water losses to the soil, hence the ‘resistor’ in the physics analogy of figure 1.3 (Steudle, 2000b).

The hydraulic resistance of the xylem is relatively small. However, xylem vessels are susceptible to cavitation, a phenomenon that occurs during strong water deficits. When it happens, the tension created by the transpiration rate and a dry soil separates the gas molecules that are dissolved in the xylem sap. Cavitation reduces the grapevine’s capacity to transport water, and if prolonged can cause irreversible damage to xylem vessels (Brodersen et al., 2010).



Isohydic genotypes like Grenache or Tempranillo can better modulate their stomatal conductance and prevent cavitation more easily than anisohydric genotypes like Syrah, Chardonnay, or Riesling (Lovisololo et al., 2010; Schultz, 2003; Vandeleur et al., 2009).

It is worth noting that isohydricity has been defined in many ways over time, which can shift the classification of cultivars on its scale. For example, Tardieu and Simonneau (1998) described it as the ability (or its lack) to maintain a constant midday water potential ( $\Psi_{MD}$ ) when  $\Psi_{soil}$  declines, while Martínez-Vilalta et al. (2014) used the slope  $\sigma$  of  $\Psi_{MD}$  and predawn water potential ( $\Psi_{PD}$ ) in their definition ( $\sigma < 1$  isohydric). Furthermore, contradicting research has recently shown that cultivars may behave differently depending on climatic conditions (Chaves et al., 2010; Hochberg et al., 2018), somewhat blurring the iso/anishohydric distinction.

## 4. Berry development and composition

### 4.1. Berry structure

The grape berry begins its development after fecundation of the ovary (fruit set). The ovary wall develops into the fruit wall or pericarp, which is in turn divided into the external exocarp (the skin), the mesocarp in the middle (the flesh), and the inner endocarp (the seeds and the tissue that surrounds them) (figure 1.4). The exocarp is made of two anatomically distinct regions, the epidermis and the hypodermis. The former is made of a single uniform layer of cells, while the latter is thicker and is the main storage organ for several secondary metabolites such as anthocyanins (responsible for the red pigmentation of certain cultivars), flavonols, and various aroma precursors (Coombe, 1987; Downey et al., 2006; Kennedy, 2002; Ollat et al., 2002).

The mesocarp represents the majority of the weight of ripe berries. Its parenchymatic cells are responsible for the storage of sugars, organic acids, and nutrients in their large vacuole, which can make up as much as 99% of the total cell volume at maturity (Jackson, 2008; Ollat et al., 2002).

The endocarp in the innermost tissue of the pericarp and represents the seeds and their surrounding locules. The seeds are made of an epiderm and a pair of integuments that encapsulate the embryo and the endosperm. The seeds only accounts for a small portion of the berry weight (Cadot et al., 2006).

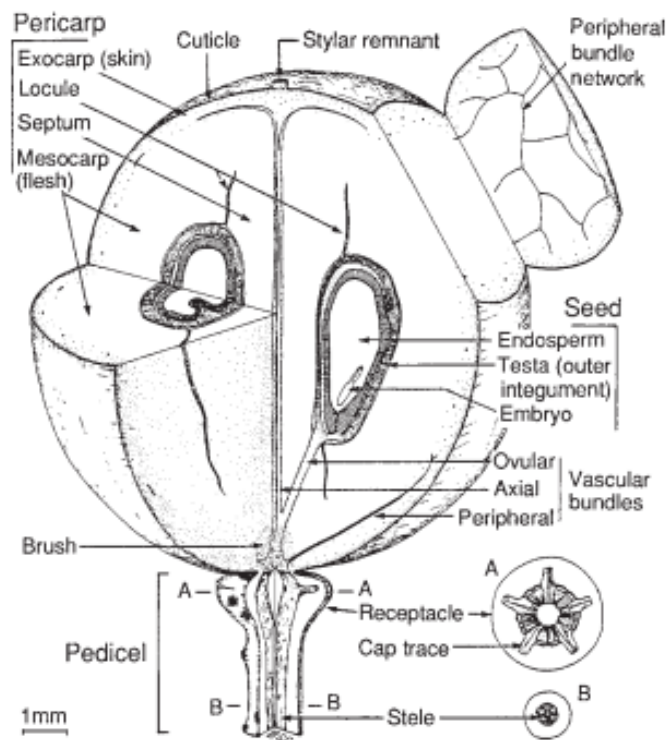


Figure 1.4. Schematic representation of the grape berry at maturity (reproduced from Coombe, 1987).

#### 4.2. Berry development

Berry growth follows a double-sigmoid curve (figure 1.5). In stage I, which typically lasts between six weeks and two months, berry cells divide rapidly and increase in size while the endosperms forms (Coombe and McCarthy, 2000; Kennedy, 2002; Ollat et al., 2002). These changes are accompanied by an accumulation of tartaric and malic acid in the mesocarp. Tartaric and malic acids are predominant at all stages of the berry development and have the largest influence on juice acidity (Sweetman et al., 2014). Phenolic compounds such as flavonoids and hydroxycinnamic acids are also accumulated (Downey et al., 2006). Chlorophyll is the major berry pigment during stage I. In fact, throughout this initial phase berries are able to produce the carbon necessary for their growth via photosynthesis (Kliewer, 1965).

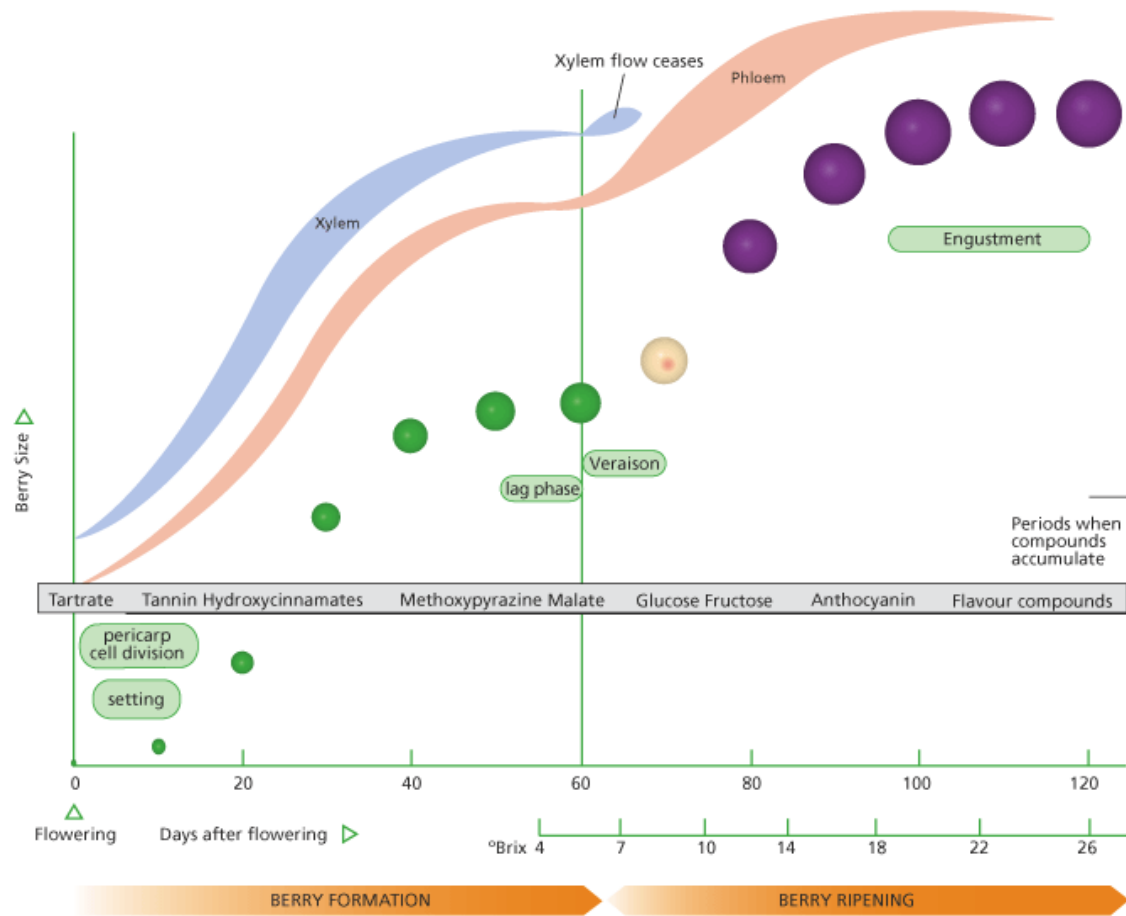


Figure 1.5. Diagram showing the double sigmoid growth curve of berry development, including the timing of accumulation of metabolites and indications of xylem and phloem inflow rates (reproduced from Kennedy, 2002).

The first growth phase is followed by a latent phase (stage II) during which the growth rate of the berry is diminished while the embryos develop and the seed coats harden (Ollat et al., 2002). This stage can vary between one to six weeks depending on the cultivar and largely decides if a cultivar has an early or late maturing character (Jackson, 2008). The second stage ends with veraison, the inflection point at which the berry begins to lose its green color. The change of color represents a fundamental shift in berry metabolism that ultimately leads to maturity (stage III); the berries lose their ability to enact photosynthesis. Instead, water and sucrose produced by surrounding leaves are imported through the phloem, which significantly increases the volume of mesocarp vacuoles (Kliwer, 1965). Sucrose is hydrolyzed to glucose and fructose inside the apoplast or the vacuole by the invertase enzyme (Conde et al., 2007). Malic acid, having reached its peak concentration just before veraison, is gradually degraded in stage III to

be used as a potential carbon source for respiration and/or gluconeogenesis (Sweetman et al., 2014). The degradation of malic acid and the dilution of tartaric acid contribute to the increase in juice pH during ripening (Kliewer, 1965).

In addition to sugar accumulation and the decrease in organic acid concentration, the third stage is also the stage at which phenolic compounds and aroma compounds are synthesized in the exocarp (Ollat et al., 2002). Phenolic compounds are derived through the phenylpropanoid pathway from the amino acid phenylalanine. The compounds produced from phenylalanine are classified as stilbens and flavonoids, the latter of which includes anthocyanins, flavonols, flavan-3-ols, and proanthocyanidins (Flamini et al., 2013).

Flavonols are a family of compounds that play a protective role against UV radiation in the plant kingdom by acting as radical scavengers (Downey et al., 2006; Haselgrove et al., 2000). They are yellow pigments that contribute directly to the color of white wine and indirectly to the color of red wine by copigmentation with anthocyanins. Flavonols mostly found in grape berries and wine are the glycosylated forms of quercetin, kaempferol, isorhamnetin, myricetin, laricitrin, and syringetin with the glycones 3-O-glycosyl, 3-O-galactosyl, and 3-O-galacturonid (Castillo-Muñoz et al., 2010). Quercetin derivatives are generally predominant in wine (Mattivi et al., 2006).

## 5. The Riesling cultivar

Riesling is believed to be native of the Rheingau valley of Germany. The oldest written reference to the cultivar, or at least one of its close progenitors, was the word 'Rieblingen' on cellar accounting documents dating from March 13, 1435 that were addressed to the Earl of Katzenelnbogen in Rüsselsheim am Main (Staab, 1991, 1986). Genetic analyses have established Riesling as having a parent-offspring relationship with Gouais Blanc, who is genetically related to at least 80 other grape varieties including Chardonnay and Gamay (Boursiquot et al., 2004). Another hypothesis suggest that Riesling may be a progeny of Traminer brought by the Romans to the Rhine region and a frost resistant, small-berry, autochthonous cultivar that was cultivated by German tribes. The resulting cultivar is thought to have been further interbred with Heunisch to increase its consistency, vitality and acid potential to become the Riesling that is cultivated today (Lott et al., 2010).

Riesling has erect shoots with fairly short internodes. Its mature trapeze-shaped leaves are dark green in color with a closed petiolar sinus. Its clusters are relatively small, compact and

cylindrical, often with one or two wings. The berries are small with a thick light-yellow skin (Schneider et al., 2012). Riesling is a late-ripening variety compared to other German cultivars like Müller-Thurgau. Its budbreak is also quite late, which makes it less prone to spring frost. In cold climate regions, Riesling needs a sufficient quantity of warmth during the growing season order to fully ripen, but excessive hot weather is generally detrimental to fruit quality (Ambrosi, 2011). Riesling is not particularly vulnerable to pests and diseases such as downy and powdery mildew, but bursting berries are frequent in years with high humidity, which may lead to higher susceptibility to *Botrytis cinerea* infections (Lott et al., 2010).

The global Riesling vineyard area is estimated around 64000 ha (OIV, 2018b). Germany accounts for 36.9% of this value, followed by Romania, the USA, France, and Australia (table 1.3). At the country scale, 23.3% of Germany's vineyard area is allocated to the variety, making it the most important cultivar in the country (Deutsches Weininstitut, 2017; OIV, 2017).

Table 1.3. Top five countries by Riesling vineyard area in 2015 (data sourced from OIV, 2018b, 2017).

Country	Riesling vineyard area (ha)	% World Riesling area	% Country vineyard area
Germany	23596	36.9	23.3
Romania	6121	9.6	3.1
USA	4605	7.2	1.0
France	4025	6.3	0.5
Australia	3157	4.9	2.0

### 5.1. Varietal aromas of Riesling

The aroma of Riesling wines reflect the soil, climate, and management practices that produced them (Douglas et al., 2001; Fischer et al., 1999). Because of this close relationship with 'terroir', it is difficult to define a very specific character for this grape variety, but some general characteristics can be extracted from the literature. For example, Riesling wines that are produced in cool climates tend to have low alcohol and a high degree of acidity, giving them a distinct brightness and clarity in the mouth (Robinson et al., 2013). On the other hand, Rieslings such as those coming from the Pfalz region of Germany are more intense and full-bodied, with aromas ranging from floral to honey-like. Other aromas that are frequently described in Riesling wines include the citrus, stone and exotic fruit families, as well as vegetal aromas like freshly cut grass and hay (Schüttler et al., 2015). When aged, Riesling wines have a uniquely

characteristic profile as they develop aromas of dried fruits and the so-called ‘kerosene’ aroma, the hallmark of the cultivar (Lott et al., 2010).

The varietal aromas of Riesling wine are derived from multiple classes of volatile compounds including monoterpenes, norisoprenoids, and volatile thiols (Schüttler et al., 2015).

#### 5.1.1. Monoterpenes

Monoterpenes are organic C<sub>10</sub> compounds that belong to a large group of chemicals known as terpenoids, which also includes norisoprenoids. The first step of monoterpene biosynthesis occurs with the formation of isopentenyl pyrophosphate (IPP) through either the cytosolic mevalonate pathway or the plastidial methylerythriol pathway (Duchêne et al., 2009). The second step involves the synthesis of geranyl phosphate (GPP) by condensation of IPP and its isomer dimethyl allyl diphosphate (DMAPP). Monoterpenes are produced from GPP through the action of terpene synthase (Robinson et al., 2014). The *Vitis vinifera* (VvTS) terpene synthase gene family is reportedly involved in the production more than 21 monoterpenes (Martin et al., 2010). Other genes like the ones coding for deoxy-D-xylulose synthase (VvDXS) are also known to be associated with the early steps of terpene production (Emanuelli et al., 2010).

Plants rely on monoterpenes for a variety of functions, from deterring animals to protecting against fungal and bacterial infections (Gershenson and Dudareva, 2007). Indeed, recent studies have shown that volatile monoterpenes and sesquiterpenes are emitted by *in vitro*-grown plants after inoculation with *P. viticola* strains, and that the nature and quantity of emitted compounds depended on the grapevine genotype (Algarra Alarcon et al., 2015). The most common monoterpenes in grapes and wine are the monoterpene alcohols linalool,  $\alpha$ -terpineol, nerol, geraniol, citronellol and hotrienol. Some of those compounds also form isomeric oxides like linalool oxide (cis-, trans- in pyranoid and furanoid forms) and nerol oxide (Bakker and Clarke, 2011). Monoterpenes are mainly found in berries as odorless glycoside conjugates. The odor-active aglycones can be released by enzymatic or acidic hydrolysis during wine fermentation and storage (Black et al., 2015). From a winemaking perspective, monoterpenes play a major role in the aroma of Muscat varieties and non-Muscat aromatic varieties like Gewürztraminer and Riesling (Mateo and Jiménez, 2000), with high levels of linalool and  $\alpha$ -terpineol being positively correlated with flowery aromas (Günata et al., 1985).

### 5.1.2. Norisoprenoids

Norisoprenoids (C<sub>13</sub>) are degradation products of carotenoids, which participate in the light harvesting activity of chloroplasts and protect the cell from photo-oxidative damage (Lashbrooke et al., 2013). Norisoprenoids consist of a megastigmane skeleton and can have various olfactory properties depending on the position of an oxygen group. Damascenones are norisoprenoids with the oxygen group bound to carbon 7, while ionones have the oxygen group bound to carbon 9 (Winterhalter et al., 1990).

Two norisoprenoids of particular importance for wine are  $\beta$ -damascenone and  $\beta$ -ionone, which contribute to fruity and flowery notes (Mendes-Pinto, 2009). The typical 'kerosene' aroma of Riesling wine is due to the norisoprenoid 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN). This compound, which is barely present in free form in berries, is obtained by the hydrolysis and rearrangement of glycosylated precursors during winemaking and aging (Sacks et al., 2012).

### 5.1.3. Volatile Thiols

Thiols are sulfur-containing molecules that are widely represented in food products beverages and are often characterized by low odor thresholds (Vermeulen et al., 2005). Several odorous thiols were identified in Sauvignon Blanc wines and later in wines from Semillon, Riesling, and Pinot Gris, and Colombard with varying concentrations (Tominaga et al., 2000). Among these thiols, 4-methyl-4-sulfanylpentan-2-one (4MSP) and 3-sulfanylhexas-1-ol (3SH) contribute respectively to Sauvignon Blanc box-tree and passion fruit aromas (Helwi et al., 2016). They are obtained by cleavage of odorless precursors, namely S-conjugates to glutathione and cysteine, during fermentation (Robinson et al., 2014; Roland et al., 2011).

## 6. Objectives of the thesis

The research objectives of the present work were to investigate the impact of grapevine age on its leaf physiology, water tolerance, vegetative growth, vine balance, and berry and wine quality. For this purpose, a dedicated vineyard planted in Geisenheim, Germany for this very purpose, with *Vitis vinifera* L. cv. Riesling grapevines of the same plant material but planted in 1971, 1995 and 2012 was studied between years 2014 and 2017.

More specifically, the four objectives of this work are were:

1. to follow the productivity and vegetative growth of the three grapevine age groups over time, and examine their response to an induced water stress;
2. to analyze and compare the canopy and cluster architectures among the three grapevine groups and assess the effects of these parameters on berry composition and enological potential;
3. to chemically analyze and sensorially evaluate wines produced from the three grapevine age groups, both from recent (2014 to 2017, all three groups) and older vintages (predating 2014, only for grapevines planted in 1971 and 1995);
4. to develop and test a non-destructive, structure-from-motion with multi-view stereo-photogrammetry-based method that could be used as a tool directly at the vineyard to complement traditional dendrology measurements.



## Chapter 2. Impact of grapevine age on water status and productivity

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Author contributions: the author designed and conducted the research experiments, analyzed the data, drafted and constructed the manuscript. ELISA analysis was performed by Dr. Elvira Bleser and Johannes Kiesgen (Department of Grapevine Breeding, HGU) and carbon discrimination analysis was performed by Olivier Mathieu (Biogeosciences, CNRS/University of Burgundy).

## 1. Abstract

Grapevines are perennial plants that can display remarkable longevity. It is often thought that some of their characteristics evolve in a positive way as they grow older, such as having a higher tolerance to water deficit and an improved balance between vegetative and reproductive growth. However, only a few studies have been conducted so far on the possible effects of age on vine productivity and water status.

An experimental vineyard was designed in the German Rheingau region to compare vines of identical planting material planted at three different times. The vineyard was established in 1971 with *Vitis vinifera* (L.) cv. Riesling vines (clone Gm 239-17) grafted on 5C Teleki and trained in double Guyot and cordon in alternating rows. In 1995 and 2012, rows of both training systems were uprooted and replanted with the same scion/rootstock combination. The aim of this study was to evaluate the impact of vine age on several physiological and reproductive parameters. Vines planted in 2012 were generally more sensitive to water deficit than vines planted in 1995 and 1971, although this depended on the amount of precipitations during the growing season. A supporting trial was carried out to create more intense drought conditions by deploying a plastic mulch around selected vines during rain events. Young vines were most affected by the treatment, suggesting that their lower tolerance to water deficit might be due to a shallower root system. Vines planted in 1995 and 1971 displayed similar response to water deficit. Canopy architecture, cluster parameters and vine balance components including pruning weight and yield were different for the youngest vines until the fifth year after planting due to their lower cropping capacity. Even though the observed yield and pruning weight per meter were lower for grapevines planted in 1971 than those planted in 1995, these variables were similar across the two age groups when missing vines were taken into account. Wood diseases were identified as the main factor behind the decline of old vines. The study suggests that the management of wood diseases is a key component in improving vineyard longevity, and that the conservation of grape yield and technological maturity parameters for vineyards in similar environmental conditions is indeed possible over the long term.

**Keywords:** old vine, water deficit, balance, vigor, capacity, trunk disease.

## 2. Introduction

Grapevines can thrive over several centuries while still producing wine (Grigg et al., 2017; Robinson et al., 2013; Vršič et al., 2015). Despite their longevity, it is not uncommon today to

see vineyards uprooted only two to three decades after planting. Sometimes the goal may simply be to replant them with cultivars that better fit wine consumption trends, but the decision can also be due to declining grape production (Bernizzoni et al., 2009; Duthie et al., 1991). Indeed, a number of external factors may, on their own or in combination, lead to significant losses in their economic viability (Kaplan et al., 2016). These factors include wood diseases such as esca, eutypa dieback, and botrytisphaeria dieback (Bertsch et al., 2013) and viral diseases that can prove damaging once their vectors are well established (Credi and Babini, 1997; Walker et al., 2004). In some cases, poor management practices may result in dense canopies and compact soils, reducing the value of the crop while escalating production costs (Morlat and Jacquet, 2003; Smart and Robinson, 1991).

While it may be difficult to manage vineyards over long periods of time, the benefits of doing so from a viticultural and enological perspective remain unclear. Nevertheless, a growing body of research on grapevine age has developed over the last 15 years, with a focus on berry metabolites and wine sensory attributes (Du et al., 2012; Heymann and Noble, 1987; Reynolds et al., 2008; Zufferey and Maigre, 2008). Other studies have investigated the influence of vine age on its vegetative and reproductive performance. For instance, Zufferey and Maigre (2007) recorded higher net photosynthesis, predawn water potential, and pruning weight for older vines, while Grigg et al. (2017) noted the tendency of old vines to form higher fruit yields, with the possible explanation of increased wood reserves. Some of these results contrasted with other research that described older vines as equally or less vigorous than young vines (Considine, 2004; Ezzili, 1992). The apparent disparities in the literature may arise from the difficulty in finding adequate plant material and designing field experiments about vine age with comparable conditions. As a consequence, the vine ages that are referred to as ‘young’ and ‘old’ vary widely among studies. In addition, the plant material of old vineyards can be difficult to acquire decades later, unless the vines are own-rooted. For grafted vines, the nature of the scion and rootstock combination can also influence vine longevity (Lider et al., 1978), which adds another layer of complexity to experimental conditions. Finally, the effects related to vine age can be difficult to separate from seasonal and site variability (Grigg et al., 2017; Reynolds et al., 2008).

A major source of climatic variation is water availability, reflected by the timing and intensity of precipitation events. High water deficits over the growing period can inhibit shoot growth and photosynthesis (Keller et al., 2016; Schultz and Matthews, 1988) and negatively affect yield

and berry composition (Marciniak et al., 2013; Romero et al., 2015). Previous research suggested that young vines were more sensitive to drought, possibly because of a less developed root system (Zufferey and Maigre, 2007), but experiments comparing groups of older vines have not yet been undertaken. In order to systematically investigate the influence of vine age on water status, this paper reports on a unique Riesling vineyard with alternating rows of vines of identical planting material, but with three planting years ranging from 1971 to 2012. It also includes two training systems, the double (bilateral) Guyot and the single spur-pruned cordon. The main goal of the present work, which focused on the Guyot vines, was to compare the three vine age groups with respect to their tolerance to water deficit and vine productivity as described by canopy architecture, cluster parameters, pruning weight, and yield from 2014 to 2017. The second part of this work was designed as a supporting experiment on the cordon trained vines to investigate drought tolerance, which was achieved by deploying a plastic mulch during rain events around a subset of vines in 2016 and 2017 and monitoring the vines' behavior with respect to the absence of precipitations.

### 3. Materials and methods

#### 3.1. Weather data

The trial was conducted in Geisenheim, Germany (49° 98' N, 07° 94' E) in an experimental vineyard managed by the Department of General and Organic Viticulture of Hochschule Geisenheim University (HGU). Daily temperature, precipitation, and wind speed data were recorded by a local weather station belonging to HGU and located around 500 m east of the vineyard. The temperature information was used to compute the growing degree-day (GDD) accumulation from April 1<sup>st</sup> to October 31<sup>st</sup> by subtracting a base temperature of 10 °C from the daily average temperature (Jones, 2005; Winkler, 1974).

#### 3.2. Experimental design and plant material

The experimental vineyard was established in 1971 with *Vitis vinifera* (L.) cv. Riesling (clone Gm 239-17) grafted on 5C Teleki rootstock (*Vitis berlandieri* × *Vitis riparia*) in well-drained, fertile clay loam soil (270 g kg<sup>-1</sup> clay, 410 g kg<sup>-1</sup> silt, 260 g kg<sup>-1</sup> sand, with a pH of 6.9, and wilting point and field capacity of 0.15 and 0.30 m<sup>3</sup> water m<sup>-3</sup> soil, respectively). The vines were planted along 68 rows in a North-South orientation coinciding with a downward slope of eight degrees, an inter-row distance of 2.00 m, and vine spacing of 1.40 m. Two pruning systems were managed on the same plot: the double (bilateral) Guyot with downward arched

canes, and the single cordon with two-node spurs. In 1995 and 2012, several rows of vines were uprooted and replanted with the same combination of clone and rootstock. The end result was a vineyard with alternating rows of vines of different ages and pruning systems. Each combined treatment (planting year  $\times$  pruning system) was available in four field replicates.

The trial on double Guyot vines started in 2014, which was the first productive year of vines planted in 2012. At that time, some vines were already missing in the rows planted in 1971 and 1995. A fully randomized block experimental design was therefore not possible to implement. Instead, a selection process was carried out at the beginning of the 2014 growing season in order to choose six double Guyot vines per age group and field replicate for seasonal measurements ( $n = 24$ ). The number of nodes retained was adjusted to 10 per meter (equivalent to 14 nodes per vine) during winter pruning. The trunk diameter measured 10 cm above the grafting point (Santesteban et al., 2010) and the bud fruitfulness of individual vines were compared to the median and mean values of their respective rows, and the three vines closest to these values were selected. Trunk size is commonly used to estimate the vigor of perennial plants. It was used at the beginning of the 2014 growing season to select vines that were most representative of their respective populations, especially for vines planted in 1971 which had the largest heterogeneity in size. Bud fruitfulness, being directly related to inflorescence number and fruit production (Sánchez and Dokoozlian, 2005) was also considered as a selection parameter. However, since it is much more sensitive to external factors such as late frost damage and is affected by the environmental conditions of the previous season (May and Antcliff, 1963) this variable only played a minor role in vine selection.

Vines close to the edges of the vineyard and those adjacent to the empty spaces left by missing vines were used as buffer and were not included in measurements. The health status of selected vines was visually appraised for trunk diseases and viral infections. In 2017, the selected vines were also inspected by Enzyme-Linked Immunosorbent Assay (ELISA) tests (Clark and Adams, 1977) for potential infections of Arabis Mosaic Virus (ArMV), Grapevine Fleck Virus (GFkV), Grapevine Leafroll-Associated Virus 1 and 3 (GLRaV-1, GLRaV-3), and Grapevine Fanleaf Virus (GFIV). The tests were made on dormant shoot cuttings that were collected prior to winter pruning.

Due to the general vigor of the site, a cover crop of grass and clover was sown in alternating rows when the vineyard was established with a weed-free strip of about 10 cm under the rows.

When the second group of vines was planted in 1995, their rows were tilled in the first two years to prevent excessive competition with cover crop during the early developmental stages of the vines (Williams et al., 1990). The same treatment was applied from 2012 to 2014 in the rows of the youngest vines. Afterwards, these rows were sown with the same cover crop combination that already existed in 1995 and 1971 rows. In order to assess the impact of cover crop over time, the soil nitrate content at depths of 0 to 30 cm and 30 to 60 cm was used as indicator for nitrogen mineralization (Stenberg et al., 1999; Wehrmann and Scharpf, 1986). It was measured from 2014 to 2017 at flowering, veraison, and harvest. Fifteen soil samples per field replicate ( $n = 4$ ) were combined with a soil homogenizer (Schäfer, Euskirchen, Germany) and analyzed according to Schaller (1988) by flow injection at 450 nm with a Tecator FIAstar Analyzer<sup>®</sup> (FOSS, Hillerød, Denmark). The fresh weight was taken at the time of measurement, after which soil samples were dried in a laboratory stove at 80 °C until mass stabilization. The difference between wet and dry weight was recorded for each sample to calculate soil water content. The leaf chlorophyll index under green excitation (SFR\_G) of three healthy, mature leaves per selected vine was also assessed every two weeks from berry set to harvest with a Multiplex Research<sup>®</sup> portable sensor array (Force-A, Orsay, France) set to leaf measurement with an aperture of 4 cm ( $n = 24$ ). The data was collected between 0900 and 1200 hr on the eastern side of the canopy on fully illuminated leaves. The vine measuring order was randomized to account for diurnal variability. Phenology was followed on 50 random vine organs per double Guyot and cordon row on a weekly basis until veraison according to the modified E-L system developed by Coombe (1995) that simplifies previous systems (Baggiolini, 1952; Lorenz et al., 1995).

### 3.3. Gas exchange measurements

The leaf CO<sub>2</sub> assimilation rate ( $A_N$ ), stomatal conductance ( $g_s$ ), and transpiration rate (E) of selected vines were followed during the 2016 and 2017 seasons using a LI-6400XT<sup>®</sup> photosynthesis system (LI-COR, Nebraska, USA). The measurements were made in a 6 cm<sup>2</sup> chamber at ambient CO<sub>2</sub> concentration and photosynthetically active radiation (PAR). Block temperature was controlled at 30 °C, which resulted in leaf temperatures between 29 and 30 °C.

### 3.4. Vine capacity and balance

Leaf and cluster distribution in the cluster zone were determined at veraison by point quadrat analysis (Meyers and Vanden Heuvel, 2008; Smart and Robinson, 1991). A measuring tape was extended along the cluster zone at a fixed height and a thin, sharpened metal rod was passed

horizontally through the canopy from end to end. A total of 50 insertions were made per row, with a distance of 20 cm between insertions. Contacts with leaves, clusters, and canopy gaps were recorded and used to calculate percent gaps (PG), leaf layer number (LLN), percent interior leaves (PIL) and percentage interior clusters (PIC). Point quadrat analysis could not be performed in 2017 due to a severe infection of downy mildew around flowering and berry set, which forced a partial defoliation of the cluster zone for all grapevines. Cluster parameters were assessed yearly around two weeks before harvest in order to ensure the availability of healthy clusters. Four clusters per row were randomly selected. After weighing and destemming, the number of berries per cluster and their average weight were recorded.

Harvest date was chosen yearly according to the technological maturity (with general targets of 23 °Brix, titratable acidity of 9 g tartaric acid eq. L<sup>-1</sup> and pH 2.9 for dry Riesling wine) and the disease status of the grapes. The double Guyot rows were harvested by hand while sorting out healthy and rotten berries. Both yield fractions were weighed separately, and only healthy berries were used in winemaking. Pruning weight per vine and average cane weight were recorded during winter pruning. The adjusted values of pruning weight and yield, both of which account for the number of missing vines per row, were also calculated.

### 3.5. Berry technological maturity and $\delta^{13}\text{C}$

Berry samples were collected at harvest from individual rows for maturity analysis and dry mass carbon isotope discrimination ( $\delta^{13}\text{C}$ ). Maturity analysis was made on fresh berries. Juice total soluble solids (TSS) was determined for each sample with a refractometer (Kübler, Karlsruhe, Germany). Titratable acidity (TA) and pH were assessed with a 719 S Titrino terminal titration unit (Metrohm AG, Herisau, Switzerland). The  $\alpha$ -amino nitrogen content was determined by o-phthaldialdehyde/N-Acetyl-L-Cysteine spectrophotometric assay (NOPA), according to Dukes and Butzke (1998). For  $\delta^{13}\text{C}$  the juice obtained at harvest was frozen until analysis, which followed the method described by Gaudillère et al. (2002). The samples were prepared at the joint research unit of Vine Ecophysiology and Functional Genomics (EGFV) of the Institute of Vine and Wine Sciences (ISVV) in Bordeaux, France, and analyzed with a Vario<sup>®</sup> elemental analyzer (Elementar, Hanau, Germany) coupled with a continuous-flow isotope mass spectrometer (Isoprime, Manchester, UK). This latter part was subcontracted to the joint research unit Biogeosciences (National Center for Scientific Research CNRS/ University of Burgundy) in Dijon, France. The instrument was calibrated with isotopic

reference materials of L-glutamic acid (USGS40;  $\delta^{13}\text{C} = -26.389 \pm 0.042 \text{ ‰}$ ) and caffeine (IAEA-600;  $\delta^{13}\text{C} = -27.771 \pm 0.043 \text{ ‰}$ ).

### 3.6. Experiment on water deficit

In 2016 and 2017, a separate field experiment on water deficit was carried out on the cordon trained rows. Eight consecutive vines were selected within these rows in 2016 with criteria similar to the double Guyot vine selection and the same number of retained nodes. White foldable plastic sheets were installed in the first week of May around four of those vines, with an approximate covering area of 20 m<sup>2</sup> (“stress”, appendix 2.1). During the growing season the plastic sheets were extended to cover the soil during rain events and were otherwise folded to avoid condensation. The other four vines remained uncovered for the duration of the experiment (“control”, appendix 2.1). The two central vines from each treatment were used for measurements (n = 8). Soil samples were manually collected at flowering, pea-size berries and two weeks after veraison for the estimation of water content for the stressed and control treatments. A Pürkauer auger was used between the center vines on the eastern and western sides of the rows, and samples were collected from 0 to 30 cm and from 30 to 60 cm. The chlorophyll index was monitored on the central vines of each treatment on the same dates as the double Guyot vines as described above.

