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Hierarchy of factors impacting grape berry mass at different scales and its direct and indirect effects on grape and wine composition

Roberta Triolo

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Roberta Triolo. Hierarchy of factors impacting grape berry mass at different scales and its direct and indirect effects on grape and wine composition. *Vegetal Biology*. Université de Bordeaux; Università degli studi (Palerme, Italie), 2016. English. NNT : 2016BORD0336 . tel-01675104v2

HAL Id: tel-01675104

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THESIS EN CO-SUPERVISION

SUBMITTED IN FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF

PhD of

BORDEAUX UNIVERSITY

and PALERMO UNIVERSITY

ECOLE DOCTORALE SCIENCE DE LA VIE ET DE LA SANTE

SPECIALITÉ ŒNOLOGIE

Roberta TRIOLO

**HIERARCHY OF FACTORS IMPACTING GRAPE BERRY
MASS AT DIFFERENT SCALES AND ITS DIRECT AND
INDIRECT EFFECTS ON GRAPE AND WINE COMPOSITION**

Under the direction of Pr. Cornelis VAN LEEUWEN and Pr. Rosario DI LORENZO

Presentation scheduled on 16 December 2016

Member of the committee:

Pr. S. Guidoni	Turin University	President
Pr. L. G. Santesteban	Navarra University	Reviewer
Pr. O. Failla	Milan University	Reviewer
Pr. P. L. Teissedre	Bordeaux University	Examiner
Eng. J. P. Roby	Bordeaux Sciences Agro	Examiner
Pr. R. Di Lorenzo	Palermo University	Examiner
Pr. S. Delrot	Bordeaux University	Invited member
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Hierarchy of factors impacting grape berry mass at different scales and its direct and indirect effects on grape and wine composition

Abstract

Final berry mass is the result of the integrated effect of several factors. They also influence berry composition. The present work was designed to study the simultaneous effect of major factors influencing berry mass and composition, to hierarchize their impact at different scales, to distinguish their direct and indirect effect on berry composition and to compare the profile of wines made from large and small berries. The study was carried out simultaneously on two vineyards located in the Saint Emilion (France) and Alcamo (Sicily) areas, during 2014 and 2015. On the first site, vines were planted on two soil types, while on the second site two different irrigation treatments were applied. Depending on the scale, some factors homogeneously impacted the berry mass and composition. At the intra-parcel scale, vine water status represented the most impacting factor, while berry seed number did not have significant effect. Opposite results were obtained when the investigation was carried out at the intra-bunch and intra-plant scales. At large scale, factors impacted directly and indirectly berry compounds and grape juices and wines produced from smaller berries were more concentrated. Neither at intra-bunch, nor at intra-plant scales, berry size effect on juice composition was significant. Only anthocyanin concentration was related to berry size at all scales. This fact was particularly obvious in berries produced under limited water conditions. Water deficit increased the skin to flesh ratio, independently of berry size. This means that small and large berries, produced from a single parcel with homogenous water uptake conditions, tend to have similar enological profiles.

Key words: *Vitis vinifera*, grape berry mass, vine nitrogen status, vine water status, carbon isotope discrimination, Yeast Assimilable Nitrogen (YAN), berry seed, grape berry sorting, grape berry composition, wine composition, polyphenols, lactones.

Hiérarchisation des facteurs impactant la masse de la baie de raisin à différentes échelles et leurs effets directs et indirects sur la composition du raisin et du vin

La masse de la baie est le résultat de l'effet intégré de plusieurs facteurs. La recherche a été dessinée afin d'étudier l'effet simultané des majeurs facteurs influençant la masse et la composition de la baie, de les hiérarchiser selon leur degré d'impact à des échelles différentes, de séparer leur effet direct et indirect sur la composition du raisin et de comparer le profil de vins élaborés à partir de petites et grosses baies. L'étude a été conduite sur deux sites expérimentaux, localisés dans les régions de Saint-Emilion (France) et Alcamo (Italie), pendant les années 2014 et 2015. Sur le premier site, les vignes sont plantées sur deux types de sols, tandis que sur le deuxième, deux traitements hydriques étaient appliqués. A l'échelle intra-parcellaire, l'état hydrique de la vigne représente le facteur le plus important, tandis que l'effet du nombre de pépins par baie n'est pas significatif. Des résultats opposés sont obtenus lorsque les relations sont étudiées à l'échelle de la grappe et de la plante. A large échelle, les facteurs impactent directement et indirectement la composition du raisin et les petites baies produisent des moûts et des vins plus concentrés. A l'inverse, à l'échelle de la grappe et de la plante, la masse de la baie n'influence pas la composition du raisin. Seulement la concentration en anthocyanes est significativement liée à la masse à toutes les échelles. Cette relation est particulièrement évidente sous conditions hydriques limitantes. Un déficit hydrique augmente le ratio pellicule:pulpe, indépendamment de la masse de la baie. Petites et grosses baies d'une parcelle ayant une condition hydrique homogène, tendent à avoir un profil similaire.