Leaf stomatal conductance was assessed on cordon vines using an AP4<sup>®</sup> porometer (Delta-T, Cambridge, UK) that was calibrated in the vineyard prior to measuring. Predawn water potential ( $\Psi_{\text{PD}}$ ) was measured on cordon vines using a Scholander pressure chamber connected to a nitrogen gas cylinder (Soil-Moisture Corporation, Santa Barbara, CA, USA; following the principle of Scholander et al., 1965) on several dates over the growing season. Measurements were made on dry, mature leaves of the two center vines of the control and stressed treatments. The negative value of the pressure required to force sap from the petiole (in MPa) was recorded as the water potential. The berry  $\delta^{13}\text{C}$  at maturity was assessed for the three age groups of vines and the two water treatments following the protocol described earlier.

### 3.7. Statistical analysis

Linear mixed-effect models (LMM) were used to evaluate the effect of vine age on the parameters of interest. Vine age and year were converted to categorical variables and used as fixed effects in all models. Water status was added as a fixed effect with two levels (control and stressed) for data related to the water deficit trial on cordon vines. For measurements that were



repeated along the growing season, random effects were structured to account for space and time variability (Schielzeth and Forstmeier, 2009). Single vines were included as a nested random effect inside blocks. The dates of measurement or phenological stages were grouped and converted to a factor that was used as a crossed random effect. A similar approach was used in the case of yearly measurements such as fruit yield and pruning weight, with both vine age and year as fixed effects and block as a random effect. Model parameters were estimated by restricted maximum likelihood (REML) in R (R Core Team, 2018) with the packages lme4 (Bates et al., 2015) and pkrtest (Halekoh and Højsgaard, 2014). The importance of fixed effects was assessed by Kenward-Roger approximations at a significance level of  $P = 0.05$  (Schaalje et al., 2002). Variation around means was reported as standard deviation (sd). Principal Component Analysis (PCA) was used to examine the relationship between point quadrat analysis, cluster parameters and grapevine balance components. PCA was performed with the packages FactoMineR (version 1.41; Lê et al., 2008) and factoextra (version 1.0.5; Kassambara and Mundt, 2017).

## 4. Results

### 4.1. Weather data

The year 2014 had the highest growing degree-day (GDD) accumulation from April 1<sup>st</sup> to October 31<sup>st</sup> (table 2.1), with high temperatures around flowering. Although mean daily temperatures in July were above average during this growing season, they were the lowest of all years in August, with elevated precipitation levels for these two months.

*Table 2.1. Growing degree-day (GDD) and precipitation data for Geisenheim Fuchsberg, Germany, from April 1st to October 31st for the growing seasons 2014-2017. The yearly average represents the period of 1981-2010.*

Growing season	GDD	Precipitations (mm)
2014	1366	447
2015	1359	239
2016	1358	358
2017	1345	363
yearly average (1981-2010)	1119	336

The year 2017 had the second highest cumulative precipitations during the growing season, but it was also the coolest on average, with low temperatures between flowering and veraison. The growing seasons of 2015 and 2016 were the warmest on average, with mean daily temperatures

close to 30 °C and maximal temperatures above 35 °C for several days in July and August (appendix 2.2). Precipitation levels in the growing season were lowest in 2015 with only 239 mm of rain. Although the year 2016 was fairly rainy compared to the long-term average, most precipitation events occurred at the beginning of the growing season, with warm and dry conditions from fruit set to harvest. Budburst occurred at its earliest on April 7<sup>th</sup> in 2014, while the latest budburst date was recorded in 2016 on April 22<sup>nd</sup>. Other phenological events, notably flowering and veraison, took place at similar times in all growing seasons. Among the three age groups and two training systems, vines planted in 2012 were consistently two to three days more advanced for budburst, flowering, and veraison compared to vines planted in 1971 and 1995.

#### 4.2. Vine selection and soil management

The trunk diameter of double Guyot vines planted in 2012 showed low variability in 2014 (figure 2.1). Trunk variability was higher for other age groups, with values ranging from 34.4 to 82.7 mm for vines planted in 1971. Although the nodes retained at pruning were limited to 14 per vine, the mean number of fruitful buds per vine in spring 2014 was 13.4, 11.5, and 7.0 for vines planted in 1971, 1995, and 2012 respectively. The bud fruitfulness of the youngest vines gradually increased to reach levels comparable to other age groups in 2016 and 2017, which were the fourth and fifth years after they were planted (figure 2.2).

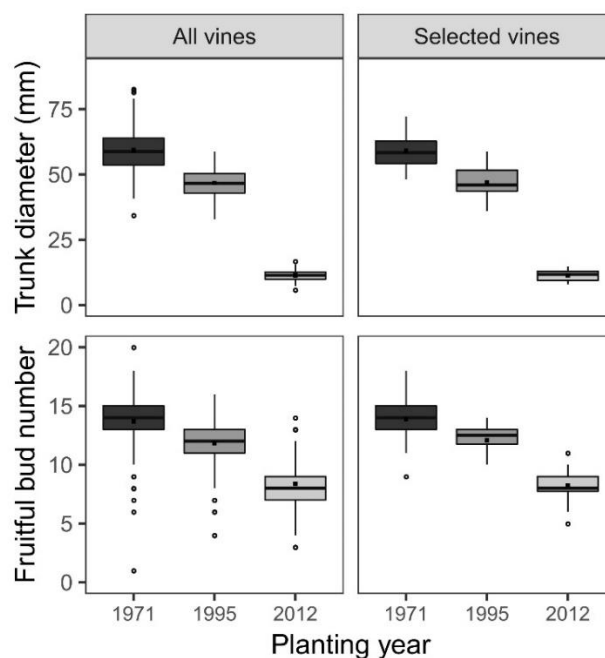


Figure 2.1. Double Guyot vine selection process in 2014 with trunk diameter and fruitful bud count for all vines and for the 24 selected vines per age group.

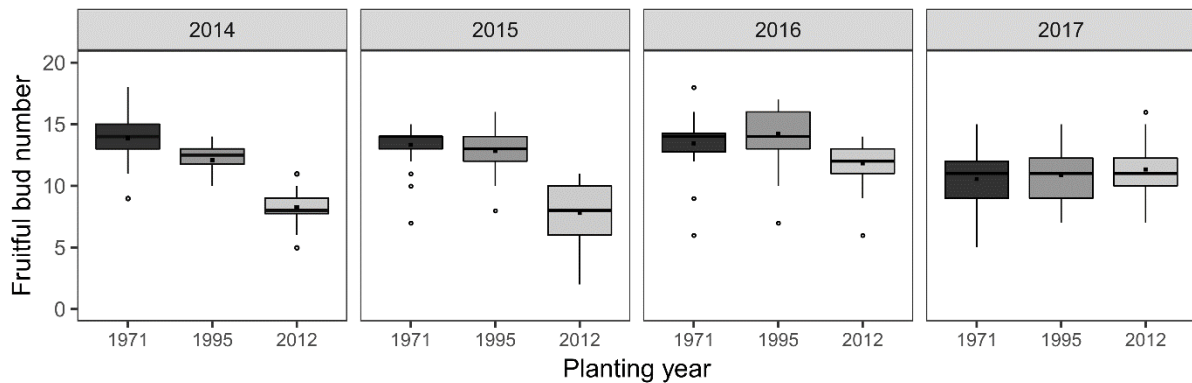


Figure 2.2. Fruitful bud count of selected double Guyot vines from 2014 to 2017 ( $n = 24$ ).

Soil cultivation in 2014 and 2015 stimulated nitrogen mineralization in rows planted in 2012, resulting in a higher soil nitrate content, as well as higher soil water content due to reduced soil transpiration compared to rows planted in 1995 and 1971 (figures 2.3 and 2.4). After sowing the cover crop in the youngest vine rows in winter 2015/2016 and halting soil cultivation practices, their soil nitrate and water content reached levels similar to those of other age groups in 2016 and 2017. The LMM for both soil  $\text{NO}_3^-$  and water content (appendix 2.3) showed significant effects for age group, growing season, and their interaction ( $P < 0.001$ ). Similarly, the leaf chlorophyll index, which is positively correlated with plant nitrogen status (Padilla et al., 2016), was consistently higher for vines planted in 2012 over the 2014 and 2015 growing seasons (figure 2.5; appendix 2.4). These differences dissipated in 2016 and 2017 once cover crop was established.

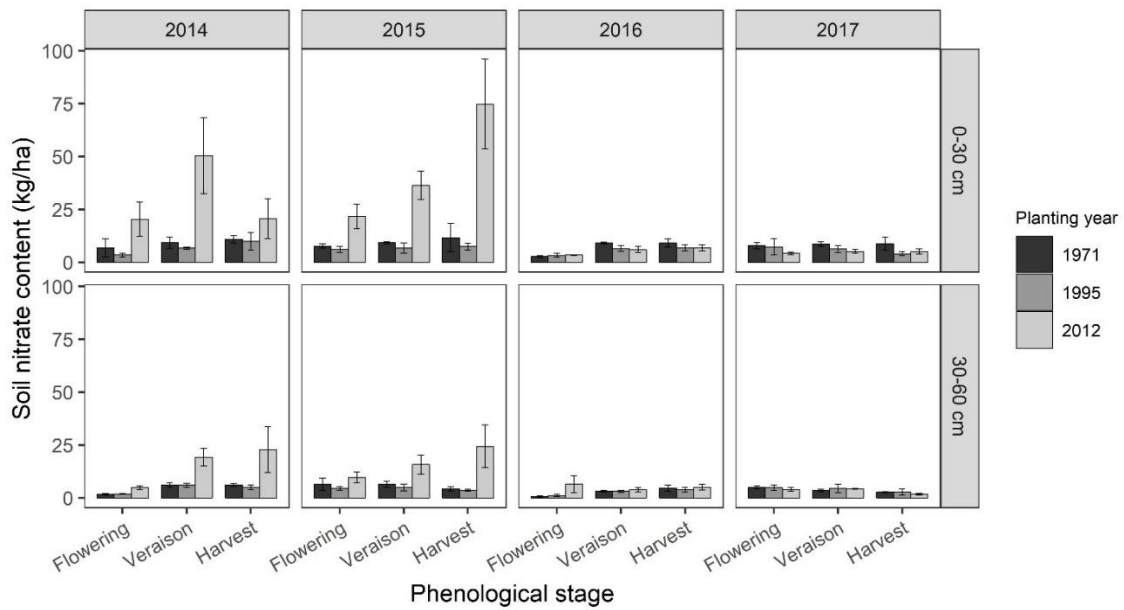


Figure 2.3. Soil nitrate content measured at horizons 0-30 cm and 30-60 cm over the growing seasons 2014-2017 for double Guyot rows planted in 1971, 1995, and 2012 (means  $\pm$  sd).

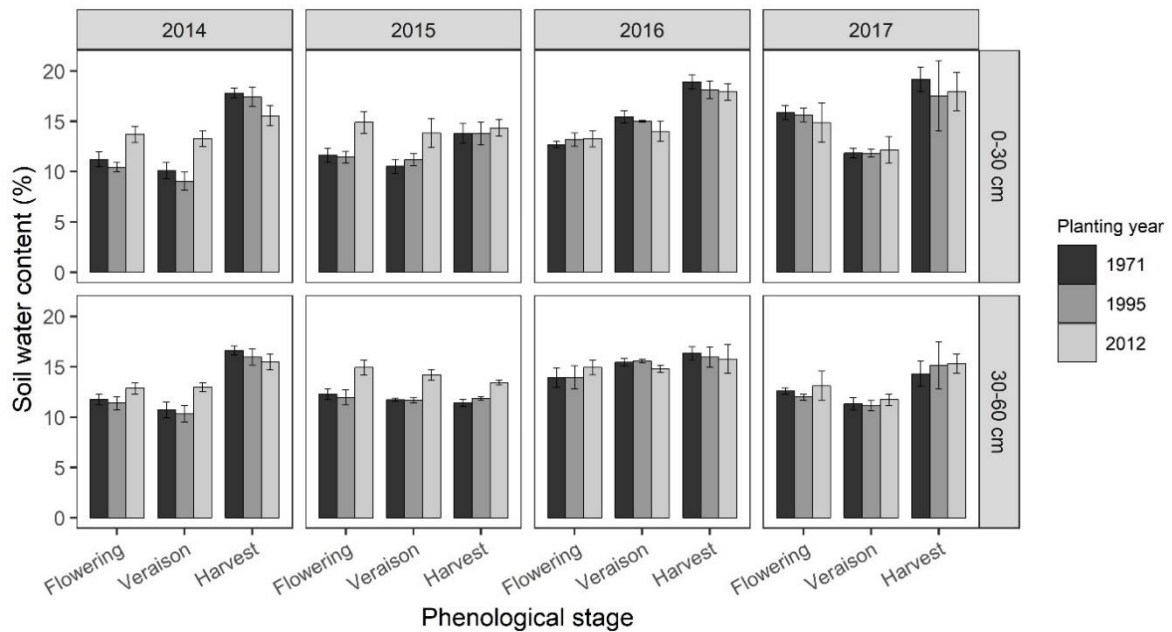


Figure 2.4. Soil water content measured at horizons 0-30 cm and 30-60 cm over the growing seasons 2014-2017 for double Guyot rows planted in 1971, 1995, and 2012 (means  $\pm$  sd).

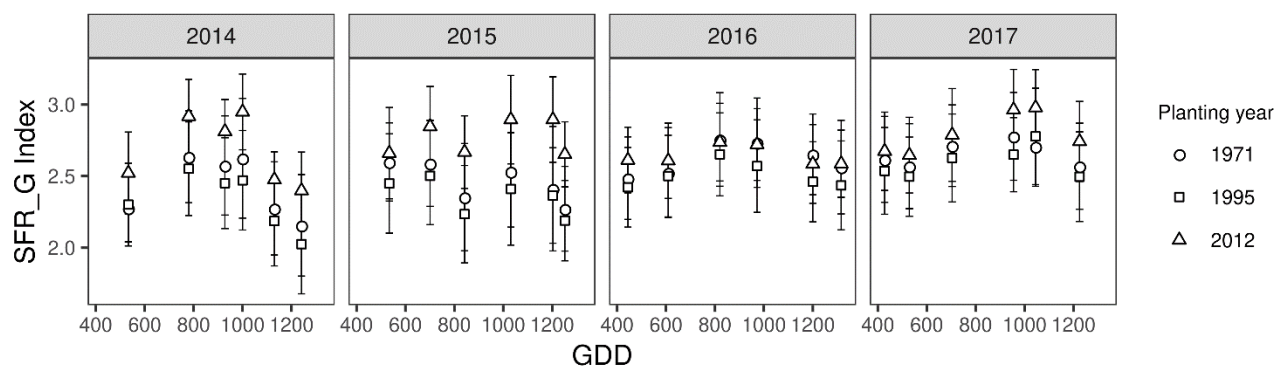


Figure 2.5. Chlorophyll fluorescence ratio under green excitation (SFR\_G) measured at regular time intervals on selected double Guyot vines from flowering to harvest over the growing seasons 2014-2017 (means  $\pm$  sd).

The health status of the vineyard was monitored since the beginning of the study. Despite continuous visual assessments of vine health, the ELISA tests revealed that from the 72 selected vines tested for five viruses, two were infected with single viruses. One vine planted in 1971 was infected with GFIV. The other belonged to the group planted in 2012 and was infected by GLRaV-1. The chlorophyll index for this latter vine was consistently high in 2015 (appendix 2.4), possibly due to interactions between the viral infection and the unusually hot and dry weather during this growing season (El Aou-ouad et al., 2016; Wolpert and Vilas, 1992). Otherwise, the observed behavior of these two vines was in line with other vines of the same age group for all studied parameters.

#### 4.3. Gas exchange measurements

Vines planted in 1995 and 1971 had greater net CO<sub>2</sub> assimilation rate, stomatal conductance, and transpiration compared to those planted in 2012 after the first measurement in 2016, differing by a factor of 1.5 to 3 close to harvest (figure 2.6; appendix 2.5). The differences among studied parameters were smaller during the majority of the 2017 growing season.

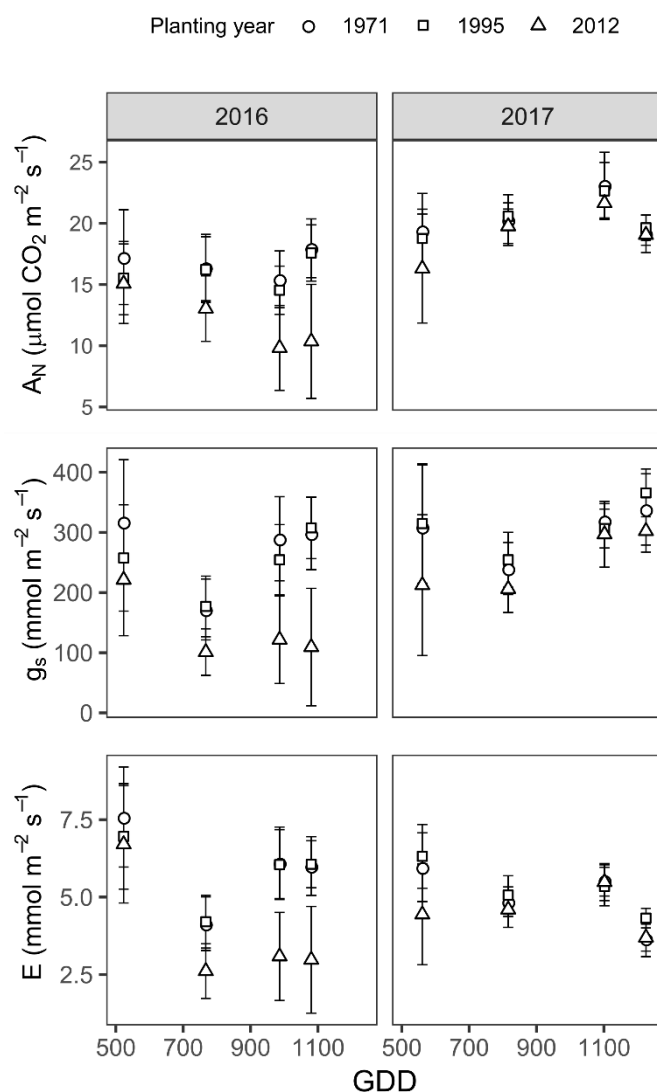


Figure 2.6. Leaf assimilation ( $A_N$ ), stomatal conductance ( $g_s$ ), and transpiration ( $E$ ) measured periodically on double Guyot vines over the growing seasons 2016 and 2017 with  $n = 24$  (means  $\pm$  sd).

#### 4.4. Vine capacity and balance

Table 2.2 shows the percentages of healthy, esca affected, and missing vines in double Guyot rows from 2015 to 2017. While only 3% of the vines planted in 1995 were missing in 2015, 27% of vines planted in 1971 had already died by that time. The percentage of vines with esca symptoms was also greater for the oldest group at 4%, which meant that only 69% of these vines were fully productive in 2015. The disease progressed over the next two years, with more vines ceasing production and more showing esca symptoms. Even though the visibility of symptoms depended on the growing season, the rate of infection slightly increased for both age groups over time.

Table 2.2. Percentages among vines planted in 1971 and 1995 of healthy vines, vines with esca symptoms, and missing vines as visually assessed at the end of each growing season between 2015 and 2017.

Planting year	Healthy vines (%)	Vines with esca symptoms (%)	Missing vines (%)
<b>2015</b>			
1971	69	4	27
1995	97	1	2
2012	98	0	2
<b>2016</b>			
1971	67	5	28
1995	95	1	4
2012	98	0	2
<b>2017</b>			
1971	65	5	30
1995	93	3	4
2012	98	0	2

The youngest vines did not reach the same number of fruitful buds than older groups until 2016. This had a direct impact on their canopy architecture, pruning weight and, yield (table 3). Within the point quadrat analysis, the percentage of gaps (PG) was considerably higher in the canopy of vines planted in 2012 in the growing seasons of 2014 and 2015 than for those planted in 1971 and 1995. Grapevines planted in 2012 also had the lowest percentage of interior leaves (PIL) and percentage of interior clusters (PIC) in 2014. Within the point quadrat analysis, the three vine ages had comparable PG, PIL and PIC in 2016. These results may be attributed in part to the growth of grapevines planted in 2012, which were in their second year after planting at the start of the trial as supported by the LMM analysis (appendix 2.5) that shows significant interactions between grapevine age and year in PG ( $P = 0.026$ ) and LLN ( $P = 0.029$ ) despite larger differences between vintages than differences due to grapevine age.

Grapevines planted in 2012 had lower cluster and berry weights in 2014 and 2015 than grapevines planted in 1995 and 1971 (table 2.3). Their average berry number per cluster was also lower in 2014. In 2016 and 2017, the three parameters were similar for all age groups. Cluster weight and number of berries per cluster were higher in 2016 compared to other growing seasons, while in 2017 clusters generally had the lowest berry numbers but the highest berry weights.

The recorded and adjusted pruning weights for vines planted in 2012 were 40-50% lower than older vines in 2014 and 2015. The average cane weight was similar across age groups ( $P = 0.12$ ) and was mostly dependent on growing season ( $P = 0.0018$ ) (appendix 2.5). The number of missing vines in the youngest rows was minimal (three vines were not able to survive after planting) so their recorded and adjusted pruning weights were almost identical. This was also relatively the case for vines planted in 1995. For vines planted in 1971, the differences between recorded and adjusted pruning weights ranged from 30 to 45%.

There were no significant differences in the recorded yield of vines planted in 1995 and 1971, but the values were consistently higher for the formers during the study. The adjusted fruit yield had relatively greater values for vines planted in 1971, slightly surpassing other vine age groups in two out of four years. In 2014 and 2015, the yield to pruning (Y/P) ratio was lowest for vines planted in 2012 with a value of 1.0. The year 2015 also had the highest yield-to-pruning ratios for all vine ages. In 2016, the lowest ratio was calculated for vines planted in 1971. In the final year of the study the values of yield to pruning weight ratios were similar among age groups. The generally lower productivity of the youngest vines was translated in the model by a strong impact of vine age on most parameters ( $P < 0.001$ ). Year was also a strong predictor for the data ( $P \leq 0.003$ ). However the interaction terms between vine age and year were significant in most cases. This was mostly attributed to the youngest vines' increase in cropping potential from 2014 to 2017. In fact, once the youngest vines were removed from the LMM, there were no significant differences in balance components among vines planted in 1995 and 1971 (appendix 2.6).

The data from point quadrat analysis and cluster parameters from 2014 to 2016 was combined with data from grapevine balance components and subjected to principal component analysis (PCA). Figure 2.7 displays the biplot of the first two principal components (PCs), which together represented 62.9% of the variance in the data. The eigenvalues from PCA and the eigenvectors for each variable in the principle components of the PCA are presented in appendix 2.7. The separation between the data points underlines the differences between grapevines planted in 2012 and the older grapevine groups in seasons 2014 and 2015, as the former had a strong positive correlation with PG and a strong negative correlation with the number of fruitful buds and yield. Percent gaps and fruitful buds were also the variables responsible for the highest variation on PC 1. The strong negative correlation between these two variables suggests that the main reason behind the high percentage gaps in the canopy of grapevines planted in 2012



was in fact due to the lower number of fruitful buds and therefore the lower capacity that is typical of young grapevines. In 2016 the four data points for young grapevines were in relative proximity to those of other age groups, indicating that their canopy displayed similar characteristics to canopies of older grapevines four years after planting.

Grapevines planted in 1995 and 1971 had similar characteristics in all growing seasons. The values of 2015 were clustered in the top right quadrant of the biplot and were positively associated with point quadrat measures PIL, PIC, LLN, and yield to pruning weight (Y/P) ratio. In contrast, the values of 2016 for the two older grapevine groups were mainly in the bottom right quadrant of the biplot, with a higher cluster weight, cane weight, and pruning weight. Data could not be obtained in 2017 for point quadrat analysis, therefore the vintage was excluded from PCA.

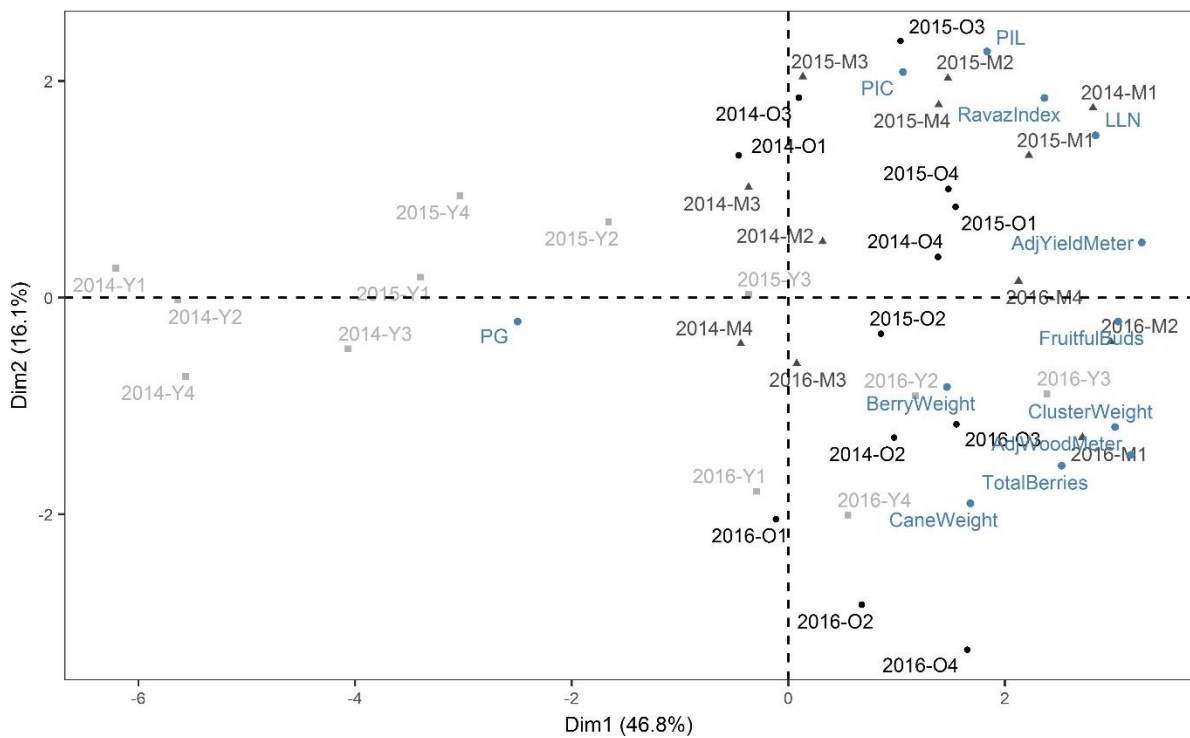


Figure 2.7. PCA biplot of point quadrat analysis, cluster architecture and grapevine balance components for 2014-2016. The point labels represent the growing season followed by the grapevine age group ('O' for old grapevines planted in 1971; 'M' for 'mature' grapevines planted in 1995; 'Y' for 'young' grapevines planted in 2012). There are four replicates per growing season per age group. They are represented by the numbers 1 to 4 next to the letter.

Table 2.3. Vine balance parameters for three age groups and four replicates over the growing seasons 2014-2017 ( $n = 4$ ). Fruit yield (which includes rotten berries) and pruning weight refer to values recorded in the field, while adjusted fruit yield and pruning weight take into account the number of missing vines per row (means  $\pm$  sd).

Planting year	% gaps (PG)	% interior leaves (PIL)	% interior clusters (PIC)	Leaf layer number (LLN)	Cluster weight (g)	Berry number (no./cluster)	Berry weight (g)	Fruitful bud number (buds/vine)	Average cane weight (g)	Pruning weight (kg/m) (P)	Adjusted pruning weight (kg/m)	Fruit yield (kg/m) (Y)	Adjusted fruit yield (kg/m)	Yield/pruning weight (Y/P)
<b>2014</b>														
1971	21.8 $\pm$ 7.4	25.3 $\pm$ 3.9	52.1 $\pm$ 7.41	1.61 $\pm$ 0.16	194.8 $\pm$ 72.4	144.0 $\pm$ 52.7	1.3 $\pm$ 0.15	13.4 $\pm$ 2.2	68.1 $\pm$ 9.2	0.48 $\pm$ 0.07	0.63 $\pm$ 0.13	1.31 $\pm$ 0.35	1.66 $\pm$ 0.24	2.7 $\pm$ 0.9
1995	9.5 $\pm$ 3.4	21.3 $\pm$ 5.0	50.1 $\pm$ 12.8	1.86 $\pm$ 0.34	187.4 $\pm$ 43.4	130.9 $\pm$ 26.8	1.3 $\pm$ 0.11	11.5 $\pm$ 1.3	71.8 $\pm$ 6.9	0.57 $\pm$ 0.09	0.60 $\pm$ 0.09	1.61 $\pm$ 0.16	1.70 $\pm$ 0.18	2.9 $\pm$ 0.5
2012	32.5 $\pm$ 11.2	11.5 $\pm$ 3.2	40.6 $\pm$ 11.6	1.07 $\pm$ 0.16	129.0 $\pm$ 33.8	104.7 $\pm$ 21.4	1.1 $\pm$ 0.15	7.0 $\pm$ 1.6	53.4 $\pm$ 13.2	0.27 $\pm$ 0.08	0.27 $\pm$ 0.07	0.26 $\pm$ 0.08	0.27 $\pm$ 0.08	1.0 $\pm$ 0.3
<b>2015</b>														
1971	21.8 $\pm$ 7.4	23.6 $\pm$ 2.0	50.5 $\pm$ 6.9	1.61 $\pm$ 0.16	213.0 $\pm$ 35.0	152.6 $\pm$ 26.1	1.1 $\pm$ 0.15	13.3 $\pm$ 1.8	65.8 $\pm$ 14.6	0.43 $\pm$ 0.03	0.62 $\pm$ 0.11	1.79 $\pm$ 0.24	2.50 $\pm$ 0.13	4.1 $\pm$ 0.7
1995	19.3 $\pm$ 2.4	22.7 $\pm$ 5.6	60.4 $\pm$ 3.9	1.69 $\pm$ 0.13	208.5 $\pm$ 32.2	153.2 $\pm$ 19.7	1.1 $\pm$ 0.15	12.8 $\pm$ 1.8	61.1 $\pm$ 12.6	0.54 $\pm$ 0.11	0.56 $\pm$ 0.11	2.39 $\pm$ 0.34	2.48 $\pm$ 0.32	4.5 $\pm$ 0.5
2012	27.3 $\pm$ 11.3	17.2 $\pm$ 3.9	57.8 $\pm$ 7.8	1.41 $\pm$ 0.15	194.1 $\pm$ 48.5	153.4 $\pm$ 33.8	0.8 $\pm$ 0.18	7.8 $\pm$ 2.6	73.5 $\pm$ 17.8	0.39 $\pm$ 0.10	0.40 $\pm$ 0.09	1.00 $\pm$ 0.51	1.01 $\pm$ 0.51	2.4 $\pm$ 0.7
<b>2016</b>														
1971	21.5 $\pm$ 6.4	13.4 $\pm$ 3.5	44.0 $\pm$ 8.0	1.40 $\pm$ 0.15	232.5 $\pm$ 49.0	172.3 $\pm$ 28.4	1.3 $\pm$ 0.16	13.5 $\pm$ 2.5	85.8 $\pm$ 18.9	0.54 $\pm$ 0.06	0.78 $\pm$ 0.06	1.27 $\pm$ 0.26	1.81 $\pm$ 0.20	2.3 $\pm$ 0.4
1995	10.0 $\pm$ 6.5	16.9 $\pm$ 2.3	49.0 $\pm$ 9.8	1.75 $\pm$ 0.24	225.6 $\pm$ 48.5	177.1 $\pm$ 41.2	1.2 $\pm$ 0.21	14.2 $\pm$ 2.3	70.4 $\pm$ 7.1	0.68 $\pm$ 0.09	0.71 $\pm$ 0.09	2.16 $\pm$ 0.45	2.23 $\pm$ 0.46	3.1 $\pm$ 0.2
2012	17.0 $\pm$ 6.9	15.2 $\pm$ 6.0	44.0 $\pm$ 5.3	1.47 $\pm$ 0.18	226.6 $\pm$ 40.6	179.8 $\pm$ 38.4	1.2 $\pm$ 0.15	11.8 $\pm$ 2.0	79.6 $\pm$ 9.4	0.66 $\pm$ 0.10	0.67 $\pm$ 0.09	2.04 $\pm$ 0.36	2.07 $\pm$ 0.33	3.1 $\pm$ 0.3
<b>2017</b>														
1971	-	-	-	-	209.1 $\pm$ 40.6	127.1 $\pm$ 28.5	1.6 $\pm$ 0.23	10.6 $\pm$ 2.0	99.7 $\pm$ 15.0	0.49 $\pm$ 0.08	0.71 $\pm$ 0.05	1.47 $\pm$ 0.36	2.14 $\pm$ 0.15	3.0 $\pm$ 0.4
1995	-	-	-	-	207.2 $\pm$ 47.9	132.3 $\pm$ 24.2	1.5 $\pm$ 0.22	10.9 $\pm$ 2.2	87.2 $\pm$ 6.2	0.65 $\pm$ 0.05	0.67 $\pm$ 0.05	1.65 $\pm$ 0.25	1.69 $\pm$ 0.26	2.8 $\pm$ 0.5
2012	-	-	-	-	174.9 $\pm$ 49.8	118.2 $\pm$ 25.0	1.4 $\pm$ 0.21	11.3 $\pm$ 2.2	70.9 $\pm$ 18.2	0.56 $\pm$ 0.14	0.57 $\pm$ 0.15	1.79 $\pm$ 0.22	1.81 $\pm$ 0.21	2.6 $\pm$ 0.6

#### 4.5. Berry technological maturity and $\delta^{13}\text{C}$

Total soluble solids (TSS), titratable acidity (TA), and pH were similar for the three age group in all growing seasons (table 2.4; appendix 2.8). However, the  $\alpha$ -amino acid content of berries of the youngest grapevines was more than double that of grapevines planted in 1995 and 1971 in the first and second years of the trial.

Table 2.4. Technological maturity parameters and dry mass carbon isotope discrimination ( $\delta^{13}\text{C}$ ) for the three grapevine age groups ( $n = 4$ ) over the growing seasons 2014 to 2017 (means  $\pm$  sd).  $\delta^{13}\text{C}$  was only measured in 2016 and 2017.

Planting year	Total soluble solids (TSS; °Brix)	Titratable acidity (TA; g tartaric acid L <sup>-1</sup> )	pH	$\alpha$ -amino acids (mg isoleucine equivalent L <sup>-1</sup> )	$\delta^{13}\text{C}$ (%)
<b>2014</b>					
1971	19.33 $\pm$ 0.41	11.85 $\pm$ 1.03	2.9 $\pm$ 0.03	68.3 $\pm$ 15.65	-
1995	19.16 $\pm$ 0.65	11.31 $\pm$ 0.29	2.9 $\pm$ 0.01	66.5 $\pm$ 13.48	-
2012	19.10 $\pm$ 0.18	10.39 $\pm$ 0.62	3.0 $\pm$ 0.02	154.3 $\pm$ 12.07	-
<b>2015</b>					
1971	20.85 $\pm$ 0.28	11.89 $\pm$ 0.25	2.9 $\pm$ 0.06	63.8 $\pm$ 16.58	-
1995	20.80 $\pm$ 0.29	12.58 $\pm$ 0.82	2.9 $\pm$ 0.03	57.0 $\pm$ 16.06	-
2012	21.30 $\pm$ 0.21	10.71 $\pm$ 0.51	3.0 $\pm$ 0.03	114.0 $\pm$ 5.94	-
<b>2016</b>					
1971	19.56 $\pm$ 0.32	8.71 $\pm$ 1.04	3.2 $\pm$ 0.10	57.5 $\pm$ 11.67	-27.78 $\pm$ 0.13
1995	19.22 $\pm$ 0.43	8.92 $\pm$ 0.32	3.1 $\pm$ 0.04	53.0 $\pm$ 11.83	-27.03 $\pm$ 1.74
2012	18.48 $\pm$ 0.50	8.79 $\pm$ 0.73	3.1 $\pm$ 0.07	68.8 $\pm$ 11.95	-26.79 $\pm$ 1.68
<b>2017</b>					
1971	19.73 $\pm$ 0.22	13.04 $\pm$ 0.46	3.0 $\pm$ 0.04	49.8 $\pm$ 9.64	-28.32 $\pm$ 0.34
1995	19.73 $\pm$ 0.65	12.90 $\pm$ 0.33	2.9 $\pm$ 0.02	48.8 $\pm$ 10.14	-28.26 $\pm$ 0.17
2012	20.01 $\pm$ 0.64	12.42 $\pm$ 0.38	3.0 $\pm$ 0.03	53 $\pm$ 9.13	-28.02 $\pm$ 0.69

The  $\delta^{13}\text{C}$  analysis that took place in 2016 and 2017 on mature berries indicated only a weak water deficit during both growing seasons. No significant differences were recorded among age groups ( $P = 0.31$ ).

#### 4.6. Experiment on water deficit

In the framework of the water deficit trial on cordon vines, the soil water content measured at flowering and pea-sized berries in 2016 was approximately 5% lower for the stressed treatment compared to the control for all age groups (figure 2.8; appendix 2.9). However, due to the dry

weather around veraison and the lack of covering, the soil water content of stress and control treatments tended to converge at the end of the season, especially in the lowest soil horizon. The frequency and intensity of precipitation events in the first half of the 2017 season led to similar water content for all treatments between flowering and pea-size, but not when measured two weeks after veraison (0 - 30 cm: Year,  $P < 0.001$ ; Water status,  $P < 0.001$ ; Year  $\times$  Water status,  $P = 0.038$ . 30-60 cm: Year,  $P < 0.001$ ; Water status,  $P < 0.001$ ; Year  $\times$  Water status,  $P = 0.54$ ). There were no differences in leaf chlorophyll between age groups in 2016 and 2017 (figure 2.9), as well as no differences between years and water treatments. The stomatal conductance measured on cordon vines in 2016 and 2017 showed significant differences across vine age groups ( $P < 0.001$ ; figure 2.10). The water treatment also affected grapevine response, but its effect depended on the year (Year  $\times$  Water status,  $P = 0.039$ ).

Over most of the 2017 season vines planted in 2012 and subjected to water deficit had the lowest stomatal conductance. During this season, the high precipitations combined with the vineyard slope led to water runoff, which affected upper and lower blocks to different extents and increased spatial heterogeneity.  $\Psi_{PD}$  on cordon vines decreased along the 2016 growing season for the cordon vines of all age groups and treatments, with the lowest  $\Psi_{PD}$  being observed for vines planted in 2012 towards harvest (figure 2.11). Values above -0.4 MPa were maintained over the 2017 growing season for all vine ages and treatments, and only the vines planted in 2012 under the stress treatment showed mild water stress around -0.3 MPa, which is usually defined as the limit for the onset of water stress for white cultivars (Schultz and Matthews, 1988).

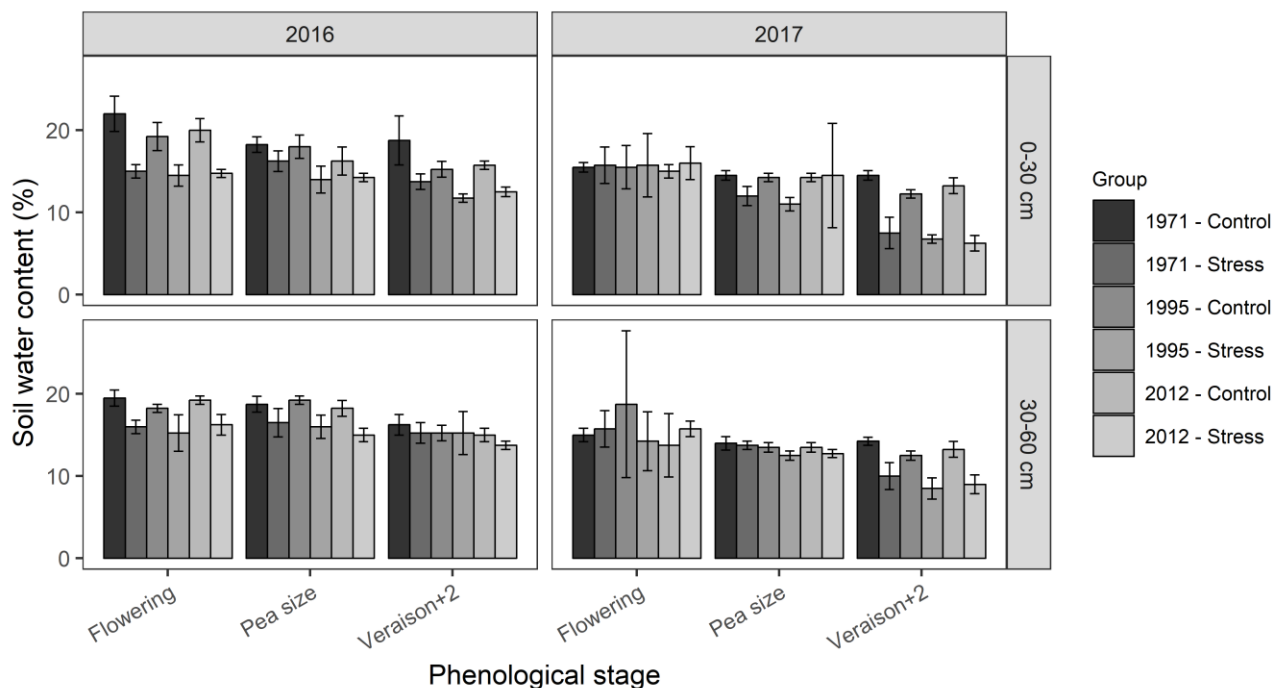


Figure 2.8. Soil water content for the water deficit experiment on cordon vines in 2016 and 2017 at flowering, pea-sized berries, and two weeks after veraison (means  $\pm$  sd) at horizons 0-30 cm and 30-60 cm.

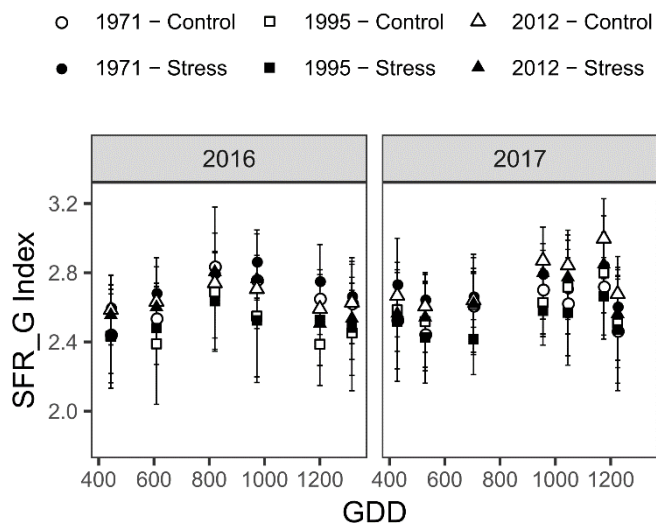


Figure 2.9. Chlorophyll fluorescence ratio under green excitation (SFR\_G) measured at regular time intervals on selected cordon vines for the water deficit experiment from flowering to harvest in 2016 and 2017 (means  $\pm$  sd).

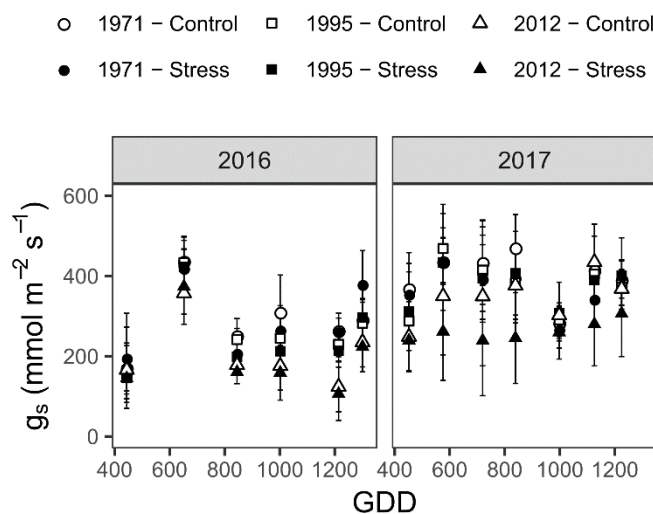


Figure 2.10. Stomatal conductance ( $g_s$ ) measured periodically on selected cordon vines for the water deficit experiment over the growing seasons of 2016 and 2017 (means  $\pm$  sd).

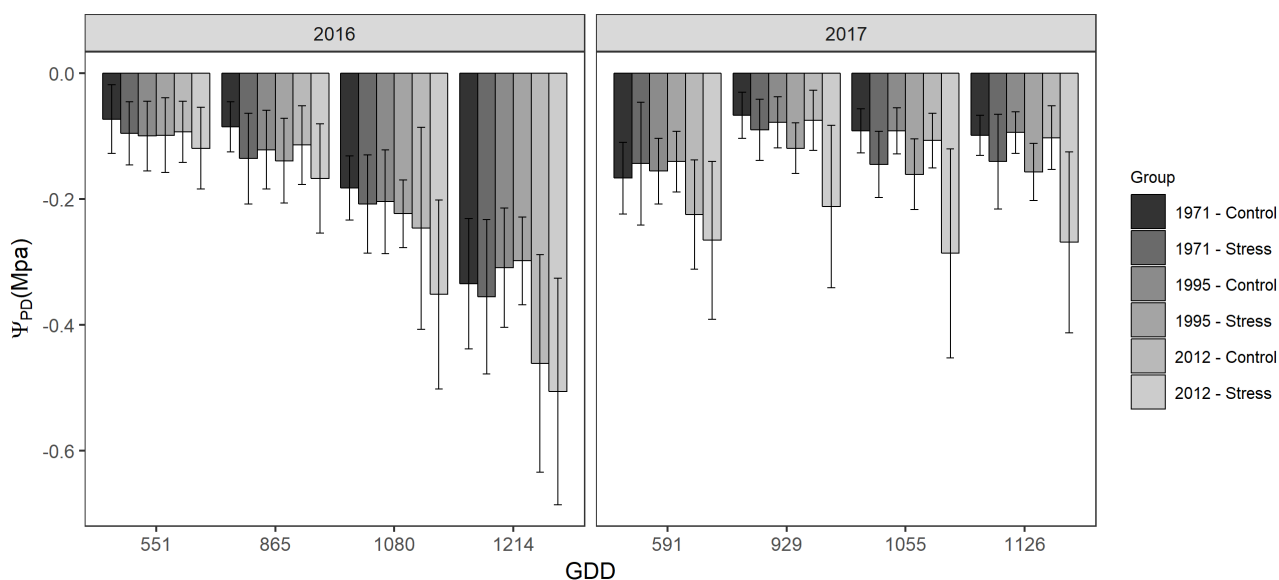


Figure 2.11. Predawn water potential ( $\Psi_{PD}$ ) measured on cordon-trained vines for the water deficit experiment over the growing seasons of 2016 and 2017 (means  $\pm$  sd).

The  $\delta^{13}C$  of cordon vines was affected by age ( $P < 0.001$ ) and year ( $P < 0.001$ ) but not by water treatment ( $P = 0.31$ ). This variable mirrored the trend observed for  $\Psi_{PD}$  in 2017. Nevertheless, neither planting year, season, nor water status were sufficiently different to warrant definitive conclusions (table 2.5).

Table 2.5. Dry mass carbon isotope discrimination ( $\delta^{13}C$ ) measured at mature berries on cordon vines for the water deficit experiment over the growing seasons of 2016 and 2017 (means  $\pm$  sd).

Planting year	Water deficit experiment (single cordon)	
	$\delta^{13}\text{C}$ – Control (%)	$\delta^{13}\text{C}$ – Stress (%)
<b>2016</b>		
<b>1971</b>	$-27.54 \pm 0.33$	$-27.97 \pm 0.41$
<b>1995</b>	$-27.68 \pm 0.13$	$-27.74 \pm 0.28$
<b>2012</b>	$-26.02 \pm 1.19$	$-25.66 \pm 1.64$
<b>2017</b>		
<b>1971</b>	$-28.23 \pm 0.69$	$-28.27 \pm 0.71$
<b>1995</b>	$-28.94 \pm 0.19$	$-28.56 \pm 0.53$
<b>2012</b>	$-28.25 \pm 0.54$	$-26.81 \pm 2.16$

## 5. Discussion

The present work is unique in that it compares field-grown vines of different planting years but identical genetic material in a single experimental vineyard. It is also, to the authors' knowledge, the only study to compare three vine groups with age gaps of around 20 years in the same environmental conditions: starting from two year old vines in their first productive vintage, nineteen year old vines representative of 'mature' vineyards, and forty-three year old vines that are reasonably considered as 'old vines' in the public opinion (Historic Vineyard Society, 2018).

Studying three age groups simultaneously can provide insight about the timing at which changes occur in vine physiology and productivity. However, a long-term trial such as this one was bound to be influenced by external factors, and vine decline was the most important. The decision was made early on not to replace dying or missing vines over time, as it would have led to increasing heterogeneity in row populations. Instead, special care was taken when selecting vines for seasonal measurements. The main purpose of the selection was to avoid vines adjacent to missing spaces and detect extreme outliers while confirming the health status of the selected vines. Despite precautions, two selected vines were tested as positive for viral infections in 2017, even though their general response was not markedly affected during the trial.

Another factor of influence was the cover crop. The Rheingau region is generally described as a cool wine region, with lower GDD accumulation and frequent precipitations over the growing season (Gladstones, 2011). Winegrowers have traditionally adapted to the constraints of this climate by preferring rootstocks that restrict scion vigor (Reynolds, 2015). Cover crops are frequently used as a complementary lever to manage vegetative growth due to their ability to

reduce the availability of water and nutrients, especially nitrogen, in upper soil layers (Agnelli et al., 2014; Celette et al., 2008). In order to prevent excessive competition with the youngest vines, the introduction of cover crop in newly established rows was postponed to the third year after planting for all vines. This treatment was applied to the entire vineyard when it was established in 1971 and for newly planted rows in 1995 and 2012. Nitrogen supplied from the soil contributes directly to berry development and has been positively correlated with yeast-assimilable nitrogen (YAN) content in must (Bell and Henschke, 2005). However, excessive nitrogen promotes vegetative growth, which can lead to dense canopies with low light interception and temperature and higher humidity. Such conditions are optimal for the development of fungal pathogens like *Botrytis cinerea* that can severely reduce grape quality and yield (Percival et al., 1994). Shaded canopies also tend to decrease the vine's water use efficiency (WUE) since inner leaves marginally contribute to photosynthesis but still transpire (Keller et al., 2016). The presence of cover crop in 2016 and 2017 reduced the soil water content for vines planted in 2012, reaching values in line with rows planted in 1995 and 1971 at both 0-30 cm and 30-60 cm horizons.

Stomatal closure is among the earliest physiological responses to water deficit, followed by a decrease in photosynthesis and transpiration (Chaves et al., 2010; Loveys et al., 2004). In the relatively warm growing season of 2016, these variables were consistently lower for double Guyot vines planted in 2012, with no apparent differences between vines planted in 1995 and 1971. In 2017, a year characterized by frequent precipitation events during the season, vines planted in 2012 had a similar response to older vine groups.

Because assimilation, stomatal conductance and transpiration were measured on leaves, these parameters were representative of WUE at leaf level and were dependent on leaf environment at the time of measurement (Tomás et al., 2014). On the other hand,  $\delta^{13}\text{C}$  is well correlated with midday stem water potential (Gaudillère et al., 2002; van Leeuwen et al., 2009) and provides an integrated assessment of the whole-vine response to water deficit when measured in berry juice at harvest (Bchir et al., 2016). Vine age did not significantly impact  $\delta^{13}\text{C}$  over 2016 and 2017, but the measurements underlined seasonal variation.

Vine productivity is often discussed in reference to vine capacity and vine balance. The term 'vine capacity' is used to describe the maximal productivity of a grapevine (Howell, 2001) while 'vine balance' is expressed as the equilibrium between vegetative growth and crop level



leading to optimal fruit quality (Gladstones, 1992). An insufficient leaf area will fail to fully ripen a crop, while excessive shoot growth affects canopy microclimate by altering light exposure, temperature, and humidity inside the canopy (Smart and Robinson, 1991). Previous studies have reported that pruning weights above  $1.0 \text{ kg m}^{-1}$  are generally detrimental to fruit quality, while optimal values varied between  $0.3$  to  $0.6 \text{ kg m}^{-1}$  depending on plant material and field conditions (Dry et al., 2004). Another common measure for vine balance is pruning weight ratio (Y/P) with an optimal range estimated between 4 and 10 (Dry et al., 2004). Ratios under 4 are often associated with excessive vigor, but they can be still be considered optimal in cool climates to compensate for lower ambient light and temperature (Kliewer and Dokoozlian, 2005).

In the present trial, even though the youngest vines had access to higher amounts of nitrogen in the first years after planting, their low bud fruitfulness had a larger impact on their canopy architecture, as shown by their high PG and low PIL, PIC, and LLN (table 3). The reduced bud count of these vines in 2014 and 2015 also translated into low pruning weight and yield. The capacity of vines planted in 2012 increased over time to become comparable to other groups in 2016 (figure 2.7), with their Y/P ratio increasing from 1.0 to 3.1 in the same period. Overall, the productivity of vines planted in 1995 and 1971 was more difficult to assess due to missing and symptomatic vines. In 2015, 27% of the vines originally planted in 1971 had already ceased production, compared to only 2% for vines planted in 1995 (table 4). In 2016, the oldest vines had the lowest recorded yield among age groups, but once adjusted to account for missing vines their yield was similar to vines planted in 2012 and 1995.

Trunk diseases such as esca, caused by complexes of xylem-dwelling fungi (Bertsch et al., 2013; Mugnai et al., 1999), can dramatically hinder canopy and yield formation. Esca specifically is becoming a widespread threat for vineyard longevity (Larignon et al., 2009). The disease precipitates vine decline and leads to apoplexy, with leaf fall and berry shriveling on shoots, and ultimately to vine death (Bertsch et al., 2013). Trunk diseases are chronic issues in old vineyards. Their cumulated incidence often correlates linearly with the age the vineyard (Surico et al., 2006), and they appeared as an inevitable constraint in this trail. The decision not to replace dying or missing vines by co-plantation meant that the remaining plants would be facing less competition and display higher vegetative and reproductive productivity, as noted in experiments that varied planting density (Bernizzoni et al., 2009). Some caution should be

applied when reviewing this data since it is possible that vines planted in 1971 could have been less productive if all vines were present.

Nevertheless, most results obtained for the main trial on double Guyot vines were in agreement with previous research that described young vines as being more sensitive to water deficit (Zufferey and Maigre, 2007), but no differences among technological maturity parameters were noted other than the high  $\alpha$ -amino acid content of the youngest vines in the absence of cover crop.

The water deficit experiment on cordon vines gave results similar to the double Guyot vines. The success of this secondary trial was based on the duration and intensity of precipitation events, which varied across growing seasons. The trial was considered in 2016 after sowing the cover crop, but its implementation in double Guyot rows was not possible due to the relatively small size of the vineyard and the inevitable interference that the plastic sheets would have with selected vines. It was therefore decided to use the adjacent cordon rows that were managed in the same way as the double Guyot rows with the addition of the water treatment (control or stress) used as a fixed LMM effect. The plastic sheets that were deployed during rain events were able to create a difference in soil moisture between stressed and control treatments at 0-30 and 30-60 cm, and this was reasonably visible in  $g_s$  and  $\Psi_{PD}$  measurements. A distinction between control and stressed vines was gradually settling over the 2016 growing season, but the high temperatures and absence of rain towards harvest reduced differences based on water treatment, and the three vine age groups followed the same patterns as double Guyot vines. In 2017, it was only possible to obtain differences between stress and control treatments for the youngest vines. This was relatively supported by  $\delta^{13}C$  measurements, with young stressed vines reaching -26.81% on average. In order to confirm the 2016 results on water deficit, it may have been desirable to increase the coverage area and/or the number of measured vines per treatment to reduce the impact of water runoff along the vineyard slope, which was responsible for increased differences between blocks. Yet, the fact that the youngest vines in both control and stress treatments experienced water stress in 2016, while only the youngest vines under the stress treatment differed from other groups in 2017, suggests that the smaller exploration of their root system is indeed the driving factor behind the youngest vines' lower tolerance to water deficit (Zufferey and Maigre, 2007).

The study underlines the special considerations needed during the establishment of young vines, as well as the ability possibility to maintain vineyards with a long-term perspective if diseases are prevented. This would create compounded benefits terms of adaptability to water deficit and yield conservation. Vine size and capacity were determinant factors in the youngest vines' productivity (Grigg et al., 2017), which gradually increased from the first to the fourth years after planting.

Future research will focus on the analysis of secondary metabolites in juice and berry skins, as well as chemical and sensory analysis of wines obtained from the three grapevine groups. It will also be interesting to study the evolution of carbohydrate reserves and root density as vines become older to learn more about the interaction of these factors with the vine's response to water deficit and its productivity.

## **6. Conclusion**

In the present work we examined the physiological behavior, productivity, and berry technological maturity of vines planted in the same vineyard in 1971, 1995, and 2012. Photosynthesis, stomatal conductance, and transpiration were generally lower for the youngest vines, even though their response was dependent on seasonal factors. One can speculate that given our results, a possible explanation is that the shallower root system of young vines and its proximity to soil surface, may prevent access to deeper water reserves in periods of drought.

The youngest vines also displayed smaller canopy size and lower productivity than older groups at the beginning of the study. However, they were able to reach similar levels of bud fruitfulness, yield, and pruning weight in the fourth years after planting. The behavior of vines planted in 1971 and 1995 did not differ for most vegetative and generative parameters including water status, canopy architecture, cluster parameters, and technological maturity. The most distinguishing factor between the two older groups was the higher incidence of trunk diseases for vines planted in 1971, which resulted in a non-negligible decrease in recorded yield despite the fact that individual vine production was similar among the two groups.

The results of this study indicate that provided they are managed in the same way, the physiological behavior, productivity, and berry composition of individual vines around 20 and 40 years old and planted in conditions of cool climate and deep soil is comparable, with yield decline being mostly due to trunk diseases.

## **7. Acknowledgments**

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# Chapter 3. Impact of grapevine age on berry structure and secondary metabolites

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Author contributions: the author designed and conducted the research experiments, analyzed the data, drafted and constructed the manuscript. Free and potential monoterpene and norisoprenoid analysis was performed by Stefanie Fritsch (Department of Microbiology and Biochemistry, HGU).

## 1. Introduction

There seems to be a general consensus about the ability of old grapevines to produce wines of superior character (Goode, 2005; Heymann and Noble, 1987; Koblet and Perret, 1982; Zufferey and Maigre, 2008), possibly due to larger wood reserves (Howell, 2001) or to an improved equilibrium between vegetative growth and yield (Zufferey and Maigre, 2008).

Previous research on grapevine age has examined berry composition of young and old vines, however most studies only focused on primary metabolites and technological maturity indicators (Grigg et al., 2017; Reynolds et al., 2008; Zufferey and Maigre, 2008). Some studies reported that old grapevine berries had a higher titratable acidity and formol index than young grapevines, while sugar content was not markedly different among grapevine age groups (Zufferey and Maigre, 2007). However, the observed trends appeared to be strongly dependent on weather conditions during the growing season, as well as vineyard location (Grigg et al., 2017; Reynolds et al., 2008).

Climate is usually a better predictor of overall grape quality than soil characteristics (van Leeuwen et al., 2004). However, the concentration of soil organic matter can still affect berry parameters, whether directly by increasing nitrogen supply to the berries, or indirectly by favoring vegetative growth (Habran et al., 2016). Canopy density is known to affect berry quality by having a direct impact on cluster light exposure, temperature, and humidity (Smart and Robinson, 1991). Vigorous grapevines with dense canopies show a tendency to have higher humidity and shade and lower temperatures in the cluster zone. This not only promotes fungal infections (Austin et al., 2009) but also affects berry composition and quality (Dry et al., 2004).

The compounds that are affected by canopy density include aromatic compounds such as monoterpenes, norisoprenoids as well as skin flavonols, especially for white cultivars. Monoterpenes have been associated with the aroma profile of Muscat varieties and non-Muscat aromatic varieties such as Gewürztraminer and Riesling (Mateo and Jimenez 2000). They are mostly present in berries as glycosidic conjugates and are gradually released during wine fermentation and aging (Black 2015). Thin canopies have also been shown to increase the expression of the terpene synthase genes and as a result the berry monoterpene content due to the higher sun exposure of clusters (Friedel et al., 2016; Skinkis et al., 2010).

Norisoprenoids are formed from the degradation of carotenoids in berry skin after they undergo chemical, photochemical, and/or oxidative reactions (Robinson et al., 2014). As such, these

reactions are highly impacted by temperature and light. Marais et al. (1999) have demonstrated early on that artificially shaded canopies, which received less light and had lower temperatures during the season, produced lower concentrations of norisoprenoids compared to control grapevines.

Flavonols are phenolic compounds located in berry skin that act as protectants against oxidation by UV radiation (Haselgrove et al., 2000). Light exposure positively impacts grape flavonols, especially quercetin glucosides (Cortell and Kennedy, 2006; Friedel et al., 2015). The effects of temperature and light are inherently difficult to separate in field experiments. Nevertheless, studies that have focused on vineyard orientation (Friedel et al., 2016) or have artificially cooled sun-exposed clusters reported that high temperatures inhibits the production of phenolic compounds such as anthocyanins without having an impact on flavonols (Spayd et al., 2002).

In order to examine the potential effects of grapevine age berry structure and composition, a vineyard planted in Geisenheim, Germany, with alternating rows of Riesling grapevines on identical rootstock that were planted in 1971, 1995 and 2012, was studied from 2014 to 2017 (Bou Nader et al., 2019). Riesling is usually described as a non-Muscat white aromatic cultivar, with a wine profile heavily influenced by monoterpenes and norisoprenoids (Günata et al., 1985; Lott et al., 2010). The two classes of compounds contribute in large part to the cultivar's typical floral and fruity aromas, in addition to its well-recognized 'kerosene' aroma due to the presence of free 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) (Mateo and Jiménez, 2000).

The aim of this work was to compare the three grapevine age groups with regards to their berry composition, with a focus on amino acids, free and potential monoterpenes and norisoprenoids, and flavonols to examine whether the nature and concentration of these compounds were affected by grapevine age.

## 2. Materials and methods

### 2.1. Experimental design and plant material

A single experimental plot located in Geisenheim, Germany, with alternating rows of *Vitis vinifera* (L.) cv. Riesling grapevines (clone Gm 239-17) on 5C Teleki rootstocks and trained in double Guyot was studied. While the vineyard was planted in 1971, some rows were uprooted in 1995 and 2012 and replanted with the same plant material in order to obtain four blocks with each grapevine age group. The vineyard is described in more detail in Bou Nader et al. (2019).

It was studied from 2014, which was the first year of harvest for grapevines planted in 2012, until 2017.

Due to the general vigor of the site, a cover crop of grass and clover was sown in alternating rows (Bou Nader et al., 2019). When the second group of grapevines was planted in 1995, their rows were ploughed in the first two years to prevent excessive competition with the cover crop during the early developmental stages of the grapevines (Williams et al., 1990). The same treatment was applied from 2012 to 2014 in the rows of young grapevines. Afterwards, these rows were sown with the same cover crop combination that already existed in 1995 and 1971 rows. Soil nitrate and water content from depths of 0 to 30 cm and from 30 to 60 cm were significantly higher in 2014 and 2015 for rows planted in 2012, but after this period the three age groups had similar soil nitrate and water contents (Bou Nader et al., 2019).

## 2.2. Berry sampling

Phenology was followed with the modified E-L system developed by Coombe (1995). No significant phenological shifts were found between grapevine age groups during the study. Veraison date was determined by the onset of berry softening and the beginning of soluble solid accumulation. Before harvest date, which was decided according to technological maturity and berry health status, randomized samples of 40 berries were collected for each treatment × replicate combination for a total of 12 samples ( $n = 4$ ). The berries were collected by cutting their pedicel with scissors and placed in plastic boxes that were saturated with CO<sub>2</sub>. This was done to limit oxidation during storage. The samples were then placed in a freezer at -20 °C.

Berries were taken out of the freezer one by one and peeled while still frozen. The pedicel, skin, seeds, and pulp were separated and weighed. The skins and seeds were returned to the freezer while the pulp was pressed in a plastic bag to extract the juice. The juice was centrifuged for 3 min at 4500 g at room temperature. Aliquots for organic acids analysis were diluted with ultrapure water from a quarter to a tenth depending on the stage of maturity before being frozen in order to prevent tartaric acid precipitation during storage.

The skins were freeze-dried for 5 days in an Alpha1-4 freeze-dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) and were weighed to determine their dry weight. The samples were then ground with the MM200 mixer mill (Retsch GmbH, Haan, Germany) for 30 seconds at a vibrational frequency of 25 s<sup>-1</sup> to obtain a fine powder.



Berry samplings for the analysis of potentially volatile monoterpene and were collected on the same dates as other berry samplings. In this case, 200 berries per replicate were collected with their pedicel and placed in liquid nitrogen under CO<sub>2</sub> atmosphere. The samples were then stored at -20 °C in sealed bags including CO<sub>2</sub> until analysis.

### 2.3. Organic and amino acids

Tartaric and malic acid concentrations were quantified with a TRAACS 800 continuous flux analyzer (Bran & Luebbe, Plaisir, France) and the AACE AutoAnalyzer software (Bran & Luebbe, Plaisir, France). It allowed measuring malic acid titration using the enzymatic spectrophotometric method at 340 nm after the reduction of NAD<sup>+</sup> to NADH by malate dehydrogenase (Merck, Darmstadt, Germany). Tartaric acid content was determined by colorimetry at 520 nm after reacting with ammonium vanadate in the presence of 2 g L<sup>-1</sup> NaOH to form the metapervanadyl tartrate complex (Torres et al., 2017).

Free amino acids in must were derived according to Hilbert et al. (2003) modified by Habran et al. (2016). The derivatization reagent was 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AccQ-Tag, supplied by Waters, Milford, MA USA). Samples were then analyzed on an UltiMate 3000 ultra-high performance liquid chromatography (UHPLC) system with a FLD-3000 fluorescence detector (Thermo Electron SAS, Waltham, MA USA). The separation occurred at 37 °C in an AccQ-Tag Ultra column of dimensions 2.1 × 100 nm and particle size 1.7 µm (Waters, Milford, MA USA). Elution rate was maintained at 0.5 mL min<sup>-1</sup> with the following elution gradient (v/v): 0 min 93% A 4.2% B 2.8% C, 6.5 min 95% A 8.4% B 5.6% C, 9 min 78% A 13.2% B 8.8% C, 11 min 71% A 17.4% B 11.6% C linear for 2 min, 14 min 60% B 40% C linear for 1 min, 15 min 93% A 4.2% B 2.8% C (eluent A, sodium acetate buffer, 140 mM at pH 5.7; eluent B, acetonitrile 100%; eluent C, ultrapure water). An ultrapure water control was added before each set of 18 samples ensure retention time and baseline consistency. Standard samples containing 20 amino-acids (Alanine, Arginine, Aspartic acid, Asparagine, Cysteine, GABA, Glycine, Glutamic acid, Glutamine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tyrosine, Valine) were used as external standards to calibrate amino-acid quantification (Sigma, St Louis, MO USA). Chromatograms were recorded for excitation at 250 nm and emission at 395 nm. The software Chromeleon 7.1 (Thermo Electron SAS, Waltham, MA USA) was used to calculate peak area.