Mots clés: *Vitis vinifera*, masse de la baie, statut azoté, régime hydrique, azote assimilable, discrimination isotopique du carbone, pépins, triage des raisins, composition des raisins, composition des vins, polyphénols, arômes, lactones

Hiérarchisation des facteurs impactant la masse de la baie de raisin à différentes échelles et leurs effets directs et indirects sur la composition du raisin et du vin

Résumé

La relation entre la taille de la baie et la composition du raisin et des vins n'est pas encore clairement établie. En effet, les résultats obtenus par des études précédentes, ne sont pas toujours en accord. Certains auteurs montrent que cette relation est significative, tandis que d'autres auteurs ne trouvent pas de différences dans la composition des petites et des grosses baies et des vins élaborés à partir de ces raisins. L'absence d'une relation linéaire pourrait être liée à l'origine de la variabilité de la taille de la baie et à l'interaction des facteurs qui l'influencent. La masse finale de la baie de raisin, en effet, est le résultat d'un effet intégré de facteurs biotiques et abiotiques qui influencent le nombre final de cellules et leur volume. Parmi eux, la variété représente un des facteurs les plus importants. D'autres facteurs, tel que le contenu en pépins par baie, sont reliés à la baie elle-même. Finalement, des déficits hydriques et azotés de la vigne peuvent jouer un rôle clé dans la croissance de la baie. Leur effet peut varier en fonction de leur intensité et/ou du stade phénologique auquel ils s'installent.

Dans la vigne, une variabilité de la taille de la baie peut être observée entre baies de la même grappe, entre grappes de la même plante, entre plantes de la même parcelle et de parcelles différentes. Ainsi, en fonction de l'échelle considérée, la hiérarchie de ces facteurs pourrait varier. A l'échelle parcellaire, la variété est probablement le facteur dominant. A l'échelle intra-parcellaire, la variabilité peut être reliée à la variation des caractéristiques du sol. Finalement, à l'échelle de la grappe, la différence parmi les baies est probablement liée à des facteurs reliés à la baie elle-même. Ainsi, étant donné le large nombre de facteurs impactant la taille finale de la baie, il est extrêmement difficile d'obtenir une vendange caractérisée par des baies de taille uniforme. Le projet s'inscrit dans la volonté de la société AMOS, à l'origine du développement et de l'industrialisation d'un appareil, le **Calibaie**, capable de trier les raisins en fonction de leur taille. Afin d'obtenir des résultats scientifiques supportant les principes de la machine Calibaie, l'étude présente a été dessinée pour :

- étudier, sous des conditions réelles de terrain, l'effet simultané des facteurs majeurs influençant la masse de la baie et de les hiérarchiser selon leur degré d'impact à des échelles différentes ;

- étudier l'effet de la taille de la baie sur la composition du raisin, en fonction du facteur responsable de la variabilité de la masse elle-même
- comparer le profil œnologique des vins issus de baies de taille différente, dans l'objectif d'étudier la possibilité d'obtenir deux vins différents d'une même parcelle
- isoler les effets de l'état hydrique de la vigne sur la taille de la baie, en se focalisant sur les relations entre les masses des différents tissus de la baie.

Cette étude a été conduite sur deux vignobles commerciaux localisés respectivement dans les régions viticoles de Saint-Emilion (Aquitaine, France) et d'Alcamo (Sicile, Italie). Les résultats obtenus sur les deux sites ont été complémentaires et ont permis de répondre aux questions posées. Un seul protocole a été dessiné pour atteindre les deux premiers objectifs. Cette partie d'étude, conduite sur le site de Saint-Emilion, a été réalisée sur deux parcelles de Cabernet franc plantées sur deux types de sol : un sol sableux, caractérisé par une disponibilité en eau (Soil Water Holding Capacity - SWHC) élevée et un sol graveleux caractérisé par une SWHC beaucoup moins importante. Au sein de chaque placette, trois blocs expérimentaux ont été choisis. Dans le sol sableux, très homogène, les trois blocs sont caractérisés par une SWHC similaire, tandis que sur le sol graveleux, moins homogène, un des trois blocs montre une disponibilité en eau supérieure par rapport aux deux autres blocs localisés sur la même parcelle.

Le dessin expérimental de cette étude a été basé en tenant compte de cette variabilité, qui pourrait impacter la masse de la baie et la composition de son moût.

L'état hydrique et azoté de la vigne ont été mesurés par différents indicateurs physiologiques : le potentiel de tige a permis de suivre l'évolution du régime hydrique au cours de la saison, tandis que la discrimination isotopique du carbone ($\delta^{13}\text{C}$) a été dosée sur les sucres à maturité, en adoptant une méthode "baie par baie", permettant d'étudier la variabilité de ce paramètre à des échelles différentes. Similairement, l'azote assimilable des moûts, indicateur du statut azoté de la vigne, a été dosé en utilisant la même approche.

La valeur moyenne de la masse finale de la baie a été estimée à maturité par la détermination de la masse de chacune des baies de plusieurs grappes par bloc. Le nombre des pépins, de chacune des baies analysées, a été enregistré au même moment.

L'impact sur la masse des baies des trois facteurs considérés (état hydrique et azoté et nombre de pépins par baie) a été quantifié à trois échelles différentes (grappe, plante et bloc par bloc) à l'aide de modèles statistiques appropriés.

La hiérarchisation des facteurs a été déterminée par le rapport de la SS (Sum of Squares) entre les variables. Il s'agit d'une approche innovante qui prend en compte la possible interaction des facteurs et leur effet combiné sur la masse de la baie.

Une seconde approche statistique, constituée par une chaîne de trois modèles différents, a été utilisée pour séparer l'effet direct des facteurs sur la composition du raisin de leur effet indirect, induit par leur impact sur la taille de la baie.

Les résultats de cette partie d'étude démontrent que, pour une échelle donnée, la variabilité de la taille de la baie reflète la variabilité du facteur le plus important. Ainsi, le nombre de pépins par baie est le facteur majeur de la variabilité à l'échelle de la grappe, où, au contraire, l'état hydrique et azoté sont très peu variables. Réciproquement, à l'échelle intra-parcellaire, tous les facteurs impactent de façon significative la masse finale de la baie, mais l'état hydrique de la vigne est le facteur le plus important, suivi par le régime azoté. Les pépins par baie n'ont qu'un effet marginal. De plus, lorsque la variabilité de la taille de la baie est liée à la variabilité du nombre de pépins par baie (ce qui est le cas à l'échelle de la grappe et de la plante), l'effet de la taille de la baie sur la composition du raisin n'est que marginal. A l'inverse, quand cet effet est étudié à l'échelle intra- ou inter-parcellaire, qui tient compte des autres facteurs (état hydrique et azoté de la vigne), l'impact de la masse de la baie sur la composition du raisin est bien plus significative. Ce fait signifie que la composition du raisin dépend plus de la façon par laquelle la taille de la baie est limitée que par la taille elle-même. Les résultats de cette deuxième analyse statistique démontrent en effet que les trois facteurs étudiés impactent la composition du raisin à la fois d'une façon directe (indépendamment de la taille de la baie) et indirect.

Un autre objectif important de cette étude était d'investiguer l'existence d'une possible différence de composition des moûts et des vins produits par des baies de taille différente, issues d'une même parcelle et triées par la machine Calibaie en fonction de leur taille. Les résultats obtenus confirment cette hypothèse. Les moûts extraits par des baies de taille plus petite sont plus riches en sucres et moins acides. Le profil des vins est également affecté. Les petites baies donnent des vins plus riches en métabolites secondaires, tels que les anthocyanes et les tannins, mais aussi les arômes appartenant à la classe des lactones. Ainsi, ces résultats démontrent que, à l'aide de la machine Calibaie, il serait possible d'obtenir deux vins différents d'une seule parcelle.

Selon un des principes de la viticulture de qualité, les meilleurs vins sont obtenus à partir de baies de petite taille. D'un point de vue géométrique, en effet, en assimilant la baie à une sphère, le rapport entre la surface et le volume diminue avec l'augmentation de la taille de la baie. Une taille

plus petite, donc, optimise le ratio entre la pellicule et la pulpe et puisque les solutés les plus importants pour la vinification en rouge sont accumulés dans les cellules de la pellicule, il semble évident qu'une augmentation progressive de la taille de la baie a pour conséquence leur dilution. Cependant, cette théorie n'a pas été confirmée par les résultats obtenus dans cette recherche, où l'analyse physique des baies montre l'absence d'une différence significative entre les masses des tissus de baies de taille différente. Cette partie d'étude a été conduite dans la parcelle expérimentale localisée dans la région viticole d'Alcamo (Sicile, Italie). L'étude des relations entre la taille de la baie et la masse de ses tissus montre que leur croissance est coordonnée, indépendamment du régime hydrique auquel la vigne est soumise. En conséquence, le rapport entre la pellicule et la pulpe reste *quasi* constant lorsque la taille de la baie augmente. Ceci signifie que la dilution des solutés de la pellicule pendant la vinification tend à être similaire entre baies de taille différente. En même temps, il a été également observé que le régime hydrique de la vigne modifie la distribution des tissus de la baie. A parité de masse de la baie, en effet, les raisins produits sous contrainte hydrique ont un rapport pellicule : pulpe plus élevé. Ceci signifie que, pour un millésime donné, petites et grosses baies, issues d'une même parcelle caractérisée par une disponibilité hydrique spatialement homogène, tendent à avoir un profil œnologique similaire. Au contraire, dans les parcelles où la variabilité spatiale des conditions hydriques est élevée, les petites baies montrent une composition différente par rapport aux grosses baies. Ceci est particulièrement évident au cours de millésimes secs et chauds.

Ainsi, un déficit hydrique contrôlé représente un outil efficace pour augmenter le ratio pellicule: pulpe des raisins, limiter la dilution des solutés de la pellicule au cours de la macération et augmenter la concentration des anthocyanes et des flavanols dans les vins.

Dans cette étude, même si la relation théorique entre la pellicule et la pulpe de la baie a été invalidée, la taille de la baie continue à représenter un facteur impactant la composition des moûts et des vins. Ainsi, l'origine de cet effet requiert encore des études supplémentaires. Cependant, la machine Calibaie a montré un grand potentiel, en offrant la possibilité d'obtenir deux vins différents d'une seule parcelle, au-delà du fait que des petites baies donneraient des vins de qualité supérieure. En effet, il n'est pas possible de définir la qualité des raisins sans faire référence au profil du vin à produire.

*"Cari giovani, non sotterrate i vostri talenti,
i doni che Dio vi ha dato!
Non abbiate paura di sognare cose grandi!"*

Papa Francesco

*"Dear young people, don't bury your talents! Bet on big
ideals, those ideals that enlarge the heart, those ideals that
will make your talents fruitful. Life is not given to us so
that we can keep it jealously for ourselves, but is given to
us so that we may donate it. Dear young people, don't be
afraid to dream great things!"*

Pope Francis

*"Chers jeunes, n'enterrez pas vos propres talents !
N'ayez pas peur de rêver de grandes choses !"*

Pape François

REMERCIEMENTS

Je remercie chaque membre du jury de thèse pour avoir accepté d'évaluer le résultat de ce travail : Silvia Guidoni, Professeur de l'Université de Turin, Président du jury ; les Professeurs Osvaldo Failla, de l'Université de Milan et Luis G. Santesteban, de l'Université de Navarre, rapporteurs de cette thèse ; l'Ingénieur Jean-Philippe Roby, de l'Ecole National Supérieure Bordeaux Sciences Agro, et le Professeur Pierre Louis Tesseidre, de l'Université de Bordeaux, examinateurs de cette étude ; Serge Delrot, Professeur de l'Université de Bordeaux, pour avoir accepté de participer à l'évaluation de ce projet ; mon Directeur de thèse, Cornelis (Kees) van Leeuwen, Professeur de l'Ecole National Supérieure Bordeaux Sciences Agro, et mon co-Directeur de thèse Rosario Di Lorenzo, de l'Université de Palerme, pour m'avoir encadrée aux cours de ces trois années.