Must sugar content was measured by enzymatic titration using a Glucose/Fructose enzymatic kit (BioSenTec, Toulouse, France). Samples were diluted with a Precision 2000 microplate preparation robot (Biotek Instruments Inc., Winooski, VT, USA) and analyzed with an ELx8000UC microplate reader (Biotek Instruments Inc., Winooski, VT, USA) by tracking the change of absorbance at 340 nm.

#### 2.4. Potentially volatile monoterpenes and C<sub>13</sub>-norisoprenoids

Potentially volatile monoterpenes and C<sub>13</sub>-norisoprenoids were analyzed by a modified solid-phase extraction (SPE) for sample preparation (Günata et al., 1985) as described in Schüttler (2012) and (Schüttler et al., 2015), followed by gas chromatography coupled with mass spectrometry (GC-MS). The method was adapted by replacing the enzymatic release of aglycones by acid hydrolysis using the procedure described by (Kotseridis et al., 1999).

Frozen berry samples were allowed to thaw overnight at 4 °C and were blended with a household blender for 30 s. The resulting must was distributed in 50 mL falcon tubes (VWR, Lutterworth, UK) and centrifuged three times at 7200 g and 4 °C in a 5430 R centrifuge (Eppendorf, Hamburg, Germany) and the clear must was analyzed.

Strata SDB-L cartridges containing 500 mg of styrene-divenylbenzene polymer (Phenomenex, Torrance, CA USA) were preconditioned with 5 mL of pentane/dichloromethane (2/1 v/v), followed by 10 mL of methanol, 10 mL of methanol/H<sub>2</sub>O (1/1 v/v) and 10 mL of H<sub>2</sub>O. Must samples of 50 mL were diluted 1:1 with ultrapure water and were spiked with octan-3-ol and 2,6-dimethylhept-5-en-2-ol (DMH) as internal standards ( $c_{(\text{octan-3-ol})} = 50 \mu\text{g L}^{-1}$ ;  $c_{(\text{DMH})} = 25 \mu\text{g L}^{-1}$ ) and were loaded on the SPE cartridges. Cartridges were washed again with 10 mL of water and dried for 1h with nitrogen under vacuum (see above). Dried samples were eluted with 10 mL of pentane/dichloromethane (2/1 v/v). The eluates were dried with anhydrous sodium sulfate and concentrated to a final volume of 50  $\mu\text{L}$  under gentle nitrogen flow, and 2  $\mu\text{L}$  were used to analyze free monoterpenes and norisoprenoids by gas chromatography.

For potentially volatile monoterpenes and norisoprenoids the SPE cartridges were eluted with 10 mL of ethyl acetate. The fractions were evaporated to dryness under vacuum with a rotary evaporator (Büchi, Flawil, Switzerland) at water bath temperature 40 °C and  $p = 138 \text{ kPa}$ . The residues were resolved in 5 mL of 0.2 M citric acid ( $\text{pH} = 2.5$ ), transferred to 10 mL head space vials, sealed, and heated in a laboratory oven at 100 °C for 60 min. After cooling down the samples on ice, the internal standard mix was added based on the initial sample volume. Three

successive liquid-liquid extractions were conducted with 2 mL, 1 mL, and 1 mL pentane/dichloromethane (2/1 v/v), combined with anhydrous sodium sulfate and concentrated under nitrogen flow to a final volume of 50  $\mu$ L. Gas chromatography coupled to mass spectrometry (GC-MS) was performed with a Thermo Fisher Trace GC Ultra gas chromatograph equipped with a PTV injector and coupled to a Thermo Fisher ITQ 900 Ion Trap MS mass spectrometric detector (Thermo Fisher Scientific, Waltham, MA USA). Samples (2  $\mu$ l) were injected in splitless mode (splitless time 90 s) at 200 °C. An Agilent DB-Wax capillary column of dimensions 30 m  $\times$  0.25 mm ID  $\times$  0.5  $\mu$ m film density (Agilent Technologies, Santa Clara, CA, USA) was used for the separation. The following temperature gradient program was used: 40 °C for 1 min, then temperature increased until 60 °C at 10 °C min<sup>-1</sup>, then from 60 °C to 200 °C at 3 °C min<sup>-1</sup>, and from 200 °C to 230 °C at 10 °C min<sup>-1</sup>. The temperature was finally maintained at 230 °C for 10 min. Helium was used as a carrier gas with a flow rate of 1.4 mL min<sup>-1</sup> and average velocity of 27 cm s<sup>-1</sup>. The interface and MS source temperatures were set to 240 °C and 200 °C respectively. Mass spectrometric data was acquired in electron impact mode (EI) with an ionization energy of 70 eV. Quantification was made with the Selected Ion Monitoring (SIM) mode.

## 2.5. Flavonol content in berry skin

The flavonol extraction from skins was done according to Hilbert et al. (2015) with 20 mg of skin powder per sample and 500  $\mu$ l of acidified methanol at 0.1% HCl (v/v). The extracts were centrifuged for 1 min at 4500 g and were filtered directly in UHPLC vials with 0.22  $\mu$ m syringe filters (GHP Acrodisc, Pall Gelman Corporation, Ann Arbor, MI, USA). The analysis was performed as described by Torres et al. (2017) with an UltiMate 3000 UHPLC system (Thermo Electron SAS, Waltham, MA USA) equipped with a DAD-3000 diode array detector at a wavelength of 360 nm (Thermo Electron SAS, Waltham, MA USA). The separation was made with a Synchronis C18 column of dimensions 2.1  $\times$  100 mm and particle size 1.7  $\mu$ m (Thermo Fischer Scientific, Waltham, MA USA) at an elution debit of 0.368 mL min<sup>-1</sup> and the following binary gradient (v/v): 0 min 92.2% A 7.8% B, 9.6 min 73% A 27% B, 14.1 min 70% A 30% B, 14.8 min 92.2% A 7.8% B (eluent A, ultrapure water and formic acid, 90/10 v/v; eluent B, acetonitrile 100%). The retention times and UV-visible spectrometric data of the samples were compared with those of pure standards for the identification and assignment of peaks. Peak area was calculated with the Chromeleon 7.1 software (Thermo Electron SAS, Waltham, MA, USA).

Individual flavonol concentrations were translated in milligrams per gram ( $\text{mg g}^{-1}$ ) of dry skin with quercetin-3-*O*-glucoside (Extrasynthèse, Genay, France) as reference.

## 2.6. Statistical analysis

The statistical analysis was performed with RStudio (version 1.1.453; Rstudio, 2018) and R (version 3.5.0; R Core Team, 2018). Linear mixed-effect models (LMM) were used to evaluate the effect of grapevine age on the parameters of interest. Grapevine age and year were converted to categorical variables and used as fixed effects in all models and field replicates were added as random effects. Model parameters were estimated by restricted maximum likelihood (REML) with the packages lme4 (Bates et al., 2015) and pkbtest (Halekoh and Højsgaard, 2014). The importance of fixed effects was assessed by Kenward-Roger approximations at a significance level of  $P = 0.05$  (Schaalje et al., 2002). Graphics were drawn with the ggplot2 package (version 2.2.1; Wickham, 2009).

Principal Component Analysis (PCA) was used to examine the various classes of chemical compounds measured in berry skin and juice. For this analysis, the current dataset was combined with data about technological maturity parameters (average berry weight, must titratable acidity, pH, total soluble solids, and  $\alpha$ -amino acids) described in Bou Nader et al. (2019) (see Chapter 2). The scaled berry composition data was also assessed by *k*-means clustering with 50 iterations to reach convergence (Hartigan and Wong, 1979). Four clusters were used based on agreement between the gap statistic and elbow methods.

PCA and *k*-means clustering were executed with the packages FactoMineR (version 1.41; Lê et al., 2008) and factoextra (version 1.0.5; Kassambara and Mundt, 2017). The point labels represent the growing season followed by the grapevine age group ('O' for 'old' grapevines planted in 1971; 'M' for 'mature' grapevines planted in 1995; 'Y' for 'young' grapevines planted in 2012). There are four replicates per growing season per age group. They are represented by the numbers 1 to 4 next to the letter.

## 3. Results and discussion

### 3.1. Berry composition

Due to the absence of cover crop in rows planted in 2012 during the growing seasons 2014 and 2015 (Bou Nader et al., 2019), the concentration of various amino acids in must was higher for the youngest grapevine age group, especially in 2015 (table 3.1). Notably, the amino acids most present in the berries from 2012 grapevines in 2015 were  $\alpha$ -ketoglutarate derivatives glutamine,

arginine, and proline, as well as phenylalanine. Glucose, fructose, malic and tartaric acid concentrations were similar for the three age groups but were susceptible to yearly changes. Free and potentially volatile monoterpenes and norisoprenoids also showed a similar trend (table 3.2), with a potential TDN content of  $8.8 \mu\text{g L}^{-1}$  for grapevines planted in 2012 compared to 4.6 and  $3.3 \mu\text{g L}^{-1}$  for grapevines planted in 1995 and 1971, respectively. The concentrations of the studied flavonols were dependent on the season, with higher values in 2017 for all age groups. This may be due to the partial defoliation that took place at fruitset as already reported (Mijowska et al., 2016; Yu et al., 2016).

The PCA showed a clear separation between data points of 2015 and those of 2016 and 2017 (figure 3.1). Young grapevines were highly represented by  $\alpha$ -amino acid content and individual amino acids in 2015, especially phenylalanine (figure 3.2; appendix 3.1). In 2016 only one replicate of grapevines planted in 2012 had higher amino acid concentration. Interestingly phenylalanine content in juice was inversely proportional with quercetin-3-galactoside and quercetin-3-glucuronide, but this correlation is not sufficient to draw a direct connection to the phenylpropanoid pathway, in which phenylalanine is used as a substrate to produce flavonoids (Flamini et al., 2013; Yin et al., 2012). As mentioned for the  $\alpha$ -amino acid content, soil ploughing and the absence of cover crop in 2015 for grapevines planted in 2012 were most likely responsible for the high increase in their berry amino acid content (Giese et al., 2015). A higher nitrogen content in berries is also known to favor the production of monoterpenes and some norisoprenoids (Linsenmeier and Löhnertz, 2016).

Young grapevines tend to be more sensitive to water stress (Bou Nader et al., 2019; Zufferey and Maigre, 2007). Seasonal water deficit may also affect monoterpene and norisoprenoid concentration (Deluc et al., 2009). In an experiment on partial rootzone drying, Bindon et al. (2007) showed that moderate water stress decreased berry weight at harvest and increased the production of norisoprenoids. The influence of water stress on berry flavonols depends on its intensity and its timing during the growing season, with post-veraion water deficits having only limited impact on flavonol content (Torres et al., 2017). In the present trial, grapevines planted in 2012 experienced a weak water stress at the end of the 2015 and 2016 growing seasons (Bou Nader et al., 2019). Therefore it is difficult to clearly differentiate between the effects of nitrogen and water status on the berry composition of the youngest grapevines.

Table 3.1. Maturity parameters and amino acid concentrations for the three grapevine age groups (n = 4) over the growing seasons 2015 to 2017.

Planting year	2015			2016			2017		
	1971	1995	2012	1971	1995	2012	1971	1995	2012
<b>Maturity parameters</b>									
Fructose (g L <sup>-1</sup> )	90.23 ± 7.13	88.8 ± 3	92.5 ± 4.6	87.7 ± 4	88.4 ± 4.6	83.3 ± 2.7	88.4 ± 3.7	89.9 ± 1.3	92.9 ± 3.7
Glucose (g L <sup>-1</sup> )	68.71 ± 10.05	65.9 ± 4.5	69.6 ± 7.4	64.4 ± 5.6	65.4 ± 6.6	56.6 ± 4.6	61.4 ± 7.1	65.3 ± 2.1	66 ± 6.2
Malic acid (g L <sup>-1</sup> )	4.51 ± 0.58	4.12 ± 0.25	4.2 ± 0.56	3.18 ± 0.2	3.0 ± 0.14	2.93 ± 0.23	4.01 ± 0.53	4.13 ± 0.21	3.75 ± 0.25
Tartaric acid (g L <sup>-1</sup> )	5.18 ± 0.68	4.93 ± 0.33	4.72 ± 0.42	3.96 ± 0.28	4.2 ± 0.31	4.5 ± 0.35	4.03 ± 0.56	4.84 ± 0.14	4.86 ± 0.14
Skin dry weight (mg)	90.3 ± 12.3	82.5 ± 5.1	77.3 ± 3.3	60.3 ± 4.7	58.8 ± 5.9	48.8 ± 3.3	65.5 ± 2.9	66.3 ± 3	59.5 ± 4.2
<b>Amino acids (mg L<sup>-1</sup>)</b>									
Aspartate									
aspartic acid	0.148 ± 0.037	0.143 ± 0.038	0.172 ± 0.037	0.349 ± 0.095	0.322 ± 0.038	0.34 ± 0.029	0.047 ± 0.015	0.037 ± 0.011	0.04 ± 0.019
asparagine	0.029 ± 0.018	0.028 ± 0.005	0.056 ± 0.013	0.025 ± 0.005	0.023 ± 0.005	0.032 ± 0.01	0.023 ± 0.009	0.02 ± 0.005	0.027 ± 0.006
methionine	0.006 ± 0.003	0.003 ± 0.001	0.019 ± 0.007	0.012 ± 0.006	0.011 ± 0.002	0.02 ± 0.016	0.019 ± 0.024	0.008 ± 0.002	0.009 ± 0.001
isoleucine	0.042 ± 0.015	0.038 ± 0.009	0.07 ± 0.008	0.051 ± 0.015	0.044 ± 0.004	0.067 ± 0.031	0.035 ± 0.024	0.043 ± 0.01	0.066 ± 0
3-phosphoglycerate									
serine	0.311 ± 0.104	0.298 ± 0.024	0.518 ± 0.101	0.374 ± 0.101	0.351 ± 0.062	0.366 ± 0.051	0.275 ± 0.052	0.25 ± 0.038	0.285 ± 0.086
glycine	0.002 ± 0.001	0.001 ± 0.001	0.005 ± 0.006	0.01 ± 0.006	0.01 ± 0.01	0.013 ± 0.01	0.012 ± 0.003	0.011 ± 0.002	0.009 ± 0.004
Pyruvate									
alanine	0.11 ± 0.062	0.098 ± 0.014	0.193 ± 0.043	0.218 ± 0.081	0.139 ± 0.016	0.181 ± 0.034	0.19 ± 0.054	0.176 ± 0.061	0.185 ± 0.093
valine	0.054 ± 0.018	0.05 ± 0.009	0.107 ± 0.014	0.084 ± 0.027	0.069 ± 0.006	0.095 ± 0.036	0.062 ± 0.012	0.058 ± 0.015	0.07 ± 0.029
leucine	0.047 ± 0.019	0.039 ± 0.006	0.082 ± 0.008	0.056 ± 0.018	0.045 ± 0.005	0.069 ± 0.038	0.074 ± 0.063	0.03 ± 0.023	0.018 ± 0.019
Shikimate									
tyrosine	0.02 ± 0.007	0.02 ± 0.003	0.053 ± 0.016	0.052 ± 0.017	0.046 ± 0.017	0.057 ± 0.011	0.037 ± 0.018	0.03 ± 0.007	0.043 ± 0
phenylalanine	0.194 ± 0.051	0.186 ± 0.041	0.298 ± 0.051	0.106 ± 0.005	0.116 ± 0.017	0.15 ± 0.09	0.106 ± 0.024	0.128 ± 0.034	0.13 ± 0.01
α-Ketoglutarate									
glutamic acid	0.36 ± 0.099	0.348 ± 0.036	0.492 ± 0.077	0.484 ± 0.113	0.444 ± 0.052	0.453 ± 0.057	0.014 ± 0.009	0.012 ± 0.004	0.016 ± 0.012
glutamine	0.65 ± 0.599	0.67 ± 0.268	1.792 ± 0.339	0.837 ± 0.314	0.639 ± 0.118	1.151 ± 0.549	0.328 ± 0.158	0.257 ± 0.096	0.315 ± 0.156
arginine	1.474 ± 1.341	1.047 ± 0.24	2.773 ± 0.38	1.504 ± 0.654	1.057 ± 0.391	2.036 ± 0.87	1.135 ± 0.606	1.002 ± 0.412	1.179 ± 0.625
proline	0.959 ± 0.249	0.859 ± 0.126	1.561 ± 0.338	1.347 ± 0.438	1.068 ± 0.151	1.093 ± 0.154	1.236 ± 0.232	0.834 ± 0.51	0.117 ± 0.017
histidine	0.126 ± 0.051	0.119 ± 0.013	0.218 ± 0.067	0.153 ± 0.042	0.14 ± 0.032	0.235 ± 0.072	0.123 ± 0.001	n.d.	0.013 ± 0.013
GABA	0.051 ± 0.011	0.044 ± 0.016	0.046 ± 0.021	0.079 ± 0.018	0.061 ± 0.015	0.066 ± 0.009	0.297 ± 0.071	0.263 ± 0.05	0.279 ± 0.10
Pyruvate									
alanine	0.11 ± 0.062	0.098 ± 0.014	0.193 ± 0.043	0.218 ± 0.081	0.139 ± 0.016	0.181 ± 0.034	0.19 ± 0.054	0.176 ± 0.061	0.185 ± 0.093
valine	0.054 ± 0.018	0.05 ± 0.009	0.107 ± 0.014	0.084 ± 0.027	0.069 ± 0.006	0.095 ± 0.036	0.062 ± 0.012	0.058 ± 0.015	0.07 ± 0.029

Table 3.2. Free and potentially volatile monoterpenes and norisoprenoids, and flavonol glycosides for the three grapevine age groups ( $n = 4$ ) from 2015 to 2017.

Planting date	2015			2016			2017		
	1971	1995	2012	1971	1995	2012	1971	1995	2012
<b>Free monoterpenes and norisoprenoids</b>									
linalool	5.8 ± 0.6	6.1 ± 0.7	8.9 ± 1.5	12.9 ± 1.3	14.1 ± 2.5	15.6 ± 3.5	11.9 ± 1.4	11.3 ± 1.6	11.8 ± 2.1
$\alpha$ -terpineol	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
nerol oxide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<i>cis</i> -linalool oxide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<i>trans</i> -linalool oxide	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
vitispirane	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
TDN	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<b>Potentially volatile monoterpenes and norisoprenoids</b>									
linalool	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
$\alpha$ -terpineol	244.0 ± 11.9	242.3 ± 15.8	330.5 ± 25.6	407.1 ± 7.3	400.6 ± 11.3	586 ± 67.7	372.8 ± 26.4	389.5 ± 31.8	516.8 ± 71.7
nerol oxide	43.9 ± 3.8	49.6 ± 3.1	69 ± 6.4	48.1 ± 3.4	59.3 ± 5.6	69.1 ± 4.4	47.5 ± 2.9	53.8 ± 3.3	61.2 ± 6.1
<i>cis</i> -linalool oxide	24.2 ± 1.7	26.4 ± 1.8	34.1 ± 1.8	24.5 ± 1.1	29.3 ± 1.4	30.5 ± 1.0	23.6 ± 1.1	24.7 ± 1.4	24.9 ± 1.8
<i>trans</i> -linalool oxide	18.7 ± 0.9	21.0 ± 1.4	25.1 ± 1.2	19.4 ± 1.1	23.2 ± 1.6	23.9 ± 1.0	19.2 ± 0.9	20.2 ± 0.8	20.8 ± 1.2
vitispirane	34.6 ± 7.8	39.6 ± 3.8	83 ± 12.4	49.4 ± 6.6	63.9 ± 11.9	106.5 ± 18.2	52.2 ± 4.7	74.4 ± 14	121.5 ± 44.7
TDN	3.3 ± 0.6	4.6 ± 0.5	8.8 ± 1.2	6.8 ± 1.6	12 ± 2.9	17.9 ± 4.2	6.3 ± 0.8	9.3 ± 1.8	13.9 ± 3.2
<b>Flavonol glycosides</b>									
quercetin-3-galactoside	0.037 ± 0.01	0.045 ± 0.013	0.029 ± 0.005	0.155 ± 0.036	0.118 ± 0.001	0.093 ± 0.022	0.265 ± 0.093	0.226 ± 0.016	0.278 ± 0.038
quercetin-3-glucuronide	0.177 ± 0.045	0.214 ± 0.083	0.189 ± 0.05	0.328 ± 0.04	0.358 ± 0.032	0.301 ± 0.063	0.499 ± 0.172	0.384 ± 0.176	0.58 ± 0.071
quercetin-3-glucoside	0.238 ± 0.063	0.328 ± 0.095	0.32 ± 0.046	0.311 ± 0.026	0.395 ± 0.067	0.468 ± 0.072	0.333 ± 0.111	0.336 ± 0.046	0.458 ± 0.072

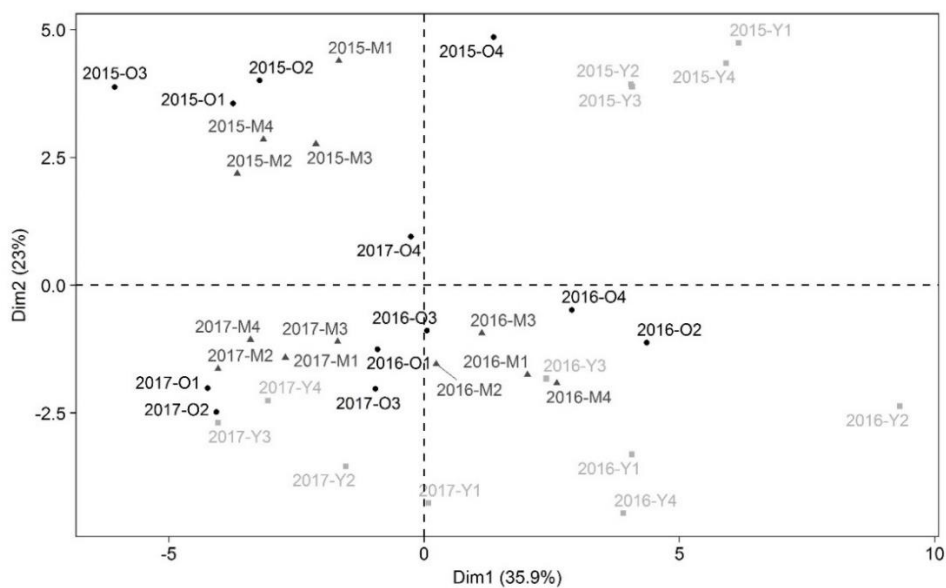


Figure 3.1. PCA score plot of technological parameters and secondary metabolites (amino acids, quercetin glucosides, and free and potentially volatile terpenes and norisoprenoids) from 2015-2017. The point labels represent the growing season followed by the grapevine age group ('O' for 'old' grapevines planted in 1971; 'M' for 'mature' grapevines planted in 1995; 'Y' for 'young' grapevines planted in 2012). There are four replicates per growing season per age group, represented by the numbers 1 to 4 next to the letter.

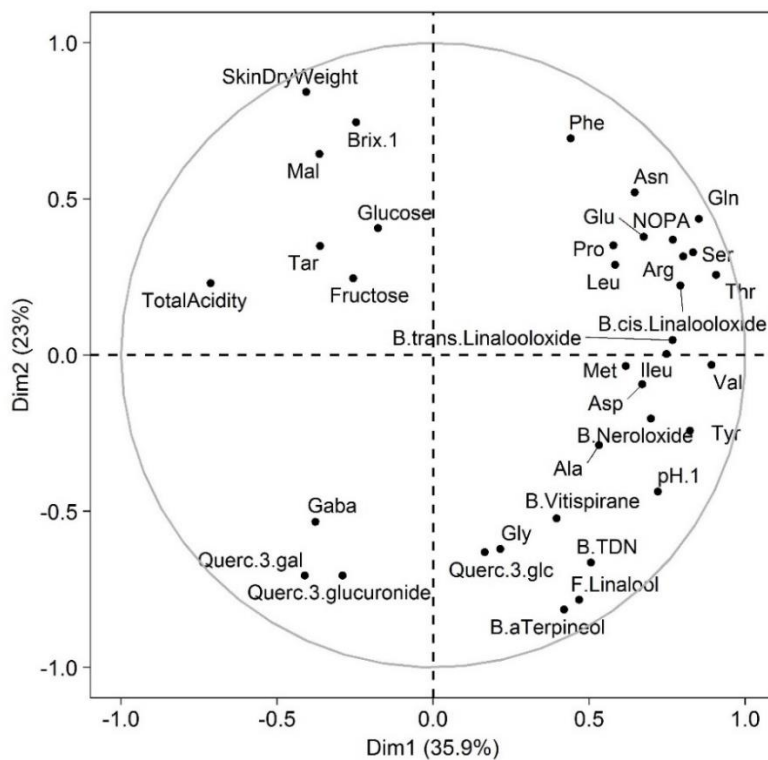


Figure 3.2. Loadings plot of technological parameters and secondary metabolites (amino acids, quercetin glucosides, and free and potentially volatile terpenes and norisoprenoids) from 2015-2017.



Multivariate *k*-means clustering was used to examine the persistence of the patterns observed in PCA for technological parameters and secondary metabolites (figure 3.3; appendix 3.2). *k*-means clustering is a partitioning method that aggregates data into a specified number of clusters such that the variance within clusters is minimized and the variance between clusters is maximized (Hartigan and Wong, 1979). The algorithm clustered the data points into four clusters containing 5, 7, 12, and 12 individuals respectively. Cluster 1 contained all 2015 juice samples of grapevines planted in 2012, with the addition of one sample from grapevines planted in 1971. Cluster 2 included the rest of the 2015 samples, i.e. three samples from grapevines planted in 1971, and all samples from grapevines planted in 1995. Clusters 3 and 4 distinguished between 2016 and 2017 juice samples for all age groups. Growing season and soil management practices were more responsible for the variability between samples than grapevine age. When the number of groups was increased beyond four, the replicates of young grapevines closest to the periphery of clusters 3 and 4 (2016-Y1, 2016-Y2, and 2016-Y3, then 2017-Y1 and 2017-Y2) became associated with new centroids without changing the overall structure of the other clusters. This suggests that these berry samples were relative outliers in their original clusters.

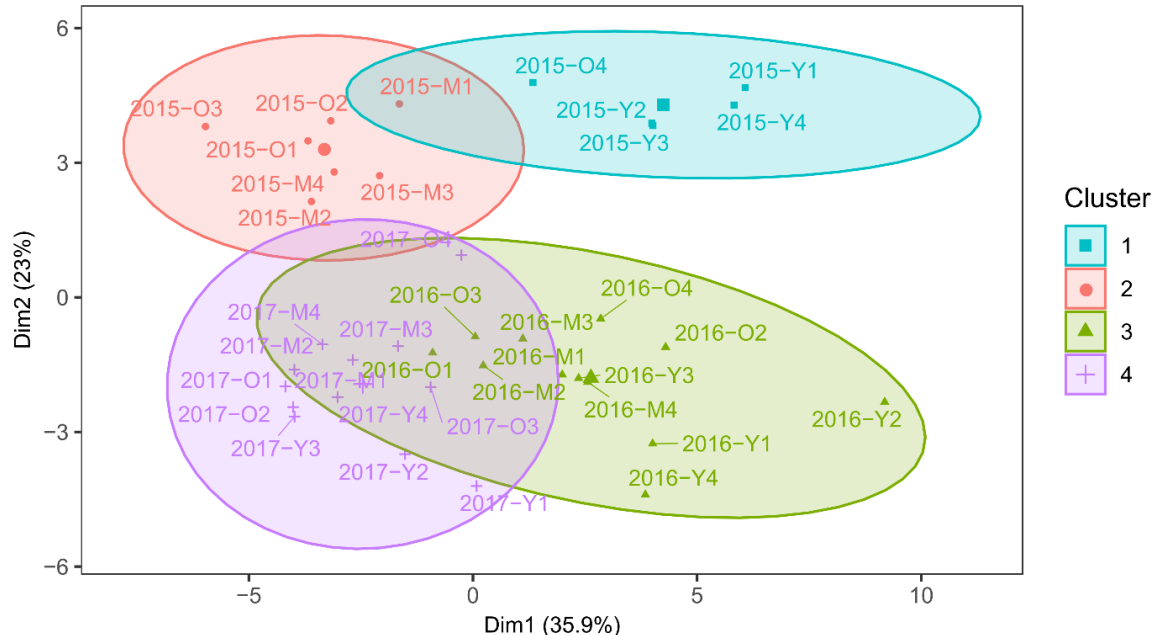


Figure 3.3. *k*-means clustering of the technological parameters and secondary metabolites, with four clusters and 50 iterations. The centroid positions were calculated as to minimize the sum of squares within each cluster. The point labels represent the growing season followed by the grapevine age group ('O' for 'old' grapevines planted in 1971; 'M' for 'mature' grapevines planted in 1995; 'Y' for 'young' grapevines planted in 2012). There are four replicates per growing season per age group. They are represented by the numbers 1 to 4 next to the letter.

### 3.2. Secondary metabolites and light interception

In 2014 and 2015, the youngest grapevines had thinner canopies and did not have a sufficient bud fruitfulness to fill their allocated space on the trellis. Bunch exposure to light positively impacts the concentration of monoterpenes, norisoprenoid and flavonols (Friedel et al., 2016; Skinkis et al., 2010; Matus et al., 2009). PCA was thus undertaken with a focus on light-sensitive compounds (monoterpenes, norisoprenoids, and flavonols) to assess the effect of canopy microclimate identified earlier between grapevines planted in 2012 and grapevines planted in 1995 and 1971 (Bou Nader et al., 2019).

Grapevines planted in 2012 were relatively more concentrated in monoterpenes, norisoprenoids, and flavonols for all growing seasons (figure 3.4; appendix 3.3), although the specific compounds varied from one season to the other. The first and second PCs were responsible for 82% of the variability in the data. The potentially volatile fractions of TDN, vitispirane and  $\alpha$ -terpineol were mostly correlated with PC 1, while PC 2 was mostly affected by quercetin-3-galactoside and quercetin-3-glucuronide concentrations. Potential monoterpenes and norisoprenoids were the most discriminant variables among samples. In 2017, only two berry samples from vines planted in 2012 were still markedly different from other age groups, which suggests that even though their characteristics were becoming similar to other age groups specifically in amino acid content, there are still some differences with regards to their phenolic and aroma composition.

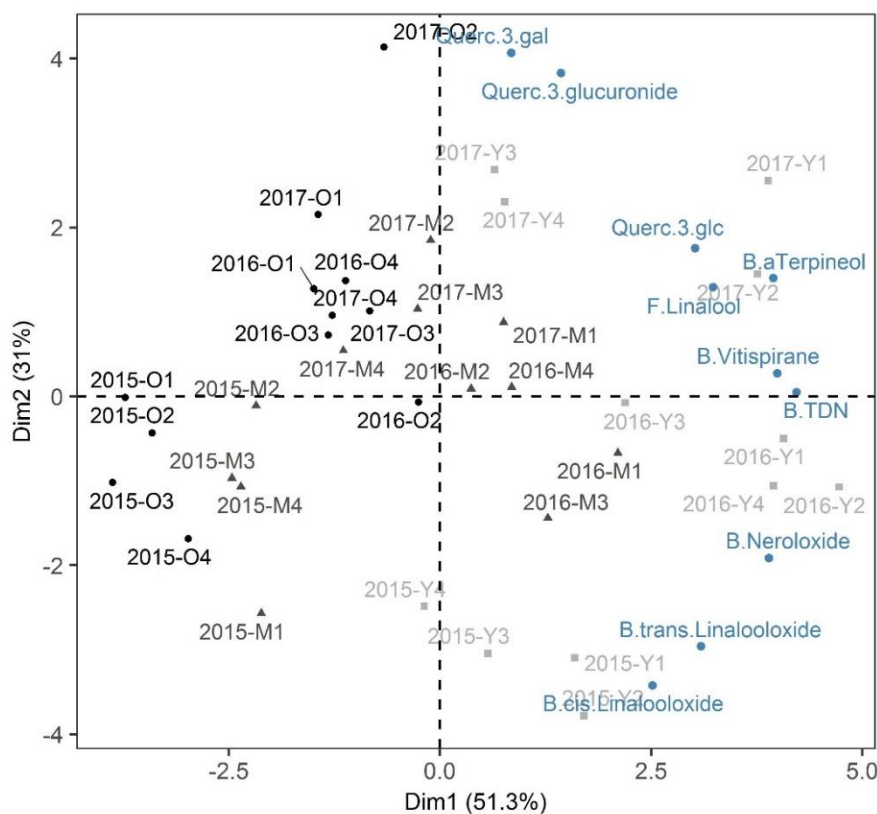


Figure 3.4. PCA biplot of bound and free terpenes and quercetin glucosides in berry must at harvest from 2015 to 2017. The point labels represent the growing season followed by the grapevine age group ('O' for 'old' grapevines planted in 1971; 'M' for 'mature' grapevines planted in 1995; 'Y' for 'young' grapevines planted in 2012). There are four replicates per growing season per age group. They are represented by the numbers 1 to 4 next to the letter.

#### 4. Conclusion

This work has focused on the canopy and cluster architecture of Riesling grapevines planted in 1971, 1995, and 2012 in the same vineyard from 2014 to 2017. It also assessed their berry composition with regards to primary metabolites, amino acids, and aroma and phenolic compounds.

Due to their young age, grapevines planted in 2012 were not subjected to the same soil treatment as older groups in 2014 and 2015 and experienced a weak water deficit at the end of 2015 and 2016. They also had a thinner canopy at the beginning of the study. These factors, which are difficult to clearly separate, had a large impact on the berry composition of the youngest grapevines in their first vintages, with higher concentrations of amino acids but also higher free and potentially volatile monoterpene and norisoprenoid content, and higher amounts of skin flavonols. Grapevines planted in 1995 and 1971 had similar canopy and cluster architectures,

as well as comparable berry compositions throughout the study. This work suggests that the impact of grapevine age is more important for young vines than fully established ones, mainly for reasons related to different bud fruitfulness and vineyard management in early years, and that these differences tend to disappear over a few years. More importantly, it was shown that grapevines that were 19 and 43 years old did not have different berry compositions for the studied compounds over a period of three to four years.