Cette thèse a été réalisée avec la collaboration des unités de recherche, de l'Institut de Sciences de la Vigne et du Vin, UMR Ecophysiologie et Génomique Fonctionnelle de la Vigne et UMR Œnologie, coordonnées respectivement par les Professeurs Serge Delrot et Philippe Darriet, de l'Université de Bordeaux. Je les remercie, ainsi que leurs équipes pour m'avoir accueillie au sein de ces laboratoires. Je tiens plus particulièrement à remercier les Docteurs Ghislaine Hilbert, Michael Jourdes, Alexandre Pons, Kleopatra Chira et Lucile Allamy, pour leur aide et leur soutien. Je remercie spécialement Sandra Vanbrabant, Christel Renaud et Claude Bonnet pour la grande attention qu'elles ont adressée à la réalisation de ce travail.

Les résultats de cette thèse, en co-tutelle avec l'Université de Palerme, sont le fruit d'une collaboration avec le département de Scienze Agrarie e Forestali. Je veux vivement remercier le Professeur Rosario Di Lorenzo, coordinateur de l'unité de viticulture, et ses collaborateurs, le Professeur Maria Gabriella Barbagallo et le Docteur Antonino Pisciotta, pour avoir suivi aux cours de ces trois ans la réalisation de ce projet au sein de leur laboratoire.

Je tiens également à remercier Antonella Plaia, Professeur de l'Université de Palerme, pour le support à la réalisation des études statistiques des données issues de cette recherche.

Ce projet naît d'une idée d'Alexandre Faupin, de la société Amos Industrie. Je les remercie, lui et toute son équipe, pour l'attention reçue au cours de sa réalisation. J'adresse un remerciement particulier à Yannique Ordeonneau.

La mise en œuvre de cette étude a été réalisée au sein de deux exploitations agricoles, Vignobles Bardet (Vignonet, France) et Tenute Rapitalà (Camporeale, Italie). Je remercie infiniment Philippe Bardet et Laurent de la Gatinais pour leur gentil accueil au sein de leurs vignobles. Je remercie également tous les

gens de ces équipes qui ont apporté à cette recherche leur aide et leur soutien : Silvio Centoze, Ignazio Arena, Lucia Cutrona, Gianfranco Paoletti et Eric Fargeot.

Je remercie également Innovin pour le montage et le suivi de ce projet et le lien entre les partenaires.

Mes remerciements vont également à toute l'équipe de VITINNOV, cellule de transfert de l'ISVV. La qualité de leur travail s'est révélée fondamentale à la réussite de cette recherche.

Cette étude a vu également la participation de nombreux étudiants, qui avec rigueur ont collaboré à atteindre les objectifs fixés. Je remercie Simona, Carmelo, Francesca, Lucie, Melissa et Cassandra.

J'adresse un remerciement particulier à Catherine et Cathy, secrétaires de l'unité EGFV. A votre professionnalisme, qui a été essentiel pour la gestion des nombreuses collaborations lors ce projet.

La qualité des résultats obtenus au cours de ces trois ans est aussi le fruit du soutien amical et familial. Nombreux sont les gens qui ont apporté leur esprit à cette thèse. C'est à eux que je dédie ce travail.

A tous les gens de l'EGFV, pour chaque sourire, pour tous les bonjours, pour chaque verre de vin bu et pour chaque gâteau partagé.

A Ghislaine, Agnès et Elisa, pour votre affection.

A Antoine, Eloïse, Isabelle, Landry, Marc et Diego, pour tous les bons moments passés ensemble.

A Noé, Pierre et Maria José. A l'amitié.

A Virginie, Laure, Coralie, Laura et Anne. A la complicité et au soutien.

A Claude et Sylvette. Au sens de la famille.

A Jean Philippe et Kees. Source infinie d'apprentissage. Au sens de la vie, au respect, à la gratitude, à l'optimisme, à l'amitié. Je recommencerais tout du début.

A Elvia, qui n'a jamais laissé ma main.

A Maxime. A sa patience. A son amour.

A Ornella, toujours à mes côtés.

Al mio papà. A toi je dédie la passion pour la science qui a fait la base de ce travail. À la rigueur et à l'ambition. T'es un enseignant extraordinaire. Un ami unique. A notre complicité.

Alla mia mamma. A tes bras. A tes plats chauds. A ton amour quotidien. A ta patience. Tu as été une source inépuisable de courage. A toi je dédie ce travail.

Tables of contents

List of figures	12
List of tables.....	15
Abbreviation	17
Preface.....	20
Chapter I: General introduction	23
Berry morphology and anatomy.....	23
From flower to berry	25
Grape berry growth	26
Hormonal control of grape berry growth and ripening	29
Chemical composition of the ripe grape berry	30
Origin of variability of grape berry mass and relationship with grape composition.....	37
Berry size and grape and wine composition.....	45
Objectives	48
Chapter II: Hierarchy of factors impacting grape berry mass. Separation of direct and indirect effects on major berry metabolites.....	510
Chapter III: Hierarchy of factors impacting grape berry mass and its consequences on grape composition at intra-bunch and intra-plant scale	787
Chapter IV: The impact of berry mass on wine quality.....	1121
Chapter V: Effect of water deficit on berry mass and skin to flesh ratio.....	1521
Chapter VI: General discussion and conclusion	1732
Literature cited	181

LIST OF FIGURES

Chapter I

Figure 1 - Grape berry structure.....	24
Figure 2 - Diagrammatic representation of the relative size and color of grape berries at 10-day intervals after bloom and of the major changes occurring during berry development.....	27
Figure 3 - Developmental changes in grape metabolites.....	32

Chapter II

Figure 1 - Seasonal trends (1 April - 31 October) of monthly temperature and rainfall recorded in 2014 and 2015; comparison with average values (1996-2015).....	59
Figure 2 - Seasonal dynamics of stem water potential (Ψ_{stem}) measured in the experimental plots in the summer of 2014 and 2015.....	60
Figure 3 - Seasonal dynamics of Yeast Assimilable Nitrogen (YAN) measured in the experimental blocks in the summer of 2014 and 2015, from veraison through ripeness.....	63
Figure 4 - Seasonal dynamics of fresh berry mass of each experimental block for 2014 and 2015.....	66
Figure 5 - Seasonal dynamics of sugar and malate concentration for the season 2014 and 2015, respectively, measured on berry juice for each experimental block.....	67

Chapter III

Figure 1 - Berry mass variability at a bunch level in 2014 and 2015.....	87
Figure 2 - Carbon isotope discrimination ($\delta^{13}\text{C}$) variability at bunch level in 2014 and 2015.....	88
Figure 3 - Yeast Assimilable Nitrogen (YAN) variability at bunch level in 2014 and 2015.....	91
Figure 4 - Berry seed content variability at bunch level in 2014 and 2015.....	91
Figure 5 - Intra-vine berry mass variability.....	96
Figure 6 - Relationship between $\delta^{13}\text{C}$ and berry mass (2015).....	99

Figure 7 - Relationship between YAN and berry mass (2015).....	99
Figure 8 - Relationship between berry seed content and berry mass (2015).....	100
Figure 9 - Relationship between average berry mass and sugar concentration (2015)...	102
Figure 10 - Relationship between average berry mass and pH (2015).....	102
Figure 11 - Relationship between average berry mass and total acidity (2015).....	103
Figure 12 - Relationship between average berry mass and malic acid (2015).....	103

Chapter IV

Figure 1 - Berry volume at harvest (calculated from diameter) for Cabernet franc vines as a function of measured berry mass (pooled data across all parcel of the 2014 growing season).....	125
Figure 2 - Seed mass per berry (g) and relative seed mass (%) as a function of berry volume (calculated from diameter) of Cabernet franc vines during the 2014 and 2015 growing seasons.....	126
Figure 3 - Flesh mass per berry (g) and relative flesh mass (%) as a function of berry volume (calculated from diameter) of Cabernet franc vines during the 2014 and 2015 growing seasons.....	127
Figure 4 - Skin mass per berry (g) and relative skin mass (%) as a function of berry volume (calculated from diameter) of Cabernet franc vines during the 2014 and 2015 growing seasons.....	128
Figure 5 - Berry surface per g of berry plotted against berry volume (calculated from diameter) of Cabernet franc vines during the 2015 growing seasons.....	129
Figure 6 - Density distribution for berry diameter of berries sampled from large and small size categories and from control of each experimental parcel, in 2014 and 2015.....	130
Figure 7 - Total anthocyanins, total tannins and total phenolic compound concentration of wines from small and large berries and from control berries at the end of malolactic fermentation, in the 2014 season.....	135
Figure 8 - Comparison of wine flavan-3-ols profiles of wines made from berry belonging to different size categories (2015).	139
Figure 9 - Total anthocyanins, total tannins and total phenolic compound concentration of wines from small and large berries compared to control berries, in the 2015 season. Wines were analyzed after 12 months of aging.....	139

Chapter V

Figure1 - Seasonal trends (1 April-30 September) of monthly temperature and rainfall recorded in 2014 and 2015; comparison with average values (1996-2015).....	158
Figure 2 - Seasonal dynamics of stem water potential (Ψ_{stem}) measured in the experimental blocks in the summer of 2014 and 2015.....	159
Figure3 - Seasonal dynamic of berry mass (g) measured in the experimental blocks in the summer of 2014 and 2015.....	161
Figure 4 - Relationship between berry mass and $\delta^{13}\text{C}$, used as indicator of vine water status, in 2014 and 2015.....	162
Figure 5 - Total skin, flesh and seed mass (g) per berry and their relative mass (g/g of berry), plotted as function of berry mass (g).....	165
Figure 6 - Skin to flesh ratio per berry as a function of berry mass.....	166
Figure 7 - Relationship between Skin to flesh ratio and $\delta^{13}\text{C}$	167

LIST OF TABLES

Chapter II

Table 1 - Influence of block on vine water and nitrogen conditions, measured with different physiological indicators, in 2014 and 2015.....	61
Table 2 - Correlations between indicators of vine water and nitrogen status used during the two years of experimentation.....	61
Table 3 - Vine and nitrogen status on the six experimental blocks.....	64
Table 4 - Effect of block on seed content per berry.....	65
Table 5 - Effect of block on berry mass, sugar and malic acid measured just prior to harvest in 2014 and 2015.....	67
Table 6 - Effects of vine water and nitrogen status and berry seed content on fresh berry mass.....	68
Table 7 - Effects of berry mass, vine water and nitrogen status and berry seed content on berry sugar concentration.....	69
Table 8 - Effects of berry mass, vine water and nitrogen status and berry seed content on berry malate concentration.....	69
Table 9 - Direct effect of vine water and nitrogen status and berry seed content on berry sugar concentration.....	70
Table 10 - Direct effect of vine water and nitrogen status and berry seed content on berry malate concentration.....	71