# Chapter 4. Impact of grapevine age on wine composition and sensory attributes

*This chapter is in preparation for publication in a modified version as:*

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Author contributions: the author designed and conducted the sensory experiment, analyzed the data, drafted and constructed the manuscript. Free and potential monoterpene and norisoprenoid analysis, organic acid analysis, volatile sulfur compound analysis were performed by Stefanie Fritsch, Heike Semmler, and Beata Beisert (Department of Microbiology and Biochemistry, HGU). <sup>1</sup>H NMR analysis was performed by Petra Kürbel (Department of Wine Analysis and Beverage Technology, HGU). Sensory analysis was performed with Doris Häge (Department of Enology, HGU).

## 1. Introduction

Grapevine age and its relationship to wine quality has been a recurring topic of interest. Old grapevines are usually held in high regard and are considered in many parts of the world as belonging to cultural heritage (Barossa Chapters, 2018; Historic Vineyard Society, 2018). Some winemaking regions even hold a register to track the age of vineyards and attribute a special denomination to their wines, which are highly regarded and have a high reputation. It is generally accepted that old grapevines have an improved balance between plant growth and yield formation, which would hypothetically lead to richer, more concentrated berries and, therefore, to an enhanced wine profile.

In the last decades, a few studies have attempted to confirm the supposed link between grapevine age and wine quality, but research on the subject is still scarce. In one of the earliest experiments to investigate wines from young and old vines, Heymann and Noble (1987) performed descriptive sensory analysis on commercial Cabernet Sauvignon wines from several regions in California, USA. The wines were produced from grapevines aged 5 to 20 years old. A positive correlation was reported between grapevine age and berry aroma, while wines from young grapevines were correlated with green bean and vegetal aromas with intensities that depended on the region.

Reynold et al. (2008) studied Cabernet Sauvignon, Cabernet Franc, Pinot Noir, and Riesling grapevines aged 4 and 14 years old over two growing seasons in Ontario, Canada. They reported a higher concentration in phenolic compounds and anthocyanins for young grapevines. Sensory analysis results for wines made from berries from grapevines of different age differed by cultivar and growing season. Pinot Noir and Pinot Meunier wines from young grapevines were rated higher for chocolate aroma, while Cabernet Sauvignon and Cabernet Franc wines from young grapevines were lower in vegetal aroma in the first season.

In the same year in Wädenswil, Switzerland, Zufferey and Maigre (2008) compared wines from three white cultivars (Chasselas, Arvine, and Pinot Blanc) and three red cultivars (Gamay, Syrah, and Humagne Rouge) with respect to grapevine age. The sensory analysis of the vintages 2002 to 2006 showed that wines produced from old Gamay, Syrah and Humagne Rouge grapevines were rated slightly better and described as having an improved tannic structure. Conversely for white cultivars, there were no significant differences among grapevine ages for

Chasselas and Arvine wines, and only wines of old Pinot Blanc grapevines were considered better than those made from young grapevines (Zufferey and Maigre 2008).

The current study aims to add to the body of work on grapevine age and wine quality by examining wines made from *Vitis vinifera* cv. Riesling grapevines of identical plant material, but planted in 1971, 1995, and 2012 in a single vineyard. A descriptive sensory analysis of wines from the 2014, 2015, and 2016 vintages was made in 2017. In parallel, the wines were analyzed chemically with a focus on potential monoterpenes and norisoprenoids, volatile sulfur compounds (VSC), organic acids, and higher alcohols. The project has also accumulated wines from vintages as old as 2002. While these wines could not be submitted to sensory analysis due to the low number of bottles remaining, they were analyzed in the same way as wines from 2014 to 2016.

## 2. Materials and methods

### 2.1. Experimental design and plant material

The study was performed in an experimental vineyard in Geisenheim, Germany, with alternating rows of *Vitis vinifera* (L.) cv. Riesling grapevines (clone Gm 239-17) on 5C Teleki rootstocks and trained in double Guyot. The vineyard was initially planted in 1971, but some rows were uprooted in 1995 and 2012 replanted with the same plant material in order to obtain four blocks with each grapevine age group. The plot is described in more detail in Bou Nader et al. (2019). It was studied from 2014, which was the first year of harvest for grapevines planted in 2012, until 2017.

A cover crop of grass and clover was sown in alternating rows, but the rows of grapevines planted in 2012 were rows were ploughed in 2014 and 2015 to prevent excessive competition during the early developmental stages of the grapevines (Williams et al., 1990). Afterwards, these rows were sown with the same cover crop combination that already existed in 1995 and 1971 rows. Soil nitrate and water content from depths of 0 to 30 cm and from 30 to 60 cm were significantly higher in 2014 and 2015 for rows planted in 2012, but after this period the three age groups had similar soil nitrate and water contents (Bou Nader et al., 2019).

Grapevines planted in 2012 did not fill their allocated space on the trellis until 2016 due to their lower bud fruitfulness in previous years (Bou Nader et al., in preparation, see chapter 3). This increased light interception in the cluster zone.

## 2.2. Winemaking

The project has accumulated wines since the 2002 vintage so the same winemaking procedure was used for wines from 2014 to 2017. A total of 200 kg of healthy grapes were harvested for each grapevine age group. The clusters were pressed without destemming and 45 mg L<sup>-1</sup> of SO<sub>2</sub> in the form of K<sub>2</sub>S<sub>2</sub>O<sub>5</sub> was added. After settling for 18 h at around 16 °C, the three must samples were racked from the lees and were transferred to one 50 L and one 25 L stainless steel tank. All batches were inoculated with dry yeast (*Saccharomyces cerevisiae*, 10g dry wt/hL) and were kept at 18 °C until the end of fermentation. After fermentation the 50 L container was topped up by combing it with the 25 L volume, sulfur dioxide was adjusted to 40 mg L<sup>-1</sup> free SO<sub>2</sub>. After six months, wines were sterile filtered, bottled in 0.75 L bottles sealed with screw caps and stored at 16°C until further analysis or sensory assessment.

## 2.3. Sensory analysis

Sensory evaluation was carried out in a laboratory equipped according to the ISO standard 8589:2007. The wine descriptive analysis (DA), which was conducted in July 2017 and was limited to wines from 2014 to 2016 (nine wines total) because of the relatively low volume of wine from previous vintages. Twelve trained panelists from the staff of Hochschule Geisenheim University (eight women and four men, ranging from 22 to 63 years) were recruited for the sensory assessment. All panelists underwent basic sensory training according to DIN EN ISO 8586:2014-05 and were familiar with Riesling wines and DA. The first few sessions were dedicated to the generation of descriptive attributes with a subset of wines from the study. The final attribute list contained one visual attribute (color intensity), nine aroma attributes (table 4.1), and three taste attributes (table 4.2). The color intensity standard consisted of a scale made with five wine glasses. The first glass contained water and the four others contained an old Riesling wine (Villa Monrepos Classic 2009, Hochschule Geisenheim University) with concentrations 100% (not diluted), 75%, 50%, and 25%. One extra aroma attribute was added to describe the general impression of the wine. Once the attributes were generated, the panelists underwent odor recognition and scale training. Most aroma references were prepared by soaking the product of interest in propanediol overnight then spiking a neutral Riesling wine with the obtained solution on the day of the tasting. This procedure allowed to precisely vary the intensity of aromas and to create standards that conformed to panelists' perception of the aromas in the wines.



The panelists practiced blind odor and taste recognition over eight one-hour sessions. Fresh references were prepared weekly and renewed whenever necessary. In the last three training sessions, subsets from the studied wines were used to conduct practice evaluations after odor and taste training. Samples were presented in a clear ISO 3591:1977 glass covered with a petri dish for aroma conservation. Each glass contained around 30 mL of wine that was cooled to 13 °C. Panelists rated the samples on an unstructured line scale of from '0 = weak' to '9 = strong'. For general impression, the four descriptors (green, floral, fruity, and ripe) were printed under the scale instead of 'weak' and 'strong'. The attributes were presented to panelists in groups according to modalities with color intensity first, followed by aroma attributes and taste attributes. Panelists were given a 20 s break after rating each sample to cleanse their palate with water and unsalted crackers (Matzo) if necessary. The data was collected on individual computers using FIZZ Acquisition version 2.50 (Biosystèmes, Couternon, France). Consensus results were shown to the panelists at the end of the training sessions with FIZZ Calculation version 2.50 (Biosystèmes, Couternon, France) so that they could assess their performance relative to the group.

The final DA evaluation session was performed in the same conditions as the training sessions. The reference standards were given to the panelists before the session to refresh their memory and remained accessible until the end of the tasting. The nine wine samples were presented in three repetitions, served nomadically in randomized order with three-digit codes. The tasting was done in triplicate (9 wines × 3 repetitions for every panelist). Each sample was followed by a 20 s break, and each flight of nine wines was followed by a 5 min break.

Table 4.1. Composition of aroma reference standards prepared using aroma standards by soaking in propanediol (Pd).

<b>Aroma references prepared by soaking in propanediol</b>		
<b>Aroma attribute</b>	<b>Reference in propanediol (Pd, 20% v/v)</b>	<b>Reference volume added to 20 mL neutral wine</b>
Citrus	0.5 g lemon zest in 20 mL Pd	2 mL
Peach	25 g peaches in syrup in 30 mL Pd	3 mL
Dry fruits	10 g dry apricots + 10 g dry prunes in 40 mL Pd	10 mL
Rose	½ white rose in 20 mL Pd	2 mL
Spicy	0.25 g black pepper + 0.75 g nutmeg in 20 mL Pd	0.5 mL
Vegetal	1 g grass in 20 mL Pd	1 mL + 1.5 mL canned white bean brine <sup>†</sup>
<b>Other aroma references</b>		
<b>Aroma attribute</b>	<b>Reference standard</b>	
Aging/kerosene	20 mL of old Riesling (Villa Monrepos Classic 2009, Hochschule Geisenheim University).	
Burnt rubber	0.2 g of burnt rubber band placed in a 10 mL vial.	
Reductive	The smell of fermented cabbage and/or rotten eggs. No reference standard produced.	
General impression	An abstract scale divided into four sections (green-floral-fruity-ripe).	

<sup>†</sup>The canned white bean brine was added directly in the wine.

Table 4.2. Taste reference standards with low and high concentrations.

<b>Taste attribute</b>	<b>Low concentration</b>	<b>High concentration</b>
Sweetness	3.0 g L <sup>-1</sup> fructose in water	7.0 g L <sup>-1</sup> fructose in water
Sourness	0.6 g L <sup>-1</sup> citric acid in water	1.0 g L <sup>-1</sup> citric acid in water
Bitterness	0.1 g L <sup>-1</sup> caffeine in water	0.3 g L <sup>-1</sup> caffeine in water

## 2.4. Wine composition

### 2.4.1. Color measurement

Wine color was assessed by tristimulus colorimetry based on CIELab parameters lightness (L\*), red/green (a\*) and yellow/blue (b\*) (CIE, 1986). The spectra were registered directly on the wine with a Unicam 500 UV/Vis spectrophotometer set to measure in the visible region ( $\lambda = 380\text{--}770$  nm) at constant intervals ( $\Delta\lambda = 2$  nm) and integrated using the software Cromalab<sup>®</sup>.

#### 2.4.2. Organic acids

Wine organic acids content were determined by HPLC according to Schneider et al. (1987). An internal standard of formic acid was added to the wine samples ( $5 \text{ g L}^{-1}$ ) and  $5 \mu\text{L}$  of the mixture were injected in a Agilent 1100 Series liquid chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a variable wavelength detector. The separation was made with an Allure Organic Acids column (Restek GmbH, Bad Homburg, Germany) with a  $5 \mu\text{m}$  particle size,  $60 \text{ \AA}$  pore size, and dimensions of  $250 \text{ mm} \times \text{i.d. } 4.6 \text{ mm}$ , in conjunction with a security guard cartridge C 18 of dimensions  $4.0 \times 3.0 \text{ mm}$  (Phenomenex, Aschaffenburg, Germany). The column was eluted with an aqueous solution of sulfuric acid ( $0.0139\% \text{ v/v}$ ) and ethanol ( $0.5\% \text{ v/v}$ ) at a flow rate of  $0.6 \text{ mL/min}$  and was operated at  $46 \text{ }^\circ\text{C}$ . Compound detection was performed at a wavelength of  $210 \text{ nm}$ .

#### 2.4.3. Potentially volatile monoterpenes and $\text{C}_{13}$ -norisoprenoids

Potentially volatile monoterpenes and  $\text{C}_{13}$ -norisoprenoids were analyzed by a modified solid-phase extraction (SPE) for sample preparation (Günata et al. 1985) as described in Schüttler (2012) and Schüttler et al. (2015), followed by gas chromatography coupled with mass spectrometry (GC-MS). The method was adapted by replacing the enzymatic release of aglycones by acid hydrolysis using the procedure described by Kosteridis et al. (1999).

Wine samples were maintained at  $4 \text{ }^\circ\text{C}$  before analysis. Strata SDB-L cartridges containing  $500 \text{ mg}$  of styrene-divenylbenzene polymer (Phenomenex, Torrance, CA USA) were preconditioned with  $5 \text{ mL}$  of pentane/dichloromethane ( $2/1 \text{ v/v}$ ), followed by  $10 \text{ mL}$  of methanol,  $10 \text{ mL}$  of methanol/ $\text{H}_2\text{O}$  ( $1/1 \text{ v/v}$ ) and  $10 \text{ mL}$  of  $\text{H}_2\text{O}$ . Must samples of  $50 \text{ mL}$  were diluted  $1:1$  with water and were spiked with octan-3-ol and 2,6-dimethylhept-5-en-2-ol (DMH) as internal standards ( $c_{(\text{octan-3-ol})} = 50 \mu\text{g L}^{-1}$ ;  $c_{(\text{DMH})} = 25 \mu\text{g L}^{-1}$ ) and were loaded on the SPE cartridges. It was washed again with  $10 \text{ mL}$  of water and dried for one hour with nitrogen under vacuum (see above). After drying the samples were eluted with  $10 \text{ mL}$  of pentane/dichloromethane ( $2/1 \text{ v/v}$ ). The eluates were dried with anhydrous sodium sulfate and concentrated to a final volume of  $50 \mu\text{L}$  under gentle nitrogen flow, and  $2 \mu\text{L}$  were used to analyze free monoterpenes and norisoprenoids by gas chromatography.

For bound monoterpenes and norisoprenoids the SPE cartridges were eluted with  $10 \text{ mL}$  of ethyl acetate. The fractions were evaporated to dryness under vacuum with a rotary evaporator (Büchi, Flawil, Switzerland) at water bath temperature  $40 \text{ }^\circ\text{C}$  and  $p = 138 \text{ kPa}$ . The residues were resolved in  $5 \text{ mL}$  of  $0.2 \text{ M}$  citric acid ( $\text{pH} = 2.5$ ), transferred to  $10 \text{ mL}$  head space vials,

sealed, and heated in a laboratory oven at 100 °C for 60 min. After cooling down the samples in ice, the internal standard mix was added based on the initial sample volume. Three successive liquid-liquid extractions were conducted with 2 mL, 1 mL, and 1 mL pentane/dichloromethane (2/1 v/v) and the recombined organic extracts were dried with anhydrous sodium sulfate and concentrated under nitrogen flow to a final volume of 50 µL and 2 µL were injected for gas chromatography. GC-MS was performed with a Thermo Fisher Trace GC Ultra gas chromatograph equipped with a PTV injector and coupled to a Thermo Fisher ITQ 900 Ion Trap MS mass spectrometric detector (Thermo Fisher Scientific, Waltham, MA USA). Two µL of sample extract were injected in splitless mode (splitless time 90 s) at 200 °C. An Agilent DB-Wax capillary column of dimensions 30 m × 0.25 mm ID × 0.5 µm film density (Agilent Technologies, Santa Clara, CA USA) which was used for the separation. During injection, the column oven was gradually heated to reach the following temperatures: 40 °C for 1 min, then heated until 60 °C at 10 °C min<sup>-1</sup>, then from 60 °C to 200 °C at 3 °C min<sup>-1</sup>, and from 200 °C to 230 °C at 10 °C min<sup>-1</sup>. The temperature was finally maintained at 230 °C for 10 min. Helium was used as a carrier gas with a flow of 1.4 mL min<sup>-1</sup> and average velocity of 27 cm s<sup>-1</sup>. The interface and MS source temperatures were set to 240 °C and 200 °C respectively. Mass spectrometric data was acquired in electron impact mode (EI) with an ionization energy of 70 eV. The quantification of aroma compounds was made with the selected ion monitoring mode (SIM).

#### 2.4.4. Volatile sulfur compounds

Volatile sulfur compounds (VSC) were analyzed by gas chromatography coupled with a pulsed flame photometric detector (PFPD) with headspace injection according to Rauhut et al. (2005) and Schüttler et al. (2015). The equipment used for the analysis included a GC Hewlett Packard (HP) 5890 Series II (Agilent, Waldbronn, Germany) equipped with cooled injection system (CIS-3) (Gerstel GmbH, Mülheim an der Ruhr, Germany), a headspace single injection system (HIS) (Gerstel GmbH, Mülheim an der Ruhr, Germany), and coupled to a PFPD Model 5380 (OI Analytical, College Station, TX, USA). The liner was packed with 25 mg Porapak Q 80/100 mesh (Sigma-Aldrich, Seelze, Germany). The separation was made on a 30 m × 0.32 mm ID × 4 µm SBP-1 Sulfur column (Supelco, Bellefonte, PA, USA). The wine samples were taken at 4 °C just before the sensory evaluation. Three mL of wine were pipetted into 5 mL GC vials that were flused with argon and contained 4 mg L<sup>-1</sup> 2,6-di-tert-butyl-4-methyl-phenol, 0.27 g mL<sup>-1</sup> NaCl, 0.2 g L<sup>-1</sup> EDTA (ethylenediamine tetra acetic acid), and 500 mg L<sup>-1</sup> propanal

to bind SO<sub>2</sub> molecules. An ethanolic internal standard of methyl-iso-propylsulfide and butylmethylsulfide (8 µg L<sup>-1</sup>) was added before closing the vial with a magnetic screwcap. The samples were preheated at 60 °C for 45 min before injection into the CIS-3. A total of 1000 µL headspace volume were injected in solvent vent mode with helium gas (Linde Gas, Würzburg, Germany). During injection the temperature was kept at 60 °C then increased to 180 °C with a gradient of 12 °C/s and was held for 8 min. Oven temperature was kept at 35 °C for 5 min and increased to 180 °C with a gradient of 10 °C/min and was kept for 10 min. The temperature of the detector was maintained at 250 °C and air and hydrogen flows were maintained at a pressure of 420 kPa.

#### 2.4.5. NMR fingerprinting

The wines were analyzed by <sup>1</sup>H NMR (nuclear magnetic resonance) for the quantification of several compounds including alcohols and aldehydes (Godelmann et al., 2016, 2013). The samples were diluted to 90% with a 1 M K<sub>2</sub>PO<sub>4</sub> buffer (pH = 2.4) in D<sub>2</sub>O, with 3-(trimethylsilyl)-propanoic acid sodium salt (TSP) as internal standard for referencing the chemical shift to 0ppm. The pH of the mixture was adjusted to 3.10 ± 0.02 with 1 M NaOH or HCl. A total of 600 µL were transferred to a 5 mm NMR tube and measured immediately. Measurements were made with a 400 MHz Avance III NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) equipped with a 5 mm 1H/D-TXI probehead with z-gradient, automated tuning and matching accessory, and BTO-2000 for temperature control. Measurements were made under water and ethanol suppression with the correction of eventual interferences. Signal-to-noise (S/N) ratio was 10:1 and recycling time was 6 s. Temperature was set to 300 ± 0.2 K without rotation. The pulse angle was maintained for the calibration and measurement of wine samples and the sweep width (SW) was 18 ppm. Compound quantification was performed by Bruker BioSpin GmbH according to Godelmann et al. (2016).

#### 2.5. Statistical analysis

The statistical analysis was performed with RStudio (version 1.1.453; Rstudio, 2018) and R (version 3.5.0; R Core Team, 2018). Analysis of variance (ANOVA) was used to assess the panelists' rating of the 2014, 2015, and 2016 wines. Principal component analysis (PCA) on mean ratings was used to examine the relationships between the sensory attributes and the wines. It was also used to assess the chemical composition of wines from older vintages. Multiple factor analysis (MFA) was used to contrast the sensory and chemical descriptions of the tasted wines. ANOVA analysis on wine sensory analysis was made with PanelCheck (Tomic et al.,

2009). PCA and MFA were performed with the packages FactoMineR (version 1.41; Lê et al., 2008) and factoextra (version 1.0.5; Kassambara and Mundt, 2017).

### 3. Results and discussion

#### 3.1. Sensory analysis

The average ratings of the 14 attributes over the three tasting replicates are presented in table 4.3. All attributes except citrus ( $P = 0.20$ ), rose ( $P = 0.51$ ), and bitterness ( $P = 0.19$ ) were significantly different across wines (appendix 4.1). There were significant interactions for wine  $\times$  replicate for the attributes of color intensity ( $P = 0.046$ ), peach ( $P = 0.033$ ), and dry fruit ( $P = 0.015$ ). The interaction for color intensity may be due to the order of tasting, with wines that are presented after a more intense wine being judged less intense than when following a light wine. Peach and dry fruit attributes were the most difficult to differentiate for panelists despite their training and experience.

PCA was used to examine the relationship between wines and attributes (figure 4.1). The first two principal components (PCs) explained 82% of the variability in the data. The first PC clustered several sensory attributes into a single group, with color intensity, peach, dry fruit, spices, aging/kerosene, general impression, and sweetness being positively correlated. This group was highly representative for the 2014 and 2015 wines from grapevines planted in 2012 (2014-Y and 2015-Y). The second PC was mostly affected by citrus, reductive, and burnt rubber aromas. The latter two attributes described the 2014 and 2016 wines from grapevines planted in 1995 (2014-M and 2016-M) and the 2016 wine from grapevines planted in 1971 (2016-O). Eigenvalues and eigenvectors are presented in appendix 4.2.

The PCA separated wines by grapevine age but the differences were mostly present between grapevines planted in 2012 on one hand and grapevines planted in 1995 and 1971 on the other. Indeed, except for the 2014 vintage, wines from grapevines planted in 1995 and 1971 were similar in sensory profile. Its results agree with the data obtained from berry composition (Bou Nader et al., in preparation, chapter 3). During 2014 and 2015, grapevines planted in 2012 were subjected to a different soil treatment (absence cover crop, ploughing) than older grapevine groups due to their young age. This is likely to have increased soil nitrogen mineralization (Davies et al., 2001), which in turn caused a higher nitrogen accumulation in the berries as reported in raspberry (Zebarth et al., 2007). In the same period, young grapevines generally had a lower bud fruitfulness than grapevines planted in 1995 and 1971. As a result, their clusters

were better exposed to light in the cluster zone and a higher production of monoterpenes, norisoprenoids, and skin flavonols (Bou Nader et al., in preparation, chapter 3). The berry composition of grapevines planted in 2012 was similar to older groups once cover crop was sown and they reached the same bud fruitfulness in 2016.

Table 4.3. Average ratings per attribute in the 2017 tasting for wines from the three grapevine age groups (vintages 2014 to 2016). The means and standard deviations were calculated from three replications with 12 panelists.

Planting date	2014			2015			2016		
	1971	1995	2012	1971	1995	2012	1971	1995	2012
color intensity	3.70 ± 1.28	3.06 ± 1.26	5.74 ± 1.33	4.02 ± 1.22	3.67 ± 1.27	6.29 ± 1.06	4.34 ± 1.25	3.47 ± 1.04	2.65 ± 0.92
citrus	2.32 ± 1.44	1.90 ± 1.39	1.93 ± 1.47	2.22 ± 1.24	2.36 ± 1.82	1.88 ± 1.37	2.15 ± 1.52	2.20 ± 1.07	2.52 ± 1.39
peach	2.28 ± 1.26	1.63 ± 1.38	2.40 ± 1.30	2.21 ± 1.49	1.89 ± 1.25	2.84 ± 1.78	1.43 ± 1.00	1.85 ± 1.52	1.87 ± 1.52
dry fruit	2.21 ± 1.62	1.10 ± 1.21	3.07 ± 1.72	2.40 ± 1.65	2.19 ± 1.54	4.35 ± 1.32	1.12 ± 1.31	0.93 ± 0.92	0.94 ± 0.93
rose	1.37 ± 0.87	1.19 ± 0.94	1.26 ± 0.98	1.40 ± 1.10	1.14 ± 1.13	1.27 ± 0.99	1.39 ± 1.17	1.49 ± 1.15	1.58 ± 1.30
vegetal	1.41 ± 1.58	1.57 ± 1.27	0.96 ± 1.21	1.17 ± 1.48	1.23 ± 1.39	0.59 ± 0.55	2.17 ± 1.47	2.07 ± 1.36	1.79 ± 1.30
spices	1.18 ± 1.06	0.71 ± 0.56	0.90 ± 0.62	1.11 ± 0.83	1.06 ± 0.81	1.25 ± 0.96	0.62 ± 0.41	0.58 ± 0.48	0.96 ± 0.93
aging/kerosene	2.42 ± 2.04	1.46 ± 1.85	3.94 ± 2.27	2.74 ± 1.8	2.83 ± 1.95	4.54 ± 2.34	1.20 ± 1.28	0.82 ± 0.75	0.84 ± 0.81
general impression	4.68 ± 2.50	2.86 ± 2.52	6.05 ± 1.92	4.77 ± 2.24	4.59 ± 2.59	7.23 ± 0.83	2.48 ± 1.88	2.38 ± 1.60	2.76 ± 1.77
reductive	0.64 ± 0.69	1.67 ± 1.85	0.60 ± 0.62	0.78 ± 1.0	0.70 ± 0.98	0.48 ± 0.56	1.28 ± 1.50	1.10 ± 1.22	1.04 ± 0.89
burnt rubber	1.14 ± 1.52	3.58 ± 2.60	0.94 ± 1.71	0.92 ± 1.27	1.04 ± 1.42	0.42 ± 0.42	3.86 ± 2.14	3.10 ± 1.98	2.77 ± 2.10
sourness	4.27 ± 1.86	4.50 ± 1.88	3.57 ± 1.68	5.38 ± 1.67	5.11 ± 1.91	3.31 ± 1.46	4.92 ± 1.66	5.05 ± 1.88	4.95 ± 1.72
sweetness	2.06 ± 1.45	1.71 ± 1.49	2.41 ± 1.40	1.77 ± 1.41	1.93 ± 1.56	4.45 ± 2.05	1.69 ± 1.19	1.57 ± 1.30	1.40 ± 1.15
bitterness	2.20 ± 1.85	2.24 ± 2.30	2.66 ± 2.25	2.68 ± 2.20	2.50 ± 1.93	1.79 ± 1.73	2.67 ± 2.30	2.46 ± 1.95	2.36 ± 2.01



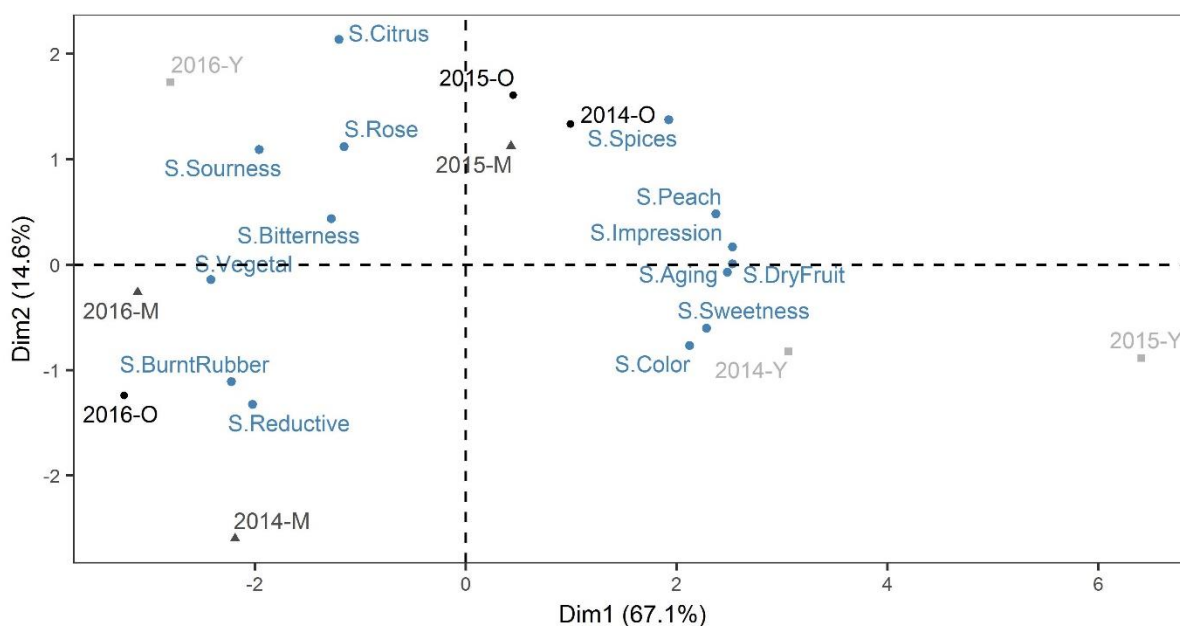


Figure 4.1. PCA biplot of the 2017 sensory analysis of grapevines from the three grapevine age groups. The point labels represent the growing season followed by the grapevine age group ('O' for 'old' grapevines planted in 1971; 'M' for 'mature' grapevines planted in 1995; 'Y' for 'young' grapevines planted in 2012). There are four replicates per growing season per age group, represented by the numbers 1 to 4 next to the letter.

### 3.2. Chemical analysis

The concentration of various compounds obtained from  $^1\text{H}$  NMR and HPLC, as well as free and potential monoterpenes and norisoprenoids and VSCs are presented in appendices 4.3-4.6. Among monoterpenes and norisoprenoids,  $\alpha$ -terpineol, hotrienol, *cis*- and *trans*-linalool oxides, and TDN were found in free form in the wines, but only hotrienol and TDN had concentrations around their odor thresholds (table 4.4). Hotrienol, with an odor threshold of  $30 \mu\text{g L}^{-1}$  (Pino and Fajardo, 2011) is characterized by a floral aroma. Its highest concentrations were reported for wines from vintages 2012, 2014, and 2015. The norisoprenoid TDN, which has an odor threshold of 2 to  $20 \mu\text{g L}^{-1}$  (Ross et al., 2014; Sacks et al., 2012) and is responsible for the typical kerosene like aroma of Riesling wines, was already present in free form at a concentration of  $1.8 \mu\text{g L}^{-1}$  for 2016 wines, and generally increased up to  $14.6 \mu\text{g L}^{-1}$  for older vintages. TDN is formed by the hydrolysis and rearrangement of carotenoid-derived precursors during wine fermentation and storage (Sacks et al., 2012). Hence, potential TDN, which represents the concentration of these precursors, was higher in younger wines, although the concentration was dependent on the vintage.

Hydrogen sulfide (H<sub>2</sub>S), dimethyl sulfide (DMS), and carbon disulfide (CS<sub>2</sub>) were the only VSCs detected in the wine samples. These low-boiling point sulfur compounds do not occur in grapes and are mostly generated during fermentation and storage from exogenous sulfate (SO<sub>4</sub><sup>2-</sup>) and sulfite (SO<sub>3</sub><sup>2-</sup>) ions and subsequently from the sulfur-containing amino acids cysteine and methionine (Smith et al., 2015). With the exception of H<sub>2</sub>S, these compounds can have a positive effect on the complexity of wine aroma by interacting with other odorant molecules. Specifically, DMS found at low concentrations can develop a blackcurrant or truffle aroma, but is related to vegetal aromas like asparagus at high concentration (Segurel et al., 2004). The wines of grapevines planted in 2012 had higher levels of H<sub>2</sub>S and DMS than wines from older grapevines in 2014 and 2015, possibly due to the higher amino acid concentration in their juice (Bou Nader et al., in preparation, chapter 3).

Table 4.4. List of the compounds analyzed in wine.

Free monoterpenes and norisoprenoids	Volatile sulfur compounds	Measured by <sup>1</sup> H NMR <sup>†</sup>
linalool	hydrogen sulfide	methanol
α-terpineol	methanethiol	2,3-butanediol
hotrienol	ethanethiol	2-phenylethanol
nerol oxide	carbon disulfide	3-methylbutanol
cis-linalool oxide	dimethyl sulfide	glycerol
trans-linalool oxide	thioaceticacid -S- methyl ester	acetaldehyde
vitispirane	thioaceticacid -S- ethyl ester	ethyl acetate
TDN	dimethyl disulfide	succinic acid
Potential monoterpenes and norisoprenoids	diethyl disulfide	galacturonic acid
linalool	dimethyl trisulfide	caftaric acid
α-terpineol	Organic acids	CIELab color space
hotrienol	tartaric Acid	L*
nerol oxide	malic Acid	a*
cis-linalool oxide	shikimic Acid	b*
trans-linalool oxide	lactic Acid	
vitispirane	acetic Acid	
TDN	citric Acid	

<sup>†</sup>Several classes of compounds.

### 3.3. MFA with chemical and sensory data

Multiple factor analysis (MFA) was used to relate the chemical data obtained for wine to the sensory attributes. MFA is a relatively recent factorial method that can analyze datasets consisting of several groups of variables defined on the same set of individuals. It allows the

group matrices to be scaled individually before concatenating them and performing principal component analysis (Escofier and Pagès, 1994).

The first and second PCAs accounted for 70% of the total variability between wine samples (figure 4.2; appendix 4.7). The separation of sensory attributes that was visible in PCA was conserved, with attributes related to ripeness grouped together on the right-hand side of the MFA loadings plot. These attributes were positively correlated with the  $b^*$  component of the CIELab color space, which takes positive values for yellow. They were also positively correlated with concentrations of free TDN, DMS, and  $H_2S$ . The left-hand side of the MFA loadings plot regrouped attributes linked to greenness such as citrus, vegetal, and rose, but also the reductive and burnt rubber aromas. Sourness was also part of this group of variables and was related to citrus aroma and the concentration of tartaric acid. Finally, the rose attribute, even though it did not significantly vary across wines, was correlated with succinic acid, methylbutanol, and phenylethanol, which have a light floral aroma (García et al., 2003; Maoz et al., 2018). The second PCA was mostly defined by potential TDN and  $\alpha$ -terpineol, as well as lactic acid. In fact, most potentially volatile monoterpenes and norisoprenoids were located on the bottom right of the loadings plot, while their free counterparts were clustered in the top right.