Chapter III

Table 1 - Variability of factors impacting on berry mass inside a bunch, measured in 2014 and 2015, on 18 different bunches.....	89
Table 2 - Relationship between berry seed content and berry mass inside a bunch.....	93
Table 3 - Effect of berry water and nitrogen status and berry seed content on berry mass inside a bunch.....	93
Table 4 - Relationship between berry mass and concentration major berry compounds, observed in 2014 and 2015, studied inside each experimental bunch.....	95
Table 5 - Variability of $\delta^{13}\text{C}$, YAN and berry seed content at plant level.....	97
Table 6 - Effect of bunch water and nitrogen status and berry seed content on berry mass within a plant.....	101

Chapter IV

Table 1 - Effect of berry size on monomeric and oligomeric flavan-3-ol concentrations (mg/L) of wines in 2012.....	124
Table 2 - Effect of berry size on anthocyanin concentrations (mg/L) of wines in 2012.....	124
Table 3 - Berry mass, berry diameter and berry volume comparison for each berry size category in 2014.....	131
Table 4 - Berry mass, berry diameter and berry volume comparison for each berry size category in 2015.....	132
Table 5 - Chemical composition of grape juice from different berry size categories in 2014.....	133
Table 6 - Chemical composition of grape juice from different berry size categories in 2015.....	133
Table 7 - Comparison of wine flavan-3-ols profiles made from berries belonging to different size categories (2014).....	136
Table 8 - Comparison of wine anthocyanin profiles made from berry belonging at different size categories (2014).....	137
Table 9 - Comparison of wine anthocyanin profiles made from berries belonging to different size categories (2015).....	140
Table 10 - Comparison of aroma compounds in wines made from berries belonging to different size categories (2014)	141
Table 11 - Comparison of wine aroma profiles made from berry belonging at different size categories (2015)	142
Table 12 - Results of sensory analysis of wines made from berries of different size in 2014.....	143
Table 13 - Results of sensory analysis of wines made from berries of different size in 2015.....	145

Chapter V

Table 1 - Vine water status in the experimental blocks, measured by Carbon isotope discrimination.....	160
Table 2 - Effect of water treatment on berry mass at harvest.....	161
Table 3 - Berry tissue mass and their relative proportion measured at harvest, in 2015...	163

ABBREVIATION

101-1MGt	<i>101-114 Millardet et De Grasset rootstock</i>
1103P	<i>1103 Paulsen</i>
196-17C	<i>196-17 Castel</i>
3309C	<i>3309 Couderc rootstock</i>
$\delta^{13}\text{C}$	<i>Carbon Isotope Discrimination</i>
Ψ_{stem}	<i>Stem Water Potential</i>

A

ABA	<i>Abscisic Acid</i>
AOC	<i>Appellation d'Origine Contrôlée</i>
ArMV	<i>Arabic Mosaic Virus</i>

B

B1	<i>[(-)-epicatechin-(4b-8)-(+)-catechin]</i>
B2	<i>[(-)-epicatechin-(4b-8)-(-)-epicatechin]</i>
B3	<i>[(+)-catechin-(4a-8)-(+)-catechin]</i>
B4	<i>[(+)-catechin-(4a-8)-(-)-epicatechin]</i>
BBB	<i>Big Bad Berry</i>

C

C	<i>(+)-catechin</i>
CFG	<i>Cabernet Franc on Gravelly soil</i>
CFS	<i>Cabernet Franc on Sandy soil</i>
cv	<i>Coefficient of variation</i>
Cy	<i>Cyanidine-3-O-glucoside</i>
Cy-ac	<i>Cyanidine-3-O-(6-acetyl) -glucoside</i>
Cy-coum	<i>Cyanidine-3-O-(6-p-coumaryl) -glucoside</i>

D

DAV	<i>Day after veraison</i>
DOC	<i>Denominazione di origine controllata</i>

Dp	<i>Delphinidine-3-O-glucoside</i>
Dp-ac	<i>Delphinidine-3-O-(-6-acetyl) -glucoside</i>
Dp-coum	<i>Delphinidine-3-O-(-6-p-coumaryl) -glucoside</i>
E	
EC	<i>(-)-epicatechin</i>
F	
FTIR	<i>Fourier Transform Infrared Spectroscopy</i>
G	
GFLV	<i>Grapevine Fan Leaf Virus</i>
GLRaV	<i>Grapevine LeafRoll associated Virus</i>
H	
HSD	<i>Tukey Honest Significant Difference</i>
HPLC	<i>High pressure Liquid Chromatography with UV detector</i>
I	
I	<i>Irrigated</i>
M	
MNG	<i>Merlot Noir on Gravelly soil</i>
MNS	<i>Merlot Noir on Sandy soil</i>
Mv	<i>Malvidine -3-O-glucoside</i>
Mv-ac	<i>Malvidine-3-O-(-6-acetyl) -glucoside</i>
Mv-coum	<i>Malvidine -3-O-(-6-p-coumaryl) -glucoside</i>
N	
NI	<i>Non-Irrigated</i>
P	
Pt	<i>Petunidine-3-O-glucoside</i>
Pn	<i>Peonidine-3-O-(-6-acetyl) -glucoside</i>
Pt-ac	<i>Petunidine-3-O-(-6-acetyl) -glucoside</i>

Pn-ac *Peonidine-3-O-(-6-acetyl) -glucoside*
Pt-coum *Petunidine-3-O-(-6-p-coumaryl) -glucoside*
Pn-coum *Peonidine-3-O-(-6-p-coumaryl) -glucoside*

R

RS *Reducing Sugar*

S

SS *Sum of Squares*

SWHC *Soil Water Holding Capacity*

T

TA *Total Acidity*

U

UV *Ultra-Violet*

Y

YAN *Yeast Assimilable Nitrogen*

PREFACE

The study of the differences in berry composition when comparing berries strictly on size leads to contrasting conclusions among researchers, because the factors impacting berry growth and development interact under field conditions. Some authors found a significant relationship between berry mass and grape and wine composition (Melo et al. 2015), whereas other authors did not find a significant difference between the composition of small and large berries (Barbagallo et al. 2011). Final berry mass, in fact, is the result of the integrated effect of biotic and abiotic factors that affect the final cell number and/or cell volume (Fernandez et al. 2006). Among them, variety is one of the major factors (Matthews and Nuzzo 2007, Attia et al. 2010, Barbagallo et al. 2011, Dai et al. 2011). Other factors, such as berry seed content, are related to the individual berry itself (Scienza et al. 1978, Roby and Matthews 2004). Finally, vine water status (Matthews and Anderson 1988, van Leeuwen and Seguin 1994, Ojeda et al. 2001, Roby et al. 2004, Chaves et al. 2007, Ferrer et al. 2008, Girona et al. 2009) and vine nitrogen status (Choné et al. 2001a, Trégoat et al. 2002, van Leeuwen et al. 2007) can play a key role in berry growth, depending on their intensity and/or the development period at which they act (Ojeda et al. 1999). However, some external factors, such as vine water status, have been shown to homogeneously inhibit berry growth in all berries (Shellie 2010). Hence, depending on the considered scale, the hierarchy of these factors may vary. In grapevine, variation in berry mass occurs between berries within the bunch, between bunches within the vine, between vines within the vineyard and between vineyards (Gray 2002). At the parcel scale, grapevine cultivar is likely to be the dominant factor. At intra-parcel scale, the variability may be related to variations in soil characteristics. Finally, at bunch scale, the differences between berries could be related to internal factors (Scienza et al. 1978, Carwthon and Morris 1982, Walker et al. 2005, Roby and Matthews 2004). Consequently, under field conditions, it is extremely difficult to obtain uniform berry diameter, even when all vineyard management practices are uniform (Pisciotta et al. 2013).

In 2011, based on these observations, Amos Industrie designed Calibaie[®], a machine able to sort the mechanical harvested berries, according to their size. This practice appeared to be a promising technological approach to reduce variability of grape composition introduced by differences in berry size, whatever the scale of their variability (intra-bunch, intra-plant, intra-parcel).

Partners were gathered in a research project in order to obtain scientific results supporting the basic principles of Calibaie[®] machine.

The present work was designed in order to:

1. Deepen the knowledges concerning the origin of berry size variability and to quantify the latter according to the factor responsible of this variation;
2. Hierarchize the factors impacting on berry size (vine water and nitrogen status and berry seed content) according to their degree of influence at different observation scales (bunch, plant, block, parcel);
3. Compare the physical and chemical composition of berries, and their corresponding wines, produced under different environmental conditions and sorted according to their size by the Calibaie[®] machine

Chapter I: General introduction

General introduction

Berry morphology and anatomy

The grape fruit belongs to the large group of fleshy fruits (Coombe 1976) and is classified as a berry which develops after fertilization of the ovary (Pratt 1971). The capacity for inflorescence initiation is a quantitative genetic character. The degree of its expression is highly affected by nutritional and environmental parameters (Kanellis and Roubelakis-Angelakis 1993). Grape berry consists of a pericarp and a maximum of four seeds (figure 1, Coombe 1987). The pericarp, which originates from the wall of the ovarium, consists of three anatomically distinct tissues: the exocarp (or skin), the mesocarp (or flesh) and endocarp (Hardie et al. 1996).

- The *exocarp* forms the grape's dermal system or "skin" which makes up between 5% and 12% of the fresh weight of mature berries (Lavee and Nir 1986). The number of cell layers in the skin of grape berries and their size and volume are cultivar specific (Kanellis and Roubelakis-Angelakis 1993). The skin is composed of the epidermis (Alleweldt et al. 1981), which has smaller cells than other dermal layers (Considine 1981) and the hypodermis (Alleweldt et al. 1981) with some flesh cells (Pratt 1971). The epidermal and sub-epidermal cell layers contain most of the color, aroma, and flavor constituents of the berries (Winkler et al. 1974).
- The *mesocarp*, which is commonly called the "flesh" or "pulp" of the grape berry consists of about 25-30 layers of large cells, where most of the constituents are stored during ripening (Gray et al. 1999). The cells of the pulp have large vacuoles containing the cell sap, which also vary greatly in size and can make up as much as 90% of the cell volume in ripe grape berries (Lavee and Nir 1986). They serve as an internal reservoir storing sugars, organic acids, and nutrients. At maturity, the mesocarp cells are approximately 75 times larger than the skin cells (Keller 2015).
- The innermost tissue of the pericarp, the *endocarp*, surrounds the seeds and consists of the inner hypodermis and also the inner epidermis (Hardie et al. 1996). The endocarp is often difficult to distinguish from the mesocarp in grapes (Mullins et al. 1992).