In 2014 and 2015 the wines from grapevines planted in 2012 were correlated with the group of ripeness attributes and potential monoterpenes and norisoprenoids, while wines from grapevines planted in 1995 and 1971 were grouped together (figure 4.3). In 2016 there was a high agreement within the sensorial panel between the three wine samples with regard to sensory and chemical profile. The trend seen in MFA mirrored the trend of berry composition, where the berry composition of grapevines planted in 2012 converged with older grapevines after cover crop was sown and they had reached their bud fruitfulness potential.

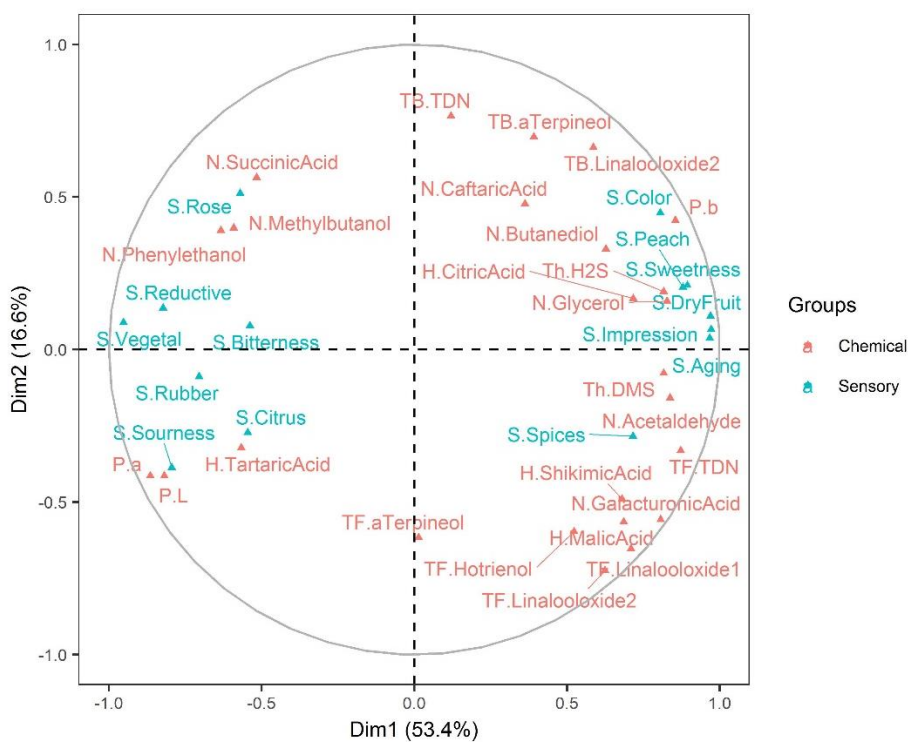


Figure 4.2. MFA Loadings plot of the sensory and chemical and sensory variables analyzed in 2017.

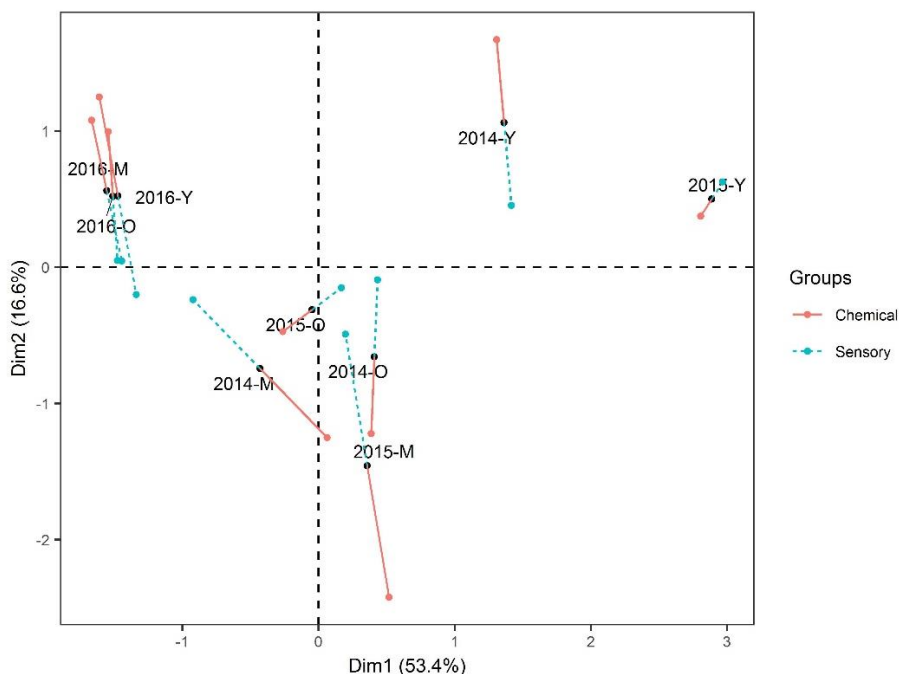


Figure 4.3. MFA partial score plot with chemical sensory variables analyzed in 2017. The point labels represent the growing season followed by the grapevine age group ('O' for 'old' grapevines planted in 1971; 'M' for 'mature' grapevines planted in 1995; 'Y' for 'young' grapevines planted in 2012). There are four replicates per growing season per age group, represented by the numbers 1 to 4 next to the letter.

### 3.4. PCA of chemical data for older wines

The current project started in 2014 but wines were already available for grapevines planted in 1971 and 1995 starting in 2002. At the time, these grapevines were 31 and 7 years old respectively, which is relatively close to the current age of grapevines planted in 1995 (22 years old) and 2012 (5 years old).

If vineyard and winemaking conditions are assumed to be comparable since 2002, it may be possible to envision how wines from the most recent vintages would evolve over time. PCA of chemical data for the available wines shows large differences between vintages (figure 4.4; appendix 4.8). This was expected as PC 1 is mostly influenced by free and potential monoterpene and norisoprenoids and VSCs, and color (figure 4.5), all of which are known to change during storage (Mateo and Jiménez, 2000; Robinson et al., 2014; Smith et al., 2015; Winterhalter et al., 1990). Nevertheless, inside vintages there were no specific trends concerning grapevine ages for the chemical compounds that were studied. Further analysis can be performed in the future by grouping the wines into subsets according to the similarity of their vintages.

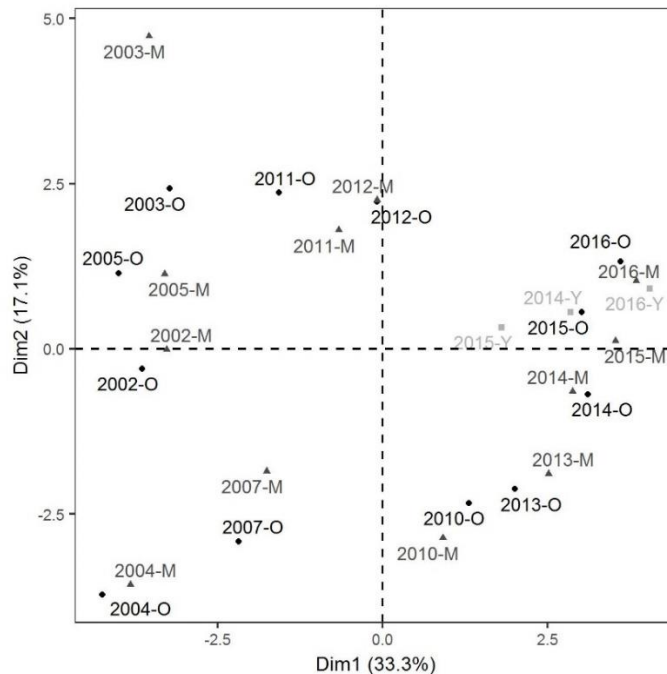


Figure 4.4. PCA score plot from the chemical analysis of wines from 2002 to 2016. Some vintages were not available for analysis. The point labels represent the growing season followed by the grapevine age group ('O' for 'old' grapevines planted in 1971; 'M' for 'mature' grapevines planted in 1995; 'Y' for 'young' grapevines planted in 2012). There are four replicates per growing season per age group, represented by the numbers 1 to 4 next to the letter.

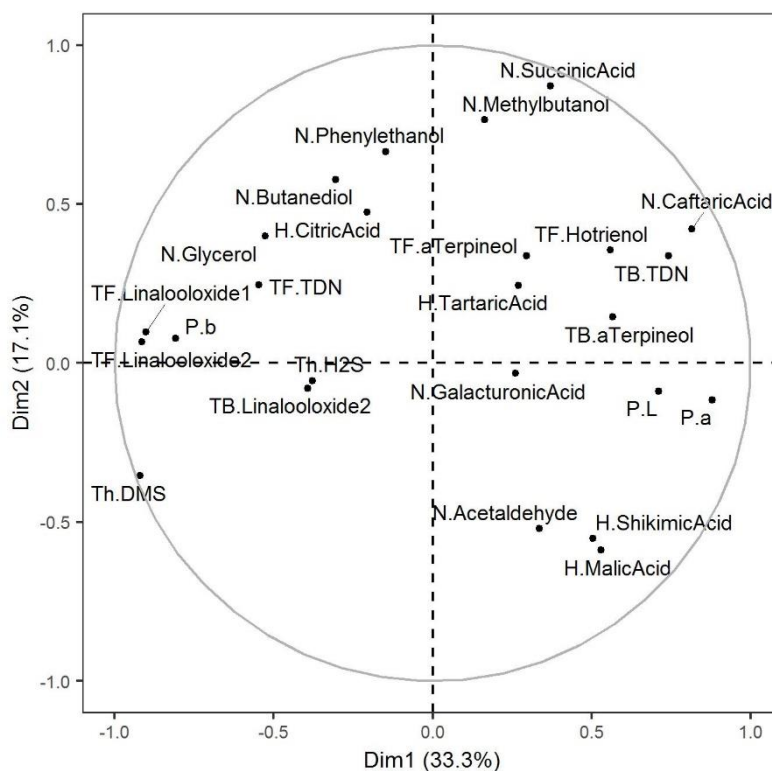


Figure 4.5. PCA loadings plot from the chemical analysis of wines from 2002 to 2016.

#### 4. Conclusion

The wines from Riesling grapevines planted in 1971, 1995, and 2012 were evaluated by sensory descriptive analysis and chemical analysis. The largest variability in the samples, both in sensory and chemical analysis, was found for wines from the first vintages of grapevines planted in 2012 and was likely caused by differences in soil management and an inherently lower bud fruitfulness. Wines produced from these grapevines were highly colored and were associated with aromas of kerosene and ripe fruits and with free and potential monoterpenes and norisoprenoids and VSC. In the third vintage, wines from grapevines planted in 2012 had similar chemical and sensory profiles than wines from older age groups. Wines from older vintages were also analyzed chemically. Vintage effects were more important than the variance due to grapevine age.

# Chapter 5. Evaluating grapevine trunk size with a handheld camera by 3D modeling

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Author contributions: the author designed the research experiment and drafted the manuscript. The experiment, analysis of the data and construction of the manuscript were conducted with Leonard Maximilian Pfahl. The modeling was performed by Leonard Maximilian Pfahl.

## 1. Abstract

**Background and aims:** Grapevine size measurements are necessary to assess their carbon reserves at single vine level and study the carbon sequestration potential of vineyards. These measurements mostly rely on traditional dendrology techniques that may be prone to errors. Here we examine the applicability of structure-from-motion with multi-view stereo-photogrammetry (SfM-MVS) to accurately measure vine trunk thickness and volume. SfM-MVS allows the creation of scaled, georeferenced 3D models based on a set of overlapping photographs.

**Methods and results:** The study was conducted on field-grown *Vitis vinifera* L. cv. Riesling vines aged 5, 22, and 46 years and pruned in a bilateral Guyot. Trunk diameter, cross-sectional area and circumference 10 cm above the grafting point were measured with traditional dendrometry and compared with values obtained from the reconstructed 3D models. SfM-MVS was also used to estimate the total trunk volume and the contribution of the vine crown to this volume. The correlations between measured and modeled metrics were close to unity ( $0.976 \leq R^2 \leq 0.988$ ). The estimates for 5-year-old vines had the largest errors due to their small trunk size ( $RMSE_{drow} = 18.59\%$ ,  $RMSE_{dper} = 14.19\%$ ,  $RMSE_{TCSA} = 20.97\%$ ,  $RMSE_{circumference} = 3.40\%$ ). In fact, the volume of 46-year-old vine trunks was 1.7 times greater than 22-year-old vine trunks and more than 10 times greater than 5-year-old trunks. The vine crown contribution to the total trunk volume was as high as 60% for the two older vine groups, and most of its size increase occurred in the early stages of grapevine development.

**Conclusions:** SfM-MVS was able to produce accurate models of the vine trunks provided adequate lighting conditions and image resolution.

**Significance of the Study:** This work serves as a proof of concept for the use of SfM-MVS to measure the trunk size of field-grown grapevines of different ages. The technique, which is relatively new, is cost-effective and easy to implement. Future research is necessary to determine specific applications where it could assist or replace traditional dendrology methods.

**Keywords:** dendrology, vine age, carbon storage, wood reserves, photogrammetry, SfM-MVS.

## 2. Introduction

Trunk dimensions and structure (Tyminski, 2013) are often assessed in the fields of forestry, ecology, and arboriculture. Their measurement at the single tree and forest scales allow the evaluation of wood volume and biomass, water limitations, carbon stocks, as well as



environmental and commercial values (Daudet et al., 2005; Houghton, 2005; Mlambo et al., 2017; Nowak et al., 2013). Traditional dendrology methods have also been applied in viticulture for determining grapevine age and annual growth rate (Tyminski, 2013). Knowledge about grapevine carbon storage capacity and reallocation dynamics is essential to study the potential of vineyards for carbon sequestration in permanent organs (trunk and roots) but also necessary to assess the effect of management strategies on grapevine performance (Miranda et al., 2017; Schreiner et al., 2006).

Grapevine perennial structure and production capacity increase with age, especially in the first years after planting (Bou Nader et al., 2019; Chiarawipa et al., 2013; Grigg et al., 2017) but their evolution depends on several environmental factors such as soil profile and depth, water availability, and climate (Keller M., 2010). Grapevines present unique challenges compared to tree species. Because of their liana nature, they can be trained into various systems to optimize vigor and canopy light interception (Reynolds and Vanden Heuvel, 2009), adapt to environmental and climatic factors (Palliotti et al., 2014), and reduce the incidence of fungal infections (Austin et al., 2009). This results in a wide variety of grapevine trunk structures, thus limiting the use of simple measurements such as trunk circumference when comparing multiple training systems. In addition, traditional dendrology methods that produce the most accurate results are often expensive, labor-intensive, and destructive, requiring plants to be uprooted for measurement, while those that are simple and cost-effective are often prone to errors (Kitahara et al., 2010).

Structure-from-motion with multi-view stereo-photogrammetry (SfM-MVS) is a recent photogrammetric approach that automatically combines overlapping 2D images to create 3D models of captured objects. It operates by identifying and matching object features across images, then using these features to infer camera location and orientation (James and Robson, 2012). The technique has already been tested with success for the description of tree structures (Liang et al., 2014; Miller et al., 2015; Morgenroth and Gomez, 2014).

The following paper is primarily intended as a proof of concept for the application of SfM-MVS in viticulture by comparing the trunk circumference and cross-sectional area measured in the field on grapevines of different ages to values derived from 3D model reconstruction. Secondly, it provides a measure of the total volume of the trunk and also gives the opportunity to divide it into parts, which might be of interest if parts of the trunk should not be taken into consideration, i.e. the crown of the trunk in the case of older grapevines.

### 3. Materials and methods

#### 3.1. Plant material and field-measured trunk diameter and circumference

The trial was conducted in an experimental vineyard at the Hochschule Geisenheim University (HGU) in Geisenheim, Germany (+49° 98' 76.59", +7° 94' 47.90"). The vineyard was initially planted in 1971 with *Vitis vinifera* (L.) cv. Riesling, clone Gm239-17, grafted on 5C Teleki rootstock. Grapevines were distributed along 68 rows in a North-South orientation and cane-pruned to form a bilateral, downward-arched Guyot. In 1995 and 2012, several rows were uprooted and replanted with the same planting material. The end result was a vineyard with identical grapevines of three different ages divided in alternating rows. The year 2014 was the first productive vintage for grapevines planted in 2012. At that time, some grapevines were already missing in the rows planted in 1971 and 1995. A fully randomized block experimental design was therefore not possible to implement. Instead, a selection process was carried out at the beginning of the 2014 growing season in order to select six grapevines per age group and field replicate (n = 24). The number of nodes retained was adjusted to 10 per meter (equivalent to 14 nodes per vine) during winter pruning. At the beginning of the growing season, the trunk diameter in the direction of the row was measured 10 cm above the grafting point with a digital caliper. This value, coupled with bud fruitfulness, was used to compare individual grapevines to the row medians and means, and the three grapevines closest to these values were selected. More weight was given to trunk diameter in the selection process because of its higher stability across growing seasons. The selected grapevines were used in a separate trial on the impact of grapevine age on productivity and water stress tolerance (Bou Nader et al., 2019). The current trial was conducted in 2017, when the yield and pruning weight of grapevines planted in 2012 was similar to those of older grapevines (table 5.1).

Table 5.1. Overview of the three grapevine ages used in the 2017 study on trunk size.

Planting year	Age (years)	Variety/ Clone	Rootstock	Pruning system	Row × grapevine distance (m)	Mean pruning weight† (kg/m)	Mean yield† (kg/m)
1971	5	Riesling/ Gm239-17	5C Teleki	Bilateral Guyot	2.0 × 1.4	0.78 ± 0.06	1.81 ± 0.20
1995	22	Riesling/ Gm239-17	5C Teleki	Bilateral Guyot	2.0 × 1.4	0.71 ± 0.09	2.23 ± 0.46
2012	46	Riesling/ Gm239-17	5C Teleki	Bilateral Guyot	2.0 × 1.4	0.67 ± 0.09	2.07 ± 0.33

†Values for the 2016 growing season.

When the trunk diameter in the row direction was measured, a thin plastic cord was tied to the trunk just under the caliper position to serve as a reference point for further measurements (figures 5.1a and 5.1b). The diameter was measured again in the direction perpendicular to the row. The two values were averaged and used to compute the trunk cross-sectional area (TCSA) by approximating the trunk to a disc (Lepsis and Blanke, 2006). The trunk circumference was measured with a flexible measuring tape at the same position.

### 3.2. Image acquisition for trunk reconstruction

The ground around the trunks was manually weeded to have a direct visual access to the trunk base. Loose bark pieces were removed by gently whisking over the trunk to prevent volume miscalculations. In order to georeference the images, a reference grid made of seven equally spaced markers (Agisoft LLC, 2014) printed on cardboard was laid down on the ground around the trunk before it was photographed (figure 5.1c). Models built with SfM-MVS initially do not have any scale or georeference, and this information can be manually supplied to the software with an object of known dimensions that is placed in the image (in this case the distance between two adjacent markers, which was equal to 10.5 cm).



Figure 5.1. Example of the experimental setup for taking the pictures (a) with a general view of grapevine with the georeference grid and the point of measurement (white arrow); (b) focus on the point of measurement of trunk circumference and cross-sectional area 10 cm above the grafting point highlighted in the field using a plastic cord; (c) focus on the georeference grid around the base of the trunk.

The photography was done under cloudy weather conditions, as bright sunlight increased the contrast between illuminated and shaded parts of the trunk, leading to a poor reconstruction of the shadowed side. The camera used was a commercial digital SLR camera (Sony  $\alpha$ 58; fixed focal length objective SAL35F18). The images were captured in several concentric circles around the grapevine. The first circle, which produced a set of around 30 images, captured the whole grapevine. It was followed by three inner circles with a focus on the low, middle, and crown parts of the trunk (figure 5.2a). In addition to these circles, some extra images were taken at certain angles to capture specific structural features such as the underside of an s-shaped trunk, leading to a total of 80 to 110 images per grapevine. It took around 5 min to prepare each grapevine and around 15 min to capture the necessary images. Since the trial was conducted in a vineyard, the operator's movement around the grapevine was limited by neighboring plants. The distance between the outer circle and the grapevine was therefore approximate to the row distance of two meters, while the inner circles had to be smaller than 1.4 m to prevent obstruction by adjacent grapevines. The RAW format was used with an ISO of 200 as well as a high aperture value and shutter speed higher than 1/250 s to prevent motion blur, which would have lowered the quality of the models.

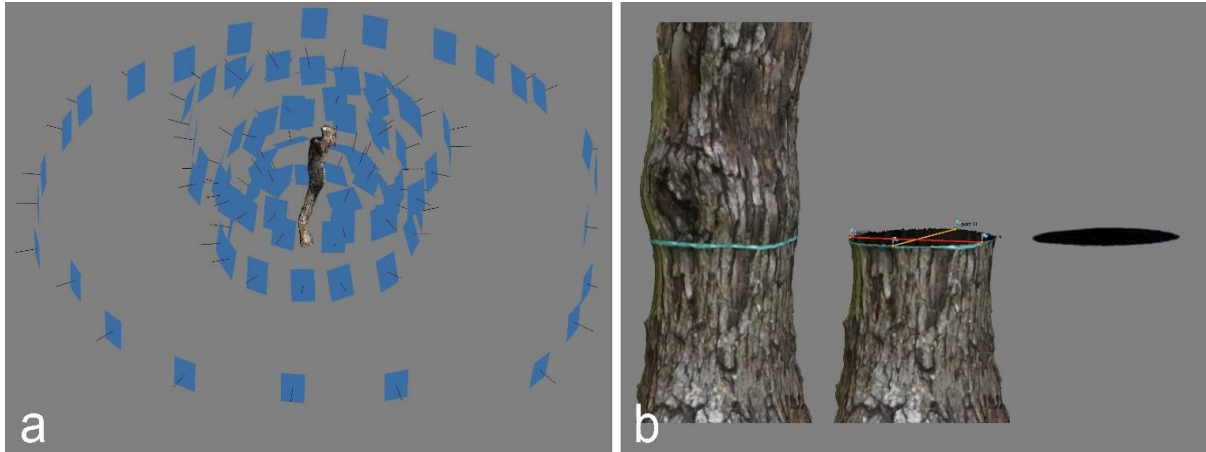


Figure 5.2. Images of the SfM-MVS reconstructed grapevine a) with squares representing camera positions when taking photographs; b) showing the intact trunk (left), the trunk cross-section 10 cm above the grafting point with the two orthogonal diameters (middle), and the modeled disc (right).

### 3.3. 3D model reconstruction by SfM-MVS

The 3D model reconstruction of individual grapevine trunks was made with the Agisoft Photoscan software (Agisoft LCC, St. Petersburg, Russia). First, the various positions of the camera were calculated by matching common points on the images (figure 5.2a) and aligning

them to produce a sparse point model. Next, a dense point model in which points are several times more numerous was generated. At this time the point cloud already resembles the original grapevine but needs to be cleaned of noise coming from background elements, the ground, trellis wires and posts, as well as false points that are sometimes caused by shadows. For the purpose of this experiment, the trunk was truncated at the grafting point and the bilateral canes were excluded. Finally, a polygonal mesh was fitted to the dense point model to form the 3D trunk models.

As noted previously, the models do not yet have a scale or geographic position. To measure distance or volume it is necessary to implement a coordinate system and a measuring scale. After specifying the distance between markers on the georeferenced grid, the software can create a coordinate system that allows data acquisition from various point. In some cases the software failed to automatically identify all markers, so missing markers were manually referenced.

#### 3.4. SfM-MVS measurements and model validation

The trunk diameters in the direction of the row and perpendicular to the row were estimated on the 3D models at the level of the plastic cord. The trunk was truncated at the measuring point, and point markers were added based on the position of the georeferenced grid relative to the trunk (figure 5.2b). For TCSA and trunk circumference it was necessary to create a surface at the cross-section. This was done with the ‘close holes’ tool in Agisoft, which generates a ‘watertight’ mesh by connecting the points at the periphery of holes with a flat surface. It was then possible to measure the trunk circumference TCSA directly on the surface without having to rely on the orthogonal diameter (figure 5.2b). The values obtained for the metrics were compared with field-measured values for validation.

#### 3.5. Estimation of trunk volume and crown contribution

The computation of the total trunk volume also required the use of the ‘close holes’ tool. However, the trunk volume that was calculated with the model could not be validated with field measurements, as it would have been necessary to uproot the grapevines to obtain an accurate volume of the trunk (Özçelik et al., 2008). This was not possible due to ongoing trials on the 72 selected grapevines.

It was apparent during measurements that the crowns of older grapevines formed more or less important part of the total trunk volume. The grapevine crown is formed over the years after

successive pruning, and depending on pruning techniques may contain dead wood. The contribution of the grapevine crown to the total trunk volume was assessed by excluding it from the model and comparing the volume obtained to the total volume. Since the grapevines were approximately trained to the same height it was possible to estimate the minimal trunk height before the crown. After visual inspection of all grapevine models, it was decided to keep 40 cm of trunk above the grafting point for measurements and omit the fraction of the trunk above this limit (figures 5.3a and 5.3b).

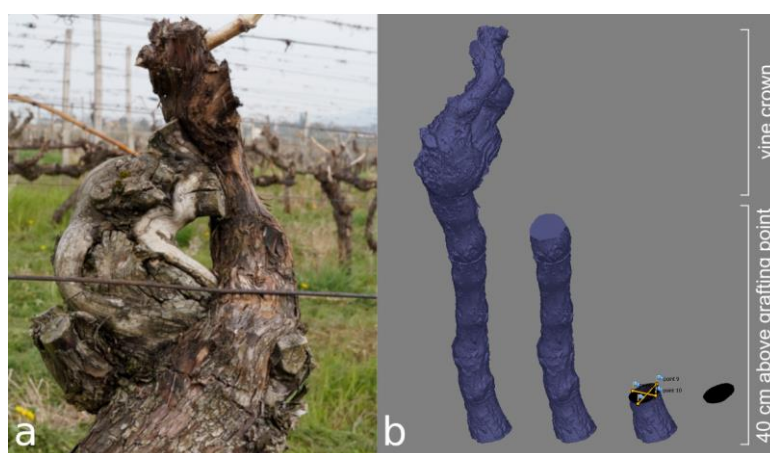


Figure 5.3. (a) Example of a complex crown on a 46 year old vine; (b) removal of the crown by truncating the trunk volume to 40 cm above the grafting point.

### 3.6. Statistical analysis

The dendrological parameters derived from the 3D models were compared to the traditional field measurements by linear regression with an estimation of  $R^2$ . In addition to this procedure, the root mean square error (RMSE) and bias as defined by equations 1 and 2 were calculated for each grapevine age according to Kankare et al. (2013) and Miller et al. (2015):

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{n}} \quad \text{Equation 1}$$

$$Bias = \frac{\sum_{i=1}^n (y_i - \hat{y}_i)}{n} \quad \text{Equation 2}$$

where  $n$  is the number of estimates,  $y_i$  is the value estimated by model reconstruction, and  $\hat{y}_i$  is the value measured directly in the vineyard. Calculations were made with R (version 3.5.1; R Core Team, 2018) and RStudio (version 1.1.419; RStudio, 2018).

## 4. Results and discussion

### 4.1. SfM-MVS measurements and model validation

SfM-MVS succeeded overall in reconstructing the trunks of grapevines of all ages. The number of pictures and their overlap were sufficient to obtain detailed 3D models of the trunks. Due to the small size of grapevines relative to trees, it was easy to capture them from low as well as high angles. Even though the two meter row distance limited movement around the grapevine of interest, the distance was large enough to obtain satisfactory results. The best results were obtained under cloudy conditions, as model reconstruction works best with diffuse light and minimal contrast between illuminated and shadowed parts (Miller et al., 2015).

Regression analysis across the three grapevine ages of the trunk diameter in the row direction ( $d_{row}$ ) and in the direction perpendicular to the row ( $d_{per}$ ) showed high correlations for both values ( $R^2 = 0.976$ ; figure 5.4). The correlation was similar for TCSA when comparing the value calculated from the two diameters in the field to the area of the reconstructed surface given by the software ( $R^2 = 0.979$ ; figure 5.4). Trunk circumference had the strongest linear relationship between measured and modeled values, approaching unity ( $R^2 = 0.988$ ; figure 5.4).

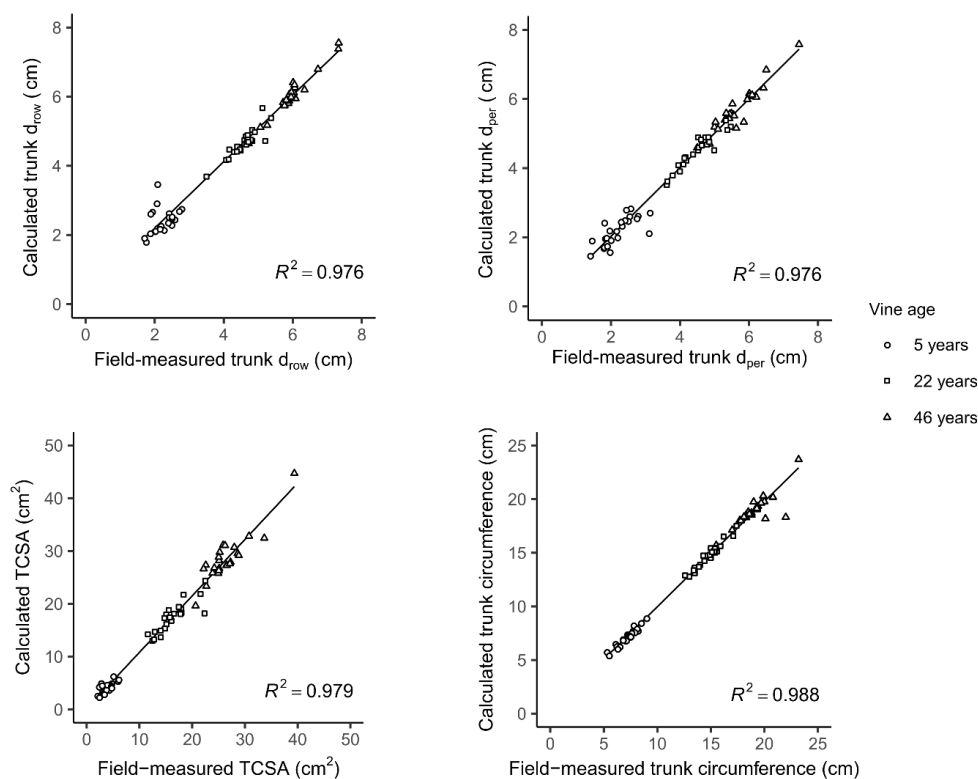


Figure 5.4. Regression analysis of the trunk diameter in the row direction ( $d_{row}$ ) and in the direction perpendicular to the row ( $d_{per}$ ), the trunk cross-sectional area (TCSA), and the trunk circumference with SfM-MVS estimates against field values for grapevines aged 5 (○), 22 (□), and 46 years (△).

Although linear regression for the three grapevine ages combined showed a good fit of the data, the RMSE and bias of the modelled data were generally higher for younger grapevines (table 5.2). This was especially true for 5-year-old grapevines, for which the impact of measurement errors was magnified due to the small size of their trunk. The decrease in accuracy for 5-year-old grapevines may also be related to the higher difficulty of applying SfM-MVS on small objects such as slender tree branches (Kankare et al., 2013; Morgenroth and Gomez, 2014). The density of point clouds generated by the software depends on image resolution, and it is therefore affected by the camera and the distance at which the images are taken (James and Robson, 2012).

Table 5.2. Field-measured and modeled metrics for 5-year-old, 22-year-old, and 46-year-old grapevines (presented as mean  $\pm$  standard deviation) and the root mean square error (RMSE) and bias of modelled data.

Metric	Mean <sub>measured</sub>	Mean <sub>modeled</sub>	RMSE <sup>†</sup>	Bias <sup>†</sup>	RMSE (%)	Bias (%)
5-year-old vines						
$d_{row}$ (cm)	2.21 $\pm$ 0.29	2.40 $\pm$ 0.36	0.41	0.19	18.59	8.67
$d_{per}$ (cm)	2.21 $\pm$ 0.46	2.20 $\pm$ 0.40	0.31	0.00	14.19	-0.20
TCSA (cm <sup>2</sup> )	3.90 $\pm$ 1.10	4.16 $\pm$ 0.97	0.82	0.26	20.97	6.61
circumference (cm)	7.23 $\pm$ 0.96	7.18 $\pm$ 0.85	0.25	-0.04	3.40	-0.60
22-year-old vines						
$d_{row}$ (cm)	4.59 $\pm$ 0.40	4.67 $\pm$ 0.40	0.19	0.07	4.22	1.61
$d_{per}$ (cm)	4.50 $\pm$ 0.52	4.50 $\pm$ 0.49	0.17	0.00	3.79	0.02
TCSA (cm <sup>2</sup> )	16.34 $\pm$ 3.80	17.45 $\pm$ 2.81	1.85	1.11	11.34	6.80
circumference (cm)	14.85 $\pm$ 2.44	14.76 $\pm$ 1.18	0.28	-0.09	1.90	-0.59
46-years-old vines						
$d_{row}$ (cm)	5.97 $\pm$ 0.57	6.03 $\pm$ 0.62	0.14	0.07	2.42	1.12
$d_{per}$ (cm)	5.65 $\pm$ 0.62	5.69 $\pm$ 0.65	0.21	0.04	3.76	0.67
TCSA (cm <sup>2</sup> )	26.64 $\pm$ 4.36	28.62 $\pm$ 4.46	2.72	1.97	10.21	7.41
circumference (cm)	19.08 $\pm$ 1.87	18.91 $\pm$ 1.42	0.89	-0.17	4.69	-0.87

<sup>†</sup>Units for RMSE and bias are the same as their respective metric.

All metrics except trunk circumference were generally overestimated by the models, as shown by their positive bias. The RMSE values for  $d_{row}$  were consistently higher than for  $d_{per}$ , possibly because of systematic errors by the operator when measuring in the two positions with the digital caliper. Nevertheless, the level of accuracy obtained in this study was comparable to the previous literature that used SfM-MVS to assess tree size (Kankare et al., 2013; Liang et al.,



2014; Miller et al., 2015). The technique was also described as an alternative to Lidar (light detection and ranging) 3D scanning, which is frequently used in crop management (Rosell and Sanz, 2012).

#### 4.2. Exclusion of the grapevine crown

The total trunk volume obtained from SfM-MVS models varied widely across grapevine ages, with 46-year-old grapevine trunks being 1.7 times larger than 22-year-old grapevines trunks and more than 10 times larger than 5-year-old grapevine trunks (table 5.3). SfM-MVS makes it possible to divide objects into specific parts and analyze each of them independently. The crown volume fraction accounted for 35% of the total volume of 5-year-old grapevines. These grapevines had a uniform, close to cylindrical trunk shape, and their crown was barely formed. In contrast, the crown fraction represented close to 60% of the total volume of 22-year-old and 46 year-old grapevine trunks. It is worth noting that the increase in the relative crown volume mostly occurred in the early years of grapevine development and remained constant thereafter.

Table 5.3. Comparison between total trunk volume and trunk volume 40 cm above the grafting point and the volume fraction formed by the grapevine crown (data presented as mean  $\pm$  standard deviation).

Planting year	Vine age	Total trunk volume (cm <sup>3</sup> )	Trunk volume 40 cm above grafting point (cm <sup>3</sup> )	Crown volume (%)
2012	5 years	327.0 $\pm$ 64.2	210.5 $\pm$ 50.5	35.1 $\pm$ 12.5
1995	22 years	2120.7 $\pm$ 349.0	875.9 $\pm$ 129.5	57.8 $\pm$ 8.3
1971	46 years	3740.9 $\pm$ 729.3	1531.8 $\pm$ 246.6	58.1 $\pm$ 8.3

Yearly pruning alters the volume of perennial wood over time, generally increasing the size and height of the trunk. At the same time, pruning can lead to the formation of cones of desiccation that restrict sap flow and cause portions of the crown wood to die (Rolshausen et al., 2010). Removing the crown and setting the trunks to the same height would allow some control over the variability of the crown size from grapevine to grapevine.

The permanent grapevine structure, which includes the trunk and roots, comprises most of the vine's nutrient reserves. Knowledge of the trunk volume may be used for the estimation of vineyard biomass and carbon sequestration potential as outlined by Chiarawipa et al. (2013) and Miranda et al. (2017), with the added advantage of being relevant across pruning systems. Another possible application of trunk volume could be to improve upon the methods that assess

vegetative and generative capacity, as increased perennial wood is known to alter yield, fruit composition, and cold hardiness (Howell, 2001).

## **5. Conclusion**

SfM-MVS was able to build realistic 3D models of grapevine trunks of different ages while providing accurate estimates of trunk diameter, area, and circumference. It was also able to accommodate complex crown structures on old Guyot grapevines and assess the relative contribution of the crown to the total trunk volume. The technique is cost-effective and easy to apply in the field, although it struggles in shadowed environments and when the object captured does not generate enough points due to low image resolution. SfM-MVS is relatively recent and its development in the areas of automation and object detection will provide various new opportunities in forestry, ecology, or viticulture.

# Chapter 6. General discussion

*“I am not young enough to know everything.”*

*- Oscar Wilde -*

The age of perennial plants is seldom investigated in relationship to the quality of their crop, and grapevines are unique in this regard. The term ‘old vines’ is often used by producers as an extrinsic indication to convey wine quality, even though there are barely any regulations to manage its use. Despite past and ongoing research, objective evidence that older grapevines produce better wines is still debated and studies on robust, specifically dedicated plots are scarce. The objective of this work was therefore to expand the current knowledge on grapevine age and its relationship with fruit and wine quality. Unlike other studies, the one described here was performed in a single vineyard, where grapevines of the same rootstock/clone combination but three different planting years (1971, 1995, and 2012) were compared in the same microclimatic and soil conditions. Having three vine age groups that were each almost 20 years apart allowed a better understanding of whether some changes occurred early on in the life of the grapevine or in later years. This strategy was supported by the use of the grapevine age variable as a three level factor instead of a continuous scale. Furthermore, the youngest grapevines were followed since their first year after planting in order to determine whether their viticultural and enological potential would become similar to older groups in the first few years after planting.

Newly planted vineyards are usually subjected to particular management strategies to ensure proper vine establishment. The years 2014 and 2015 of this study were indeed marked by different management practices for grapevines planted in 2012. The removal of cover crop in their rows led to a higher nitrogen mineralization in the upper soil layers, which resulted in a higher nitrogen accumulation in their leaves and berries as shown by green fluorescence and berry analysis (Chapters 2 and 3; Giese et al., 2015; Habran et al., 2016). Over the same time period, the bud load of grapevines planted in 2012 was not sufficient to achieve the same number of shoots compared to grapevines planted in 1995 and 1971, which resulted in an overall lower vegetative and fruit production. Specifically, the canopy density in the cluster zone was significantly lower for vines planted in 2012 than for vines planted in 1995 and 1971 (Chapter 2). No differences were found in the number of berries or their weight among age groups, which led to the conclusion that most differences in early vintages were caused by the lower shoot number of vines planted in 2012. In terms of statistical analysis, the change in cover crop management and the gradual increase in productivity of the youngest grapevines until 2016 resulted in interactions between grapevine age and growing season in several LMMs. Part of this limitation could have been avoided if the soil management had been the same for all age

groups since the beginning of the trial, but doing so would have increased the risk that the cover competes too severely with young grapevines, stunting their development in the long term. It is therefore recommended to re-examine the data in the future by separating the 2014 and 2015 vintages from later ones.

Stomatal conductance and carbon isotope discrimination measurements suggested that grapevines planted in 2012 were more sensitive to water deficit as indicated in previous literature (Zufferey and Maigre, 2007). However, due to the frequent summer precipitations and perhaps the limited soil area that was covered around the grapevines, only a mild water stress was reported in a single growing season (Chapter 2). Seasonal variations present challenges in every field trial, and conducting this study over a longer period of time that includes more extreme weather conditions could potentially lead to greater significance in the results. One can hypothesize that in drier vintages, the more developed root system of old vines may be advantageous compared to younger grapevines. It is also worth noting that the trial was conducted in a deep loam-clay soil, where grapevines could exploit a large soil volume. If the experiment was performed in a shallower soil with a higher content of sand and coarse elements, the soil volume available for root colonization would have been limited, and older grapevines might have been more exposed to water deficit. In this situation the rooting density may have become a higher differentiator among grapevine age groups (Smart et al., 2006).

In order to have a better overview of the evolution of grapevine-soil interactions over time, future work on root distribution would greatly add to the value of the present study. Seguin (1972) and Champagnol (1984) suggested that depending on soil characteristics, young grapevines reach their maximal rooting distribution in the first decade after planting. They also suggested that further soil colonization happened mostly by increasing the root density in the already explored soil volume, a process that becomes less efficient as they grow older. However, literature on the long-term dynamics of grapevine rooting is limited, and these results have yet to be confirmed. In addition, it is possible that the aging of the root system may have less to do with vine age than with viticultural practices, with parameters such as soil compaction leading to losses in vegetative and fruit production by limiting root growth (Ferree and Streeter, 2004; Unger and Kaspar, 1994). The aging of the root system may also be influenced by soil microbiota, a topic that has been scarcely explored for grapevines (Schreiner and Mihara, 2009).

Technological parameters such as TSS, TA, and pH recorded at harvest were not influenced by grapevine age and depended mostly on weather conditions during the growing season. Due to the absence of cover crop in 2014 and 2015, the berry  $\alpha$ -amino acid content of grapevines planted in 2012 and single amino acid concentrations were significantly higher than for older grapevines (Chapters 3). At the same time the production of monoterpenes, norisoprenoids, and flavonols in berries was highest for grapevines planted in 2012. This may be correlated with the higher nitrogen concentration in berries, but also with the higher sun exposition of berries from these grapevines due to the thinner canopy (Friedel et al., 2016; Linsenmeier and Löhnertz, 2016; Schüttler et al., 2015). In the following years, once the soil management practices became identical for all three treatments and grapevines planted in 2012 matched the bud load of older grapevines, the previously reported differences decreased rapidly. At the end of the study, grapevines planted in 2012 had a similar berry composition than older grapevines.

The differences in grapevine physiology were indicative of wine sensory and chemical profiles. Nitrogen supply in the vineyard is known to influence the chemical and sensory profile of wine both directly and indirectly, whether through its involvement in the metabolic pathways of various aroma compounds in berries (Linsenmeier and Löhnertz, 2016) or by providing yeast with added nutrition (Bell and Henschke, 2005). Wines from grapevines planted in 2012 were deeper in color and were more concentrated in free and potential monoterpenes and norisoprenoids as well as volatile sulfur compounds in 2014 and 2015 (Chapter 4). Their aroma profiles were associated with ripeness, spices, and aging aromas. In 2016 the wines made from the three age groups had almost overlapping profiles, with a high agreement between sensory and chemical data in the MFA. When wines from vintages 2002 to 2013 were added to the comparison of chemical profiles, most of the variability among samples could be explained by the 'vintage' variable. This result may be due to various rearrangements that monoterpenes and norisoprenoids undergo during wine fermentation and storage (Robinson et al., 2014). From this point of view the trial could have benefited from the analysis of other aromatic compounds such as esters, which are typically associated with fruity notes (Maoz et al., 2018). Other alternatives to descriptive sensory analysis, for example triangular tests that compare the three grapevine age groups for every vintage, could have been undertaken. However triangular tests require a much higher wine volume due to the higher number of panelists needed and the pairwise comparisons of the samples. This would have not been possible with the low yield of the youngest grapevines in 2014. Nevertheless, descriptive analysis coupled with multivariate

techniques such as PCA and MFA has repeatedly proven its ability to reveal differences between wine products (Heymann and Noble, 1987; Le Dien and Pagès, 2003).

Throughout the study the main contrasting factor between grapevines planted in 1971 and 1995 was the higher incidence of wood diseases for the former group. Pruning wounds can become entry doors to the xylem-dwelling fungi that are responsible for the diseases (Rolshausen et al., 2010). The higher incidence of wood diseases for grapevines planted in 1971 resulted in a significant number of missing grapevines even before the start of the study. The decision was made not to replace missing grapevines early on in order to maintain the same grapevine age inside the rows. As a consequence the remaining grapevines were subjected to less competition than grapevines planted in 1995 (Bernizzoni et al., 2009). It was therefore difficult to rule out the possibility of obtaining different results if missing grapevines were still present. An alternative could have been to replace missing vines in 2012 when the youngest grapevines were planted. However, these grapevines due to their smaller root system would not have competed as intensely over resources as if they were of the same age. Furthermore, several vines planted in 1995 and 1971 were infected by trunk diseases and died after the beginning of the trial, and this would have still influenced the end results.

An important factor to consider when studying perennial plants over time is their increasing capacity to store reserves in wooden parts, reflected in the growing trunk and the expanding root system. Changes in the perennial wood and carbohydrate reserves are known to affect grapevine vegetative and reproductive growth. Research by Koblet and Perret (1982) and Reynolds et al. (1994) reported higher yields and improvements in technological maturity parameters for grapevines with a large amount of perennial wood. These results were in line with the findings of Grigg et al. (2017) who reported a correlation between vine productivity and size. Carbohydrate reserves play a significant role in the adaptability and ecological resilience of grapevines. Like other perennials, they rely on their overwintering carbon reserves to supply the nutrition necessary for growth reestablishment at the beginning of the season. This dependency only ceases around flowering, when leaves are sufficiently developed to meet the carbon needs of the grapevine (Zapata et al., 2004). Carbohydrate reserves can also be mobilized in response to environmental stresses such as adverse weather conditions or pathogens (Holzapfel et al., 2010). Contrary to expectations, grapevines planted in 1995 and 1971 displayed similar physiological characteristics at the single vine level, including yield, pruning weight, berry structure and composition, and ultimately wine profile. This was despite

the significant difference in their trunk size as measured in Chapters 2 and 5. Further experiments can be carried out to potentially isolate the effects of vine age from vine size. Some vines from the three age groups could be cut off and allowed to regrow, and then compared to vines without changes in above-ground storage capacity (Grigg, 2017).

The results of this thorough investigation on grapevine age go against the common belief that old grapevines produce wines that are different from young grapevines. While this study was centered on Riesling in a cool climate setting, there has been other research on the sensory aspects of grapevine age. Studies that focused on red cultivars reported higher fruit character for old grapevine wines and improved tannic structure (Heymann and Noble, 1987; Zufferey and Maigre, 2008) although results also depended on the cultivar studied (Reynolds et al., 2008). The dependence on cultivar was also found for white cultivars (Zufferey and Maigre, 2008).

A clear solution to the puzzle of old grapevines and wine quality is difficult to find. Site selection and experimental designs are often difficult to replicate, and the ages considered vary widely among studies. Finding old grapevines in a commercial setting can already be a problem, as the only grapevines that are not prematurely replaced are grapevines that already produce acceptable yield and wine quality. A large number of external factors can also have compound effects on grapevine physiology, from climate to soil but also pathogens and management practices (Duthie et al., 1991; Lider et al., 1978). Some old practices that were habitual in previous decades such as pruning with a wood saw instead of shears or relying on environmentally harmful pesticides may have been abandoned, but they can arguably leave a long-standing mark on grapevines that are still alive today.

Nevertheless, the project brings significant contributions to the body of knowledge regarding the effects of grapevine age. It has taken a multidisciplinary approach that combined physiological aspects with berry composition and wine sensory and chemical analysis. Furthermore, it has successfully applied new photogrammetric methods for the three-dimensional assessment of grapevine size, regardless of the complexity of its trunk. It has shown that in similar environmental conditions single grapevine yield, berry composition and wine profile of grapevines older than 40 years grapevines were equivalent to those of 22-year-old grapevines, and that only wood diseases were responsible for lower production associated with the older group. This constitutes a strong argument for managing vineyards with a long-term perspective to extend their longevity.



From a consumer perspective it is usually impossible to verify the age of grapevines or what percentage of the original vineyard had been replanted with new grapevines over time when tasting a wine made from ‘old vines’. Extrinsic attributes such as protected denominations of origins (PDO) create clear expectations about wine characteristics and style. PDOs are closely related with the concept of ‘typicality’ which allows to recognize a typical wine with respect to the expression of the terroir (Canuti et al., 2017). Regions that lack a traditional background in wine production have attempted to overcome the absence of established traditions that lend themselves to regional branding (Duarte Alonso and Northcote, 2009). The concept of old grapevines was first mentioned in an Old World country, but interestingly the only two regions to have codified its use, California in the USA and the Barossa Valley in Australia, are both in the New World.

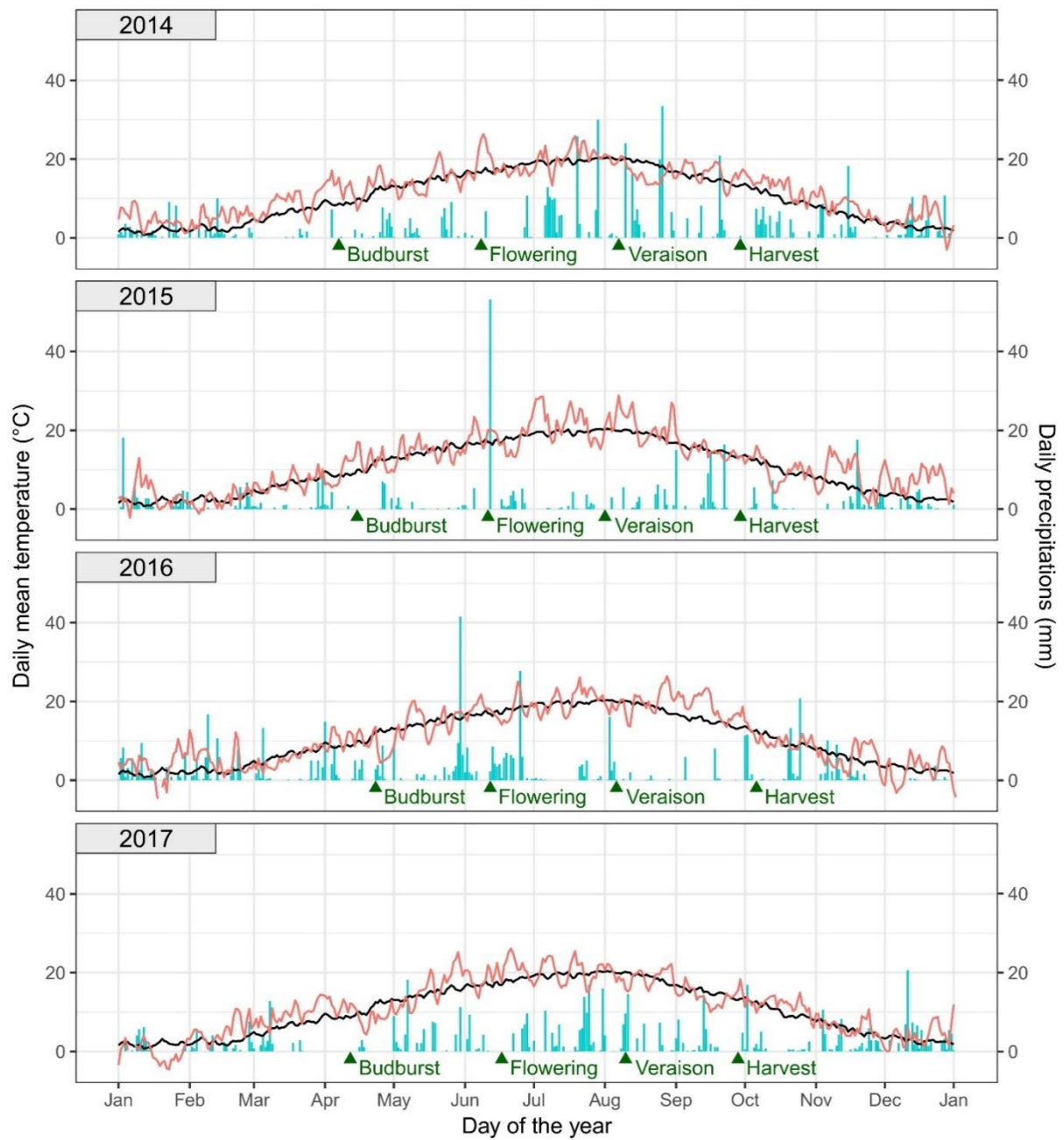
Instead of typicality, the term ‘old vine’ may be more associated with the concept of ‘authenticity’. As noted by Peterson (2005), concerns about authenticity are only brought up when authenticity is itself questioned. ‘Old vines’ can be found in all corners of the world, yet not all wines made from grapevines of a certain age carry the denomination. If a wine is qualitative enough and already representative of a region and a terroir, there should be little need to assert its authenticity. And even if grapevine age is actually an intrinsic attribute of wine quality, i.e. that grapevine age does indeed have an impact on wine quality, the current literature and the present work suggest that its effect would be less influential on wine profile than growing season, region, or management practices (Grigg et al., 2017; Heymann and Noble, 1987; Reynolds et al., 2008).

This is not to say that the term should be prohibited or that bottles that display it be treated with suspicion. In the end, it all comes down to whether the increasing costs of managing older grapevines, which have a compounding exposure to wood diseases and long-term stresses, are justly reflected in the price of the bottle. When presented with a bottle of ‘old vines’, consumers should not shy away from asking about the age of the grapevines and their history. Because even if there is no direct relationship between grapevine age and wine quality, the fact that they still produce wine definitely reflects the care and dedication that generations of viticulturists have given them.

# Appendices



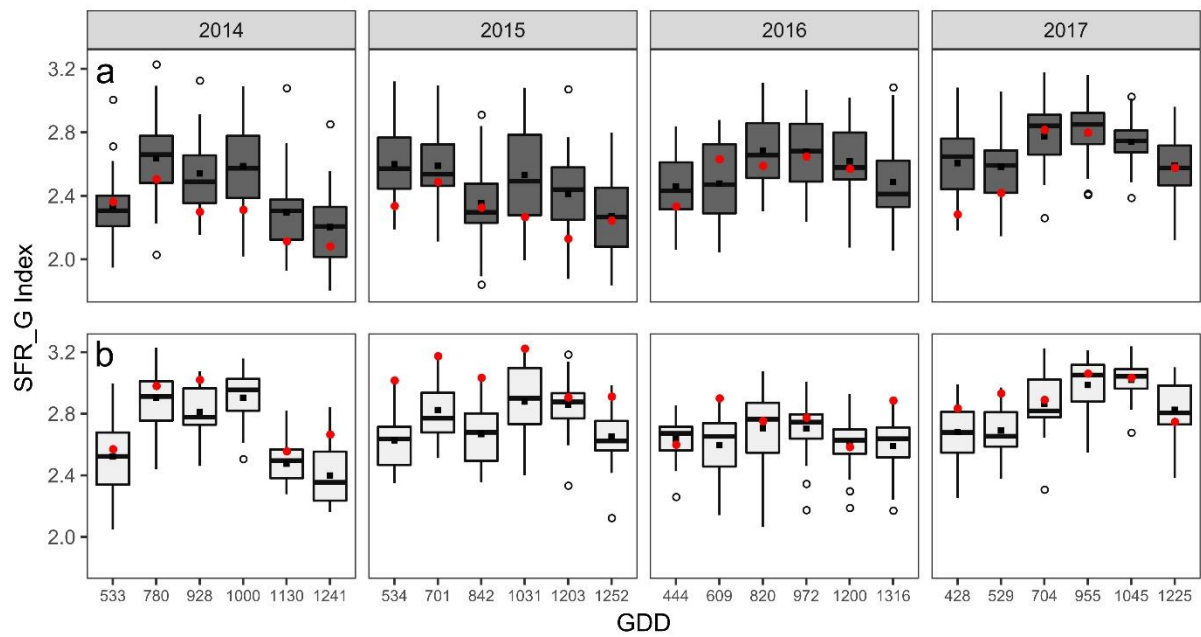
*Appendix 2.1. Images of the plastic covers when retracted and deployed (approximate surface of 20 m<sup>2</sup>) around the four vines with the “stress” treatment.*



Appendix 2.2. Daily mean temperatures, precipitations, and main phenological stages for the years 2014-2017. The black line represents the global average of daily temperatures from 1981 to 2010.

Appendix 2.3. LMM results for double Guyot including soil  $\text{NO}_3^-$  and water contents from 0-30 cm and 30-60 cm ( $n = 4$ ), leaf chlorophyll index, assimilation, stomatal conductance, transpiration ( $n = 24$ ), and carbon isotope discrimination ( $n = 4$ ). The data represents three age groups and four replicates over the growing seasons 2014-2017. Three measurements were made for soil  $\text{NO}_3^-$  and water contents per vintage (flowering, veraison, and harvest), while six measures were taken between 400 GDD and harvest for the chlorophyll index depending on weather conditions. Assimilation, stomatal conductance and transpiration measured four times during the season.

Variable	Num. df	Denom. df	F	P
<b>Soil <math>\text{NO}_3^-</math> at 0-30 cm</b>				
Planting year	2	133	22.08	< 0.001
Year	3	133	12.30	< 0.001
Planting year $\times$ Year	6	127	16.60	< 0.001
<b>Soil <math>\text{NO}_3^-</math> at 30-60 cm</b>				
Planting year	2	133	27.68	< 0.001
Year	3	133	13.33	< 0.001
Planting year $\times$ Year	6	127	9.55	< 0.001
<b>Soil water at 0-30 cm</b>				
Planting year	2	133	3.60	0.030
Year	3	133	21.87	< 0.001
Planting year $\times$ Year	6	127	2.96	0.0097
<b>Soil water at 30-60 cm</b>				
Planting year	2	133	8.87	< 0.001
Year	3	133	27.24	< 0.001
Planting year $\times$ Year	6	127	2.50	0.025
<b>SFR_G index</b>				
Planting year	2	142	17.33	< 0.001
Year	3	5081	198.77	< 0.001
Planting year $\times$ Year	6	5081	25.05	< 0.001
<b>A<sub>N</sub></b>				
Planting year	2	34	17.42	< 0.001
Year	1	236	221.31	< 0.001
Planting year $\times$ Year	2	233	89.36	< 0.001
<b>g<sub>s</sub></b>				
Planting year	2	34	25.19	< 0.001
Year	1	236	84.85	< 0.001
Planting year $\times$ Year	2	233	12.22	< 0.001
<b>E</b>				
Planting year	2	34	21.73	< 0.001
Year	1	236	0.47	0.49
Planting year $\times$ Year	2	233	17.51	< 0.001



Appendix 2.4. Leaf chlorophyll index (SFR\_G) for selected vines planted in 1971 (a) and 2012 (b) from 2014 to 2017. The red points represent the vines R#61-15 and R#56b-2, which were found to be positive for GLRaV-1 (grapevine leafroll-associated virus 1) and GFLV (grapevine fan-leaf virus) respectively.

Appendix 2.5. LMM results for double Guyot vine balance parameters for three age groups and four replicates over the growing seasons 2014-2017 ( $n = 4$ ).

Planting year	Num. df	Denom. df	F	P
<b>% gaps (PG)</b>				
Planting year	2	2	6.76	0.13
Year	2	22	3.17	0.062
Planting year $\times$ Year	4	18	3.56	0.026
<b>% interior leaves (PIL)</b>				
Planting year	2	2	6.98	0.13
Year	2	22	5.78	0.0096
Planting year $\times$ Year	4	18	4.85	0.079
<b>% interior clusters (PIC)</b>				
Planting year	2	2	1.16	0.46
Year	2	22	5.68	0.010
Planting year $\times$ Year	4	18	1.47	0.25
<b>Leaf layer number</b>				
Planting year	2	2	4.21	0.19
Year	2	22	0.21	0.81
Planting year $\times$ Year	4	18	6.03	0.029
<b>Cluster weight (g)</b>				
Planting year	2	2	3.74	0.21
Year	3	33	15.52	< 0.00
Planting year $\times$ Year	6	27	2.50	0.047
<b>Berry number (no./cluster)</b>				
Planting year	2	2	0.98	0.51
Year	3	33	32.52	< 0.00
Planting year $\times$ Year	6	27	2.68	0.035
<b>Berry weight (g)</b>				
Planting year	2	2	5.72	0.15
Year	3	33	53.08	< 0.00
Planting year $\times$ Year	6	27	2.99	0.023
<b>Fruitful bud number</b>				
Planting year	2	2	16.96	< 0.001
Year	3	33	5.49	0.0030
Planting year $\times$ Year	6	27	9.71	< 0.001
<b>Average cane weight</b>				
Planting year	2	2	2.56	0.12
Year	3	33	6.01	0.0018
Planting year $\times$ Year	6	27	2.24	0.063

Appendix 2.5. cont.

<b>Planting year</b>	<b>Num. df</b>	<b>Denom. df</b>	<b>F</b>	<b>P</b>
<b>Pruning weight</b>				
Planting year	2	2	8.72	< 0.001
Year	3	33	9.36	< 0.001
Planting year × Year	6	27	3.10	0.016
<b>Adjusted pruning weight</b>				
Planting year	2	2	17.94	< 0.001
Year	3	33	12.28	< 0.001
Planting year × Year	6	27	2.15	0.074
<b>Fruit yield</b>				
Planting year	2	2	8.77	< 0.001
Year	3	33	6.24	0.0014
Planting year × Year	6	27	12.01	< 0.001
<b>Adjusted fruit yield</b>				
Planting year	2	2	13.14	< 0.001
Year	3	33	8.40	< 0.001
Planting year × Year	6	27	14.13	< 0.001
<b>Yield/pruning weight</b>				
Planting year	2	2	5.08	< 0.001
Year	3	33	7.56	< 0.001
Planting year × Year	6	27	12.18	< 0.001

Appendix 2.6. LMM results for double Guyot vine balance parameters for vines planted in 1995 and 1971 with four replicates over the growing seasons 2014-2017 ( $n = 4$ ).

Planting year	Num. df	Denom. df	F	P
<b>Fruitful bud number</b>				
Planting year	1	24	0.75	0.40
Year	3	24	12.45	< 0.001
Planting year $\times$ Year	3	21	2.91	0.059
<b>Average cane weight</b>				
Planting year	1	24	3.71	0.066
Year	3	24	11.84	< 0.001
Planting year $\times$ Year	3	21	1.37	0.28
<b>Pruning weight</b>				
Planting year	1	24	20.53	< 0.001
Year	3	24	4.01	0.019
Planting year $\times$ Year	3	21	0.24	0.87
<b>Adjusted pruning weight</b>				
Planting year	1	24	4.25	0.051
Year	3	24	8.13	< 0.001
Planting year $\times$ Year	3	21	0.14	0.93
<b>Fruit yield</b>				
Planting year	1	24	18.70	< 0.001
Year	3	24	5.89	< 0.001
Planting year $\times$ Year	3	21	2.34	0.061
<b>Adjusted fruit yield</b>				
Planting year	1	24	0.0008	0.98
Year	3	24	11.62	< 0.001
Planting year $\times$ Year	3	21	4.72	0.011
<b>Yield/pruning weight</b>				
Planting year	1	24	1.91	0.18
Year	3	24	19.76	< 0.001
Planting year $\times$ Year	3	21	2.86	0.061



Appendix 2.7. Eigen values and Eigen vectors from principal component analysis presented in Figure 2.7.

<b>Eigenvalues</b>	<b>PC 1</b>	<b>PC 2</b>	<b>PC 3</b>	<b>PC 4</b>	<b>PC 5</b>	<b>PC 6</b>	<b>PC 7</b>	<b>PC 8</b>
Eigenvalue	5.817	2.509	1.506	0.55	0.398	0.115	0.081	0.023
Variance (%)	52.886	22.808	13.695	5.004	3.619	1.043	0.733	0.212
Cumulative (%)	52.886	75.694	89.389	94.393	98.012	99.055	99.788	100
<b>Eigenvectors</b>								
ClusterWeight	0.8958	-0.2858	0.3276	0.0769	0.0286	0.0205	-0.0171	-0.0343
TotalBerries	0.7627	-0.4000	0.4568	0.0682	-0.0996	0.0799	-0.1562	0.0653
BerryWeight	0.3987	-0.3459	-0.8085	0.1394	0.1353	0.1570	0.0530	0.0506
PG	-0.8028	0.0665	0.3718	0.3422	0.3058	0.0285	0.0218	-0.0304
LLN	0.8297	0.3571	-0.2867	-0.3037	-0.0729	-0.0405	-0.0215	-0.0477
PIL	0.5102	0.7333	-0.1922	0.0320	0.3698	-0.1062	-0.1216	0.0345
PIC	0.3439	0.7848	0.3917	-0.2243	0.0883	0.2238	0.0637	0.0000
AdjWoodMeter	0.8829	-0.4058	-0.1135	0.0871	0.1597	0.0482	-0.0202	-0.0842
AdjYieldMeter	0.9355	0.1358	0.0146	0.3007	-0.1065	-0.0448	0.0445	-0.0230
RavazIndex	0.7921	0.4953	0.1293	0.2677	-0.1274	-0.0694	0.1265	0.0432
CaneWeight	0.5301	-0.6584	0.3101	-0.3002	0.2694	-0.1016	0.1223	0.0378
FruitfulBuds	0.8344	-0.060	-0.3340	-0.3178	0.1481	-0.1010	-0.0444	-0.2070

Appendix 2.8. LMM results for double Guyot berry technological maturity for the three age groups and four replicates over the growing seasons 2014-2017 ( $n = 4$ ).

<b>Planting year</b>	<b>Num. df</b>	<b>Denom. df</b>	<b>F</b>	<b>P</b>
<b>Total soluble solids</b>				
Planting year	2	2	0.46	0.68
Year	3	33	44.0	<0.001
Planting year $\times$ Year	6	27	3.62	0.0092
<b>Titrateable acidity</b>				
Planting year	2	2	9.51	0.098
Year	3	33	51.64	<0.001
Planting year $\times$ Year	6	27	1.54	0.2
<b>pH</b>				
Planting year	2	2	15.45	0.06
Year	3	33	15.69	<0.001
Planting year $\times$ Year	6	27	1.72	0.14
<b><math>\alpha</math>-amino acids</b>				
Planting year	2	2	17.80	0.055
Year	3	33	13.14	<0.001
Planting year $\times$ Year	6	27	32.14	<0.001
<b><math>\delta^{13}\text{C}</math></b>				
Planting year	2	17	1.25	0.31
Year	1	17	8.8	0.0084
Planting year $\times$ Year	2	15	0.43	0.66

Appendix 2.9. LMM results for the water deficit trial on cordon vines. Soil water content from 0-30 cm and 30-60 cm ( $n = 4$ ), leaf chlorophyll index ( $n = 8$ ), stomatal conductance ( $n = 8$ ), predawn water potential ( $n = 8$ ), and carbon isotope discrimination ( $n = 4$ ). The data represents three age groups, two water deficit treatments, and four replicates over the growing seasons 2016-2017. Three measurements were made for soil water content per vintage (flowering, pea size, and two weeks after veraison). Six measures were taken between 400 GDD and harvest for chlorophyll index and stomatal conductance in 2016 and seven measures in 2017, depending on weather conditions. Four measures were taken for predawn water potential during the same period. Carbon isotope discrimination was measured on berry samples at harvest.

	Planting year	Num. df	Denom. df	F	P
<b>Soil water at 0-30 cm</b>					
	Planting year	2	134	4.60	0.012
	Year	1	134	74.90	< 0.001
	Water status	1	134	87.48	< 0.001
	Planting year $\times$ Year	2	129	1.98	0.14
	Planting year $\times$ Water status	2	129	0.95	0.39
	Year $\times$ Water status	1	129	4.40	0.038
	Planting year $\times$ Year $\times$ Water status	2	127	0.024	0.98
<b>Soil water at 30-60 cm</b>					
	Planting year	2	134	1.75	0.18
	Year	1	134	86.38	< 0.001
	Water status	1	134	34.38	< 0.001
	Planting year $\times$ Year	2	129	0.0016	1
	Planting year $\times$ Water status	2	129	0.69	0.51
	Year $\times$ Water status	1	129	0.45	0.54
	Planting year $\times$ Year $\times$ Water status	2	127	1.26	0.29
<b>SFR_G index</b>					
	Planting year	2	43	2.59	0.086
	Year	1	1680	1.67	0.20
	Water status	1	43	0.03	0.87
	Planting year $\times$ Year	2	1677	5.67	0.0035
	Planting year $\times$ Water status	2	41	0.74	0.48
	Year $\times$ Water status	1	1677	3.28	0.070
	Planting year $\times$ Year $\times$ Water status	2	1676	4.93	0.0073
<b>g<sub>s</sub></b>					
	Planting year	2	571	42.92	< 0.001
	Year	1	571	187.18	< 0.001
	Water status	1	571	9.81	0.0018
	Planting year $\times$ Year	2	566	0.81	0.45
	Planting year $\times$ Water status	2	566	3.68	0.026
	Year $\times$ Water status	1	566	4.28	0.039
	Planting year $\times$ Year $\times$ Water status	2	564	3.77	0.02
<b><math>\Psi_{PD}</math></b>					
	Planting year	2	41	10.40	< 0.001
	Year	1	332	51.21	< 0.001
	Water status	1	41	10.39	0.0025
	Planting year $\times$ Year	2	329	0.015	0.99
	Planting year $\times$ Water status	2	39	2.62	0.085
	Year $\times$ Water status	1	329	3.52	0.062
	Planting year $\times$ Year $\times$ Water status	2	327	1.65	0.19

Appendix 2.9. cont.

	<b>Planting year</b>	<b>Num. df</b>	<b>Denom. df</b>	<b>F</b>	<b>P</b>
$\delta^{13}\text{C}$					
	<b>Planting year</b>	2	40	13.37	< 0.001
	<b>Year</b>	1	40	16.61	< 0.001
	<b>Water status</b>	1	40	1.08	0.31
	<b>Planting year <math>\times</math> Year</b>	2	35	1.93	0.16
	<b>Planting year <math>\times</math> Water status</b>	2	35	1.75	0.19
	<b>Year <math>\times</math> Water status</b>	1	35	1.60	0.21
	<b>Planting year <math>\times</math> Year <math>\times</math> Water status</b>	2	33	0.19	0.83

Appendix 3.1. Eigenvalues and eigenvectors from principal component analysis presented in Figure 3.2.

<b>Eigenvalue</b>	<b>PC 1</b>	<b>PC 2</b>	<b>PC 3</b>	<b>PC 4</b>	<b>PC 5</b>	<b>PC 6</b>	<b>PC 7</b>	<b>PC 8</b>	<b>PC 9</b>
Eigenvalue	12.903	8.371	3.648	3.074	1.887	1.327	0.874	0.678	0.555
Variance (%)	35.841	23.254	10.134	8.540	5.241	3.685	2.426	1.883	1.543
Cumulative (%)	35.841	59.095	69.229	77.768	83.009	86.694	89.121	91.004	92.546
<b>Eigenvector</b>									
SkinDryWeight	-0.407	0.849	0.024	-0.020	0.124	0.014	0.127	0.122	-0.068
Fructose	-0.262	0.269	0.468	0.186	0.753	-0.007	0.026	0.086	-0.107
Glucose	-0.190	0.413	0.279	0.100	0.797	-0.052	0.056	0.138	-0.057
Mal	-0.353	0.669	0.334	0.308	-0.301	-0.078	0.091	-0.093	0.169
Tar	-0.343	0.380	0.433	-0.433	-0.255	-0.087	0.111	0.280	0.272
Asp	0.649	-0.137	-0.612	-0.283	0.133	0.071	-0.012	0.147	0.016
Glu	0.660	0.347	-0.490	-0.335	0.160	0.118	-0.051	0.138	0.049
Asn	0.654	0.543	0.312	0.155	-0.037	-0.038	-0.199	-0.043	0.045
Ser	0.828	0.341	0.044	0.211	0.184	0.230	0.032	0.022	0.148
Gly	0.214	-0.617	0.041	0.214	0.205	0.072	0.547	0.031	0.249
Gln	0.853	0.445	0.045	0.050	-0.044	0.090	-0.114	-0.009	0.019
Arg	0.806	0.341	0.198	0.249	-0.210	0.118	-0.053	-0.005	0.035
Thr	0.907	0.257	-0.023	0.140	-0.124	0.041	-0.046	0.027	0.121
Ala	0.532	-0.260	0.091	0.570	-0.147	0.373	0.220	-0.053	0.008
Pro	0.560	0.319	-0.479	0.398	0.099	0.025	0.109	-0.094	0.129
Tyr	0.830	-0.229	0.033	0.302	0.237	-0.112	-0.048	0.109	0.118
Val	0.899	0.007	0.107	0.243	-0.054	0.240	0.064	0.127	-0.133
Met	0.619	-0.055	0.106	0.492	-0.072	-0.496	-0.162	0.213	-0.078
Ileu	0.755	0.021	0.129	-0.002	-0.201	0.449	0.101	0.107	-0.275
Leu	0.575	0.258	-0.202	0.500	-0.128	-0.432	-0.066	0.199	0.068
Phe	0.470	0.729	0.314	0.089	-0.108	-0.088	-0.016	0.010	-0.190
F.Linalool	0.464	-0.788	-0.052	0.055	0.003	-0.054	-0.006	0.020	-0.266
B.Linalooloxide1	0.804	0.235	0.235	-0.309	0.106	-0.117	-0.007	-0.280	0.045
B.Neroloxide	0.718	-0.170	0.508	-0.316	-0.027	-0.177	0.058	-0.164	-0.006
B.Linalooloxide2	0.780	0.063	0.306	-0.311	0.120	-0.206	0.025	-0.259	0.017
B.Vitispirane	0.418	-0.462	0.694	-0.196	-0.097	-0.022	0.129	0.068	-0.066
B.aTerpineol	0.427	-0.795	0.284	-0.084	-0.062	-0.087	-0.003	0.121	-0.037
B.TDN	0.519	-0.641	0.370	-0.300	-0.009	-0.167	0.194	0.079	0.018
Brix	-0.237	0.779	0.278	-0.027	0.064	0.278	-0.138	-0.048	0.026
pH	0.719	-0.456	-0.327	-0.189	0.112	-0.038	-0.060	-0.066	0.051
TotalAcidity	-0.693	0.230	0.430	0.431	-0.118	-0.001	0.111	-0.064	-0.013
BerryWeight	-0.223	-0.628	-0.264	0.512	0.143	-0.140	0.001	-0.208	0.032
NOPAFresh	0.762	0.367	-0.052	-0.136	0.219	-0.013	0.001	-0.282	-0.002
Querc.3.gal	-0.408	-0.692	0.283	0.416	0.031	0.074	-0.151	-0.117	0.004
Querc.3.glucuronide	-0.284	-0.686	0.340	0.268	0.094	0.224	-0.360	-0.033	0.099
Querc.3.glc	0.180	-0.608	0.364	-0.209	0.135	0.277	-0.344	0.149	0.255

Appendix 3.2. Mean values per cluster from the k-mean clustering in Figure 3.3.

Variable	Cluster 1	Cluster 2	Cluster 3	Cluster 4
SkinDryWeight	0.630	1.533	-0.867	-0.289
Fructose	0.272	0.329	-0.583	0.279
Glucose	0.344	0.584	-0.396	-0.089
Mal	1.016	0.662	-1.126	0.317
Tar	0.357	0.903	-0.672	-0.004
Asp	-0.046	-0.276	1.232	-1.052
Glu	0.874	0.253	0.798	-1.310
Asn	2.041	-0.330	-0.216	-0.442
Ser	1.644	-0.509	0.272	-0.659
Gly	-0.574	-0.997	0.450	0.371
Gln	1.822	-0.369	0.251	-0.794
Arg	1.741	-0.628	0.079	-0.438
Thr	1.445	-0.550	0.429	-0.710
Ala	0.451	-1.182	0.217	0.285
Pro	1.021	-0.294	0.347	-0.601
Tyr	0.565	-1.177	0.748	-0.297
Val	1.113	-0.885	0.411	-0.359
Met	0.497	-0.817	0.250	0.020
Ileu	1.016	-0.584	0.286	-0.369
Leu	0.884	-0.369	0.173	-0.326
Phe	1.886	0.294	-0.503	-0.454
F.Linalool	-0.773	-1.339	0.900	0.204
B.Linalooloxide1	1.546	-0.483	0.323	-0.685
B.Neroloxide	0.953	-0.954	0.318	-0.158
B.Linalooloxide2	1.172	-0.606	0.373	-0.507
B.Vitispirane	0.186	-1.028	0.117	0.405
B.aTerpineol	-0.658	-1.284	0.681	0.342
B.TDN	-0.278	-1.080	0.617	0.129
Brix	1.261	0.984	-0.947	-0.152
pH	0.108	-1.108	1.147	-0.545
TotalAcidity	-0.216	0.552	-1.181	0.949
BerryWeight100	-1.224	-0.920	0.363	0.684
NOPAFresh	1.774	-0.559	0.443	-0.856
Querc.3.gal	-1.100	-0.968	-0.165	1.188
Querc.3.glucuronide	-1.000	-0.855	-0.047	0.963
Querc.3.glc	-0.648	-0.611	0.387	0.239

Appendix 3.3. Eigenvalues and eigenvectors from principal component analysis presented in Figure 3.4.

<b>Eigenvalues</b>	<b>PC 1</b>	<b>PC 2</b>	<b>PC 3</b>	<b>PC 4</b>	<b>PC 5</b>	<b>PC 6</b>	<b>PC 7</b>	<b>PC 8</b>	<b>PC 9</b>
Eigenvalues	5.126	3.098	0.687	0.454	0.364	0.095	0.076	0.058	0.027
Variance (%)	51.266	30.983	6.877	4.541	3.643	0.952	0.761	0.581	0.273
Cumulative (%)	51.266	82.249	89.126	93.667	97.311	98.263	99.024	99.606	99.880
<b>Eigenvectors</b>									
F.Linalool	0.722	0.289	-0.487	0.373	0.049	-0.087	0.002	0.084	0.005
B.Linalooloxide1	0.562	-0.764	0.176	0.184	0.157	-0.017	0.054	-0.007	0.002
B.Neroloxide	0.869	-0.428	0.109	-0.122	0.104	0.034	0.042	0.073	-0.114
B.Linalooloxide2	0.690	-0.660	0.132	0.139	0.175	0.059	-0.054	-0.059	0.077
B.Vitispirane	0.891	0.060	0.020	-0.406	0.024	-0.172	0.035	0.030	0.060
B.aTerpineol	0.881	0.312	-0.246	-0.037	-0.124	0.084	0.140	-0.141	-0.007
B.TDN	0.943	0.010	-0.161	-0.133	-0.133	0.080	-0.202	-0.006	-0.013
Querc.3.gal	0.189	0.908	0.069	-0.082	0.308	0.157	0.029	0.077	0.036
Querc.3.glucuronide	0.320	0.855	0.283	0.125	0.194	-0.119	-0.068	-0.105	-0.049
Querc.3.glc	0.675	0.391	0.465	0.197	-0.359	0.019	0.027	0.064	0.023

Appendix 4.1. ANOVA P-values from the sensory descriptive analysis in 2017.

Attribute	Assessor	Replicate				
	(A)	Wine (W)	(R)	A × W	W × R	A × R
color intensity	< 0.001	< 0.001	0.624	0.001	0.046	< 0.001
citrus	< 0.001	0.199	0.544	0.217	0.352	0.002
peach	< 0.001	0.029	0.955	< 0.001	0.033	< 0.001
dry fruit	< 0.001	< 0.001	0.240	0.009	0.015	0.583
rose	< 0.001	0.508	0.003	0.054	0.624	0.043
vegetative	< 0.001	0.001	0.845	0.389	0.128	< 0.001
spice	< 0.001	< 0.001	0.067	0.127	0.951	0.401
aging/kerosene	< 0.001	< 0.001	0.024	0.002	0.887	0.422
burnt rubber	< 0.001	0.019	0.543	0.02	0.056	0.053
reductive	< 0.001	< 0.001	0.984	0.001	0.773	0.007
sourness	< 0.001	< 0.001	0.954	< 0.001	0.731	0.001
sweetness	< 0.001	< 0.001	0.018	< 0.001	0.793	0.382
bitterness	< 0.001	0.19	0.60	0.028	0.729	< 0.001
overall	< 0.001	< 0.001	0.124	< 0.001	0.118	0.399

Appendix 4.2. Eigenvalues and eigenvectors from principal component analysis presented in Figure 4.1

Eigenvalue	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8
Eigenvalue	9.398	2.048	1.157	0.832	0.228	0.213	0.094	0.030
Variance (%)	67.129	14.628	8.266	5.944	1.629	1.519	0.673	0.214
Cumulative (%)	67.129	81.756	90.022	95.966	97.595	99.114	99.786	100.000
<b>Eigenvector</b>								
Color	0.832	-0.301	-0.130	0.404	0.164	0.073	0.063	0.016
Citrus	-0.472	0.838	0.099	-0.081	0.201	-0.110	0.042	-0.073
Peach	0.931	0.189	0.177	0.139	-0.178	-0.003	-0.123	0.013
DryFruit	0.992	0.003	-0.045	0.055	0.038	0.090	0.028	0.008
Rose	-0.452	0.439	0.491	0.572	-0.156	0.087	0.055	-0.017
Vegetal	-0.947	-0.055	0.125	0.190	0.181	-0.038	-0.029	0.116
Spices	0.756	0.539	0.119	-0.283	-0.047	0.033	0.185	0.081
Aging	0.973	-0.028	-0.225	-0.002	0.006	0.005	0.033	-0.017
Reductive	-0.793	-0.520	0.084	-0.210	-0.163	0.103	0.110	-0.019
BurntRubber	-0.871	-0.436	0.182	0.062	0.058	0.011	0.100	-0.028
Sourness	-0.768	0.428	-0.251	-0.141	0.035	0.372	-0.071	0.007
Sweetness	0.895	-0.236	0.283	0.041	0.178	0.165	0.007	-0.046
Bitterness	-0.500	0.172	-0.764	0.356	-0.071	-0.026	0.072	-0.011
Impression	0.992	0.066	-0.099	0.016	-0.030	-0.008	0.030	-0.008



Appendix 4.3. Concentrations of free and potentially volatile monoterpenes and norisoprenoids ( $\mu\text{g L}^{-1}$ ) measured in available wines from 2002 to 2016.

Wine	$\alpha$ -terpineol	hotrienol	<i>cis</i> -linalool oxide	<i>trans</i> -linalool oxide	TDN
<b>Free monoterpenes and norisoprenoids</b>					
2002-O	34.0	64.8	133.0	64.8	7.5
2002-M	37.4	63.8	122.0	60.5	8.8
2003-O	31.3	69.2	136.0	67.9	14.6
2003-M	22.1	65.3	128.2	66.4	12.4
2004-O	9.8	29.2	113.9	60.1	6.0
2004-M	10.9	33.5	117.0	59.6	10.1
2005-O	82.7	75.9	127.5	65.5	1.5
2005-M	77.2	66.7	113.7	56.0	1.6
2007-O	31.6	80.7	107.4	57.6	4.6
2007-M	27.1	68.4	124.5	63.2	6.0
2010-O	36.7	57.0	83.4	42.0	4.2
2010-M	39.3	70.3	92.6	47.7	4.8
2011-O	65.0	90.0	127.2	62.4	13.1
2011-M	59.9	83.6	121.8	63.4	9.0
2012-O	85.4	104.4	106.5	47.9	2.8
2012-M	75.0	113.4	113.3	53.3	6.4
2013-O	28.1	50.7	50.7	22.4	3.5
2013-M	24.3	59.0	57.8	26.0	5.2
2014-O	54.9	145.8	70.0	33.1	3.1
2014-M	45.9	132.2	70.0	33.5	3.4
2014-Y	28.6	125.2	56.4	25.9	3.8
2015-O	29.9	121.4	53.2	24.6	4.3
2015-M	191.9	128.6	77.1	38.2	4.5
2015-Y	60.8	107.0	66.7	28.5	5.2
2016-O	55.5	90.1	23.2	11.5	1.8
2016-M	54.7	79.7	22.7	11.4	2.1
2016-Y	73.2	62.9	23.4	11.5	1.9
<b>Potentially volatile monoterpenes and norisoprenoids</b>					
2002-O	15.6	n.d.	n.d.	10.1	6.1
2002-M	18.3	n.d.	n.d.	10.8	9.0
2003-O	11.0	n.d.	n.d.	9.0	7.9
2003-M	8.7	n.d.	n.d.	8.3	7.3
2004-O	6.8	n.d.	n.d.	9.0	0.8
2004-M	6.6	n.d.	n.d.	8.4	3.4
2005-O	12.9	n.d.	n.d.	9.6	3.9
2005-M	13.5	n.d.	n.d.	9.1	0.7
2007-O	9.3	n.d.	n.d.	8.9	0.8
2007-M	8.7	n.d.	n.d.	9.1	3.7
2010-O	19.8	n.d.	n.d.	9.7	7.2
2010-M	21.5	n.d.	n.d.	11.5	8.6
2011-O	15.6	n.d.	n.d.	10.4	11.4
2011-M	17.5	n.d.	n.d.	10.5	11.2
2012-O	22.1	n.d.	n.d.	7.9	6.6
2012-M	19.3	n.d.	n.d.	8.3	8.3
2013-O	21.9	n.d.	n.d.	7.9	9.5
2013-M	17.0	n.d.	n.d.	7.9	9.6

Appendix 4.3. cont.

<b>Wine</b>	<b><math>\alpha</math>-terpineol</b>	<b>hotrienol</b>	<b><i>cis</i>-linalool oxide</b>	<b><i>trans</i>-linalool oxide</b>	<b>TDN</b>
<b>Potentially volatile monoterpenes and norisoprenoids</b>					
2014-O	23.7	n.d.	n.d.	8.1	11.2
2014-M	19.7	n.d.	n.d.	7.7	10.9
2014-Y	82.2	n.d.	n.d.	10.1	19.6
2015-O	20.6	n.d.	n.d.	8.1	9.7
2015-M	18.4	n.d.	n.d.	7.8	10.1
2015-Y	38.0	n.d.	n.d.	9.3	14.0
2016-O	26.9	n.d.	n.d.	7.6	11.3
2016-M	28.0	n.d.	n.d.	8.3	14.1
2016-Y	35.6	n.d.	n.d.	8.7	17.9

Appendix 4.4. Volatile sulfur compounds ( $\mu\text{g L}^{-1}$ ) measured in available wines from 2002 to 2016. Only  $\text{H}_2\text{S}$  and DMS were detected in wine.

<b>Wine</b>	<b><math>\text{H}_2\text{S}</math></b>	<b>DMS</b>
2002-O	7.8	31.2
2002-M	15.5	27.6
2003-O	10.2	23.5
2003-M	20.0	20.6
2004-O	24.1	36.8
2004-M	16.7	33.1
2005-O	29.0	28.3
2005-M	25.9	25.7
2007-O	32.3	28.7
2007-M	27.8	25.3
2010-O	7.8	18.4
2010-M	8.6	19.2
2011-O	17.7	15.5
2011-M	10.6	13.7
2012-O	26.4	18.8
2012-M	20.7	17.1
2013-O	24.5	16.8
2013-M	7.7	13.8
2014-O	11.8	10.2
2014-M	21.9	10.1
2014-Y	26.1	11.7
2015-O	12.0	7.3
2015-M	10.3	6.5
2015-Y	27.5	10.8
2016-O	6.4	4.2
2016-M	6.0	3.7
2016-Y	9.6	4.4

Appendix 4.5. Chemical compounds by <sup>1</sup>H NMR and CIELab parameters measured in available wines from 2002 to 2016.

Wine	Butane- diol	Phenyl- ethanol	Methyl- butanol	Acet- aldehyde	Caftaric Acid	Ethyl- acetate	Galactu- ronic Acid	Glycerol	Methanol	Succinic Acid	L*	a*	b*
2002-O	300	53.0	132	18.8	19.5	n.d.	467	6534	102	453	96.85	-2.50	20.04
2002-M	265	62.4	141	26.5	27.0	n.d.	478	6530	92	471	96.39	-2.29	20.82
2003-O	461	61.2	157	19.5	24.6	n.d.	362	7524	56	576	98.55	-2.17	11.71
2003-M	518	85.9	182	17.9	28.0	n.d.	343	7512	51	657	98.34	-2.01	12.11
2004-O	256	n.d.	n.d.	20.9	n.d.	n.d.	364	6011	87	214	98.14	-2.54	14.41
2004-M	248	n.d.	n.d.	29.6	n.d.	n.d.	348	5584	99	216	97.65	-2.07	15.23
2005-O	408	27.8	127	20.1	19.8	n.d.	425	8840	52	498	97.85	-2.79	16.10
2005-M	349	44.9	152	12.6	21.5	n.d.	414	8486	53	528	98.30	-2.72	14.23
2007-O	365	n.d.	n.d.	38.8	n.d.	n.d.	243	6659	57	263	99.41	-1.30	6.16
2007-M	279	n.d.	110	34.2	16.0	n.d.	246	6565	60	327	99.28	-1.39	6.94
2010-O	217	44.0	152	49.3	29.6	n.d.	661	7587	87	441	99.38	-1.27	6.49
2010-M	237	38.7	133	49.3	29.0	n.d.	615	7000	100	371	99.19	-1.46	7.34
2011-O	285	70.3	163	25.9	32.3	50.6	513	9302	56	570	98.78	-1.72	9.33
2011-M	264	69.4	149	31.9	40.3	52.5	463	8392	49	536	99.03	-1.55	8.16
2012-O	328	73.9	209	23.7	39.3	n.d.	525	7138	84	718	98.47	-1.97	11.31
2012-M	389	93.4	179	26.3	40.4	n.d.	493	6638	83	626	98.81	-1.86	9.59
2013-O	225	36.0	118	36.4	44.7	n.d.	475	5505	76	412	99.30	-1.45	6.77
2013-M	191	55.0	109	33.7	48.3	n.d.	451	5363	67	386	99.48	-1.23	5.50
2014-O	300	25.5	104	36.3	44.2	59.5	576	5228	63	447	99.27	-1.01	5.69
2014-M	258	27.9	108	32.0	42.4	52.0	468	5006	57	489	99.41	-0.99	4.95
2014-Y	272	31.5	127	26.6	42.7	60.1	476	6030	51	629	98.63	-1.58	9.59
2015-O	261	35.6	141	23.6	40.7	60.5	476	6165	55	660	99.14	-1.07	6.16
2015-M	261	n.d.	112	31.4	41.5	54.5	764	6126	86	504	99.27	-0.98	5.51
2015-Y	547	n.d.	100	53.4	50.7	85.6	720	8196	66	479	98.70	-1.56	9.54
2016-O	306	31.3	137	19.8	44.2	n.d.	282	5507	36	710	99.17	-1.01	5.89
2016-M	301	31.8	131	21.5	46.4	n.d.	266	5299	36	624	99.40	-0.89	4.84
2016-Y	299	31.9	125	24.1	44.5	n.d.	254	5120	36	603	99.54	-0.81	4.09

Appendix 4.6. Organic acids measured by HPLC ( $g L^{-1}$ ) in available wines from 2002 to 2016.

<b>Wine</b>	<b>Tartaric Acid</b>	<b>Malic Acid</b>	<b>Shikimic Acid</b>	<b>Lactic Acid</b>	<b>Acetic Acid</b>	<b>Citric Acid</b>
2002-O	1.96	2.60	52.63	0.16	0.25	0.11
2002-M	2.00	2.43	51.12	0.14	0.22	0.10
2003-O	2.41	1.27	32.66	0.11	0.28	0.11
2003-M	2.78	0.97	28.68	0.13	0.23	n.d.
2004-O	2.66	2.33	46.35	0.15	0.34	n.d.
2004-M	2.70	2.36	43.63	0.14	0.30	0.11
2005-O	1.64	1.79	37.15	0.22	0.28	n.d.
2005-M	1.67	1.65	37.10	0.23	0.26	0.13
2007-O	2.97	1.59	43.30	0.58	0.45	0.12
2007-M	2.97	1.94	45.39	0.19	0.34	0.12
2010-O	0.96	4.21	67.41	0.24	0.34	0.14
2010-M	0.98	4.64	70.71	0.18	0.29	0.14
2011-O	2.95	1.49	39.75	0.11	0.41	0.11
2011-M	3.10	1.40	39.24	n.d.	0.46	0.12
2012-O	1.88	2.16	51.37	0.16	0.30	0.14
2012-M	2.22	1.91	53.09	0.12	0.29	0.12
2013-O	1.16	3.84	58.35	n.d.	0.40	0.15
2013-M	1.82	3.74	61.02	n.d.	0.38	0.14
2014-O	2.37	2.67	57.21	0.11	0.49	0.12
2014-M	2.84	2.80	54.00	0.10	0.38	0.12
2014-Y	1.14	2.82	55.41	0.25	0.33	0.13
2015-O	3.88	3.13	55.31	0.10	0.47	0.14
2015-M	4.08	3.27	54.00	0.11	0.45	0.14
2015-Y	2.83	3.01	52.44	0.13	0.58	0.21
2016-O	3.62	2.28	44.36	0.11	0.25	0.13
2016-M	3.98	2.30	42.29	0.14	0.27	0.12
2016-Y	3.82	2.25	42.41	0.14	0.29	0.14

Appendix 4.7. Eigenvalues and eigenvectors from principal component analysis presented in Figure 4.2.

<b>Eigenvalue</b>	<b>PC 1</b>	<b>PC 2</b>	<b>PC 3</b>	<b>PC 4</b>	<b>PC 5</b>	<b>PC 6</b>	<b>PC 7</b>	<b>PC 8</b>
Eigenvalue	1.938	0.598	0.373	0.283	0.185	0.125	0.052	0.020
Variance (%)	54.212	16.728	10.434	7.923	5.177	3.501	1.462	0.562
Cumulative (%)	54.212	70.940	81.374	89.297	94.475	97.976	99.438	100.000
<b>Eigenvector</b>								
Color	0.785	-0.478	-0.019	0.063	0.337	-0.026	-0.187	0.039
Citrus	-0.524	0.334	0.234	0.577	-0.434	-0.133	-0.075	0.122
Peach	0.879	-0.218	0.144	0.231	-0.222	0.176	0.032	-0.159
DryFruit	0.968	-0.134	0.053	0.176	0.085	0.047	-0.029	0.021
Rose	-0.577	-0.422	0.427	0.334	-0.314	0.310	0.015	-0.016
Vegetal	-0.956	-0.042	0.104	-0.142	0.046	-0.034	-0.221	-0.014
Spices	0.739	0.292	0.176	0.387	-0.333	0.177	0.042	0.207
Aging	0.965	-0.088	-0.141	0.174	0.097	-0.028	-0.012	0.032
Rubber	-0.711	0.096	-0.079	-0.613	0.207	0.073	0.224	0.073
Reductive	-0.837	-0.109	0.071	-0.493	0.173	-0.046	0.015	0.090
Sourness	-0.769	0.450	-0.008	0.385	0.175	0.084	0.121	-0.067
Sweetness	0.886	-0.204	0.368	-0.114	0.154	-0.006	-0.027	0.016
Bitterness	-0.545	-0.112	-0.638	0.457	0.258	-0.056	-0.058	0.033
Impression	0.971	-0.105	-0.045	0.208	-0.018	0.027	-0.005	0.024
Butanediol	0.620	-0.268	0.710	-0.134	0.137	0.036	-0.034	0.044
Phenylethanol	-0.647	-0.403	-0.388	0.020	-0.150	0.494	0.034	-0.008
Methylbutanol	-0.602	-0.367	-0.251	0.512	0.371	0.186	0.075	-0.014
Acetaldehyde	0.846	0.160	0.404	-0.290	-0.107	0.008	-0.004	0.027
CaftaricAcid	0.347	-0.412	0.776	-0.250	0.000	-0.038	-0.145	-0.150
Ethylacetate	0.918	0.267	-0.205	-0.047	-0.006	0.186	0.077	-0.019
GalacturonicAcid	0.829	0.521	-0.020	0.045	0.044	-0.182	-0.060	-0.010
Methanol	0.661	0.691	-0.126	0.061	0.012	-0.252	-0.019	-0.033
SuccinicAcid	-0.538	-0.534	-0.189	0.414	0.443	0.091	0.044	0.108
L	-0.798	0.468	0.210	-0.098	-0.268	0.016	0.125	-0.058
a	-0.841	0.476	0.180	0.043	-0.172	0.025	0.021	-0.034
b	0.833	-0.477	-0.152	0.040	0.211	-0.033	-0.087	0.037
TartaricAcid	-0.542	0.441	0.508	0.327	0.315	-0.037	0.198	-0.084
MalicAcid	0.709	0.522	-0.283	0.209	0.215	-0.001	0.225	-0.065
ShikimicAcid	0.699	0.400	-0.525	-0.027	-0.106	0.238	-0.069	0.030
LacticAcid	0.262	-0.767	-0.419	0.049	-0.186	-0.346	0.026	-0.103
AceticAcid	0.824	0.449	0.179	0.075	-0.055	0.279	0.021	-0.022
CitricAcid	0.714	-0.112	0.572	0.076	0.256	-0.054	0.221	0.165
Ethanol	0.664	0.114	0.385	0.313	0.535	0.081	-0.034	-0.080
F.Linalooloxide1	0.732	0.579	-0.293	-0.185	-0.094	-0.025	0.009	-0.013
F.Linalooloxide2	0.649	0.647	-0.334	-0.178	-0.104	-0.075	-0.005	-0.022
F.Hotrienol	0.545	0.501	-0.568	-0.171	-0.058	0.188	-0.247	-0.034
F.aTerpineol	0.034	0.627	0.159	0.235	0.044	-0.722	-0.025	0.033
F.TDN	0.887	0.298	-0.100	0.124	0.199	0.009	0.234	-0.073

Appendix 4.7. cont.

<b>Eigenvector</b>	<b>PC 1</b>	<b>PC 2</b>	<b>PC 3</b>	<b>PC 4</b>	<b>PC 5</b>	<b>PC 6</b>	<b>PC 7</b>	<b>PC 8</b>
B.Linalooloxide2	0.553	-0.716	-0.109	0.152	-0.277	-0.163	0.182	-0.099
B.aTerpineol	0.350	-0.784	-0.398	0.071	-0.180	-0.254	0.035	0.028
B.TDN	0.078	-0.807	-0.054	0.050	-0.422	-0.334	0.217	0.003
H2S	0.797	-0.268	-0.205	-0.393	-0.026	0.009	0.304	0.058
DMS	0.812	-0.022	-0.383	-0.337	-0.214	0.178	0.013	0.049

Appendix 4.8. Eigenvalues and eigenvectors from principal component analysis presented in Figure 4.4.

<b>Eigenvalue</b>	<b>PC 1</b>	<b>PC 2</b>	<b>PC 3</b>	<b>PC 4</b>	<b>PC 5</b>	<b>PC 6</b>	<b>PC 7</b>	<b>PC 8</b>	<b>PC 9</b>
Eigenvalue	9.021	5.312	3.408	2.872	1.809	1.646	1.483	1.078	0.749
Variance (%)	30.069	17.707	11.360	9.572	6.031	5.486	4.943	3.593	2.498
Cumulative (%)	30.069	47.776	59.135	68.707	74.739	80.225	85.168	88.761	91.259
<b>Eigenvector</b>									
Butanediol	-0.268	-0.564	0.049	0.338	0.161	0.293	0.068	0.294	-0.209
Phenylethanol	-0.150	-0.408	0.466	-0.531	0.423	-0.017	0.211	0.059	0.055
Methylbutanol	0.167	-0.547	0.565	-0.399	0.202	-0.008	0.293	-0.014	-0.130
Acetaldehyde	0.382	0.670	0.230	0.323	0.231	0.041	-0.026	0.061	-0.301
CaftaricAcid	0.824	-0.281	0.309	-0.159	0.126	0.082	0.113	0.087	0.172
Ethylacetate	0.505	-0.142	0.331	0.649	0.102	0.069	-0.280	0.020	0.206
GalacturonicAcid	0.296	0.243	0.786	0.326	-0.060	-0.149	0.144	0.205	0.068
Methanol	-0.361	0.595	0.466	-0.011	-0.256	-0.311	0.048	0.222	0.038
SuccinicAcid	0.365	-0.809	0.271	-0.277	0.028	0.069	0.110	0.033	-0.016
L	0.733	0.099	-0.355	0.099	0.329	-0.024	0.271	-0.027	-0.279
a	0.884	0.089	-0.263	0.024	0.164	-0.214	-0.067	-0.017	-0.143
b	-0.825	-0.075	0.336	-0.082	-0.284	0.082	-0.170	0.023	0.234
TartaricAcid	0.267	-0.472	-0.502	0.321	0.032	-0.467	-0.197	-0.018	0.127
MalicAcid	0.506	0.708	0.278	-0.171	-0.217	-0.070	0.028	0.178	-0.012
ShikimicAcid	0.485	0.682	0.405	-0.114	-0.205	-0.057	0.063	0.112	-0.081
LacticAcid	0.111	0.547	-0.216	-0.218	0.295	0.376	0.391	-0.084	0.391
AceticAcid	0.462	0.248	0.043	0.744	0.272	0.041	0.026	0.000	0.212
CitricAcid	-0.359	-0.117	-0.227	0.054	0.134	0.110	-0.060	0.770	-0.027
Ethanol	-0.548	-0.491	0.250	0.444	-0.062	0.058	0.188	-0.139	0.093
F.Linalooloxide1	-0.870	-0.020	0.287	0.205	0.213	-0.110	0.080	-0.089	-0.087
F.Linalooloxide2	-0.884	-0.005	0.217	0.203	0.224	-0.130	0.048	-0.098	-0.117
F.Hotrienol	0.596	-0.369	0.321	0.411	-0.113	0.008	0.078	-0.047	-0.068
F.aTerpineol	0.300	-0.338	0.266	0.309	-0.264	-0.343	0.397	-0.186	0.035
F.TDN	-0.510	-0.125	0.178	0.040	0.599	-0.256	-0.336	0.134	0.116
B.Linalooloxide2	-0.351	0.225	0.511	-0.054	0.186	0.117	-0.447	-0.346	-0.242
B.aTerpineol	0.577	-0.127	0.268	-0.089	-0.217	0.546	-0.378	-0.052	-0.019
B.TDN	0.755	-0.271	0.231	-0.159	0.100	0.156	-0.416	0.003	0.088
H2S	-0.342	0.005	-0.100	0.480	-0.117	0.650	0.269	-0.014	-0.093
DMS	-0.928	0.329	-0.021	0.049	-0.097	0.079	0.010	-0.008	-0.017

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