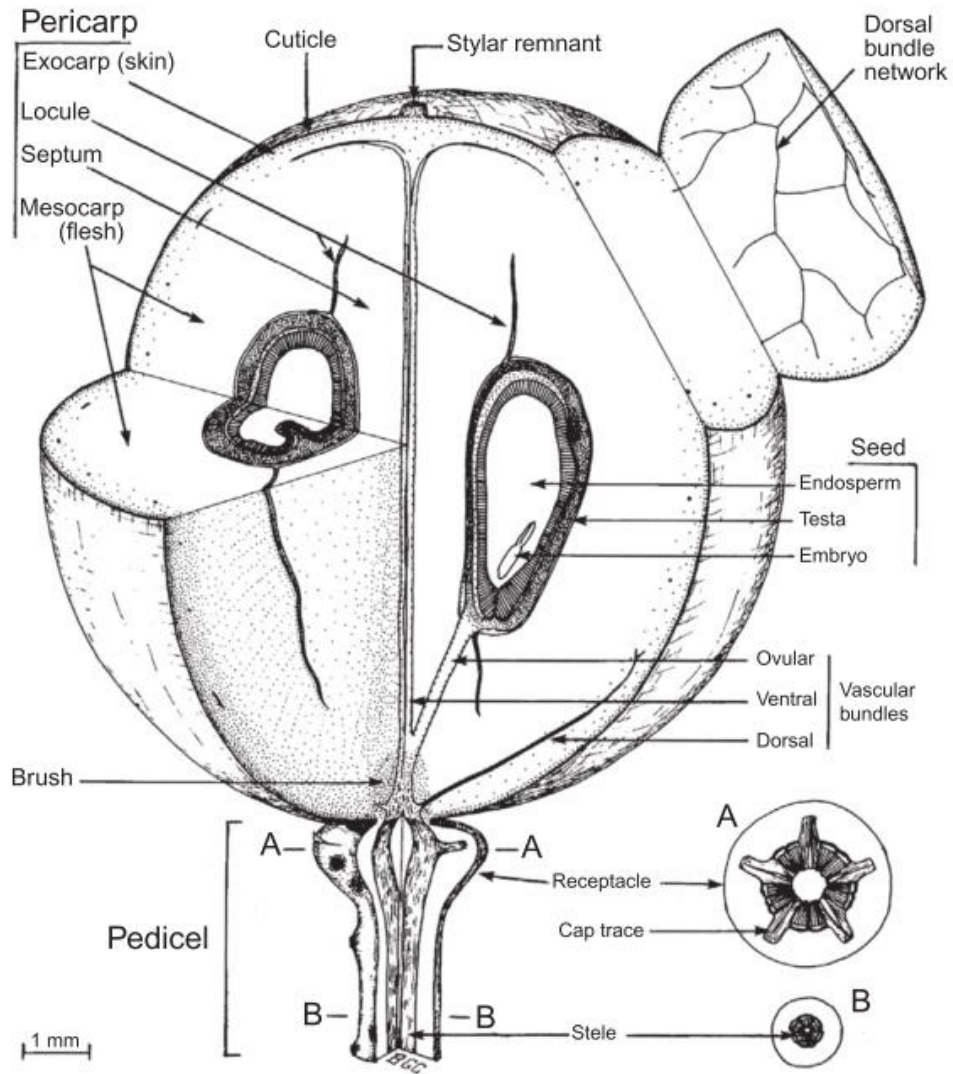


Figure 1 - Grape berry structure (Coombe 1987)

From flower to berry

The biology of grape berry development is explained in terms of ovule/seed development, fruit set and berry growth. All three phenomena are essential for a grape berry to achieve its maximum potential size. Limitations to any, or all, of these lead to variation between berries (Gray 2002).

Berry development after fertilization

The development of grape berries is usually dependent on pollination, fertilization, and development of at least one seed (Pratt 1971). The normal ovary in *Vitis vinifera* has two carpels, each with two ovules (Coombe 1976). Many ovules fail to develop into functional seeds. Among the ovules that are not fertilized, some remain in the form of ovules. They are soft and are difficult to find among the tissue of mature berries. This is called *parthenocarpic* fruit development. A second group of ovules grows minimally to become so-called seed-traces. These are the seeds present in *stenospermocarpic* grapes. Their integuments remain soft but show slight browning (May 2004). Ovaries that do not contain at least one fertilized ovule, or whose seed development is aborted early, are abscised from the fruit cluster (Nitsch et al. 1960). Later degeneration of the embryo and endosperm (about 42 days after flowering) can result in apparently normal seeds. They are externally indistinguishable in size and color from functional seeds. However, as they are hollow, they float on water (“floaters”), while the functional seeds sank (“sinkers”) (Ebadi et al. 1996).

Fruit-set and poor fruit-set

Fruit set is a development stage common to many plant families occurring after fertilization of the ovule and prior to significant fruit enlargement (Gray 2002). Fruitset is normal when the framework provided by the bunch peduncle is filled with berries that have reached full size. This phenomena is strongly modulated by environmental conditions (Keller et al. 2001), carbohydrate availability and by chance (May 2004). The proportion of flowers that develop into berries following anthesis is typically in the range of 20-50% (Keller 2015). It is the results of a large number of factors. Some of these are inherent in the plant, others are related to the environmental conditions before, during and immediately after anthesis. These factors can be classified into four groups: (a) anomalous or defective flower development, (b) physiological phenomena, (c) environmental factors, (d) pathological interventions (Kozma 1961a).

Poor fruit set due to excessive abortion of flowers and ovaries is termed *coulure*. Fertilization of at least one of up to four ovules is required for the ovary to develop into a berry; hence, the abscised ovaries typically do not contain any fertilized ovules. Normal fruit set despite inadequate seed development in a portion of the fertilized berries, on the other hand, is called “millerandage” and results in clusters having the appearance of “hens and chicks” (May 2004). The term “hens” refers to large, normal berries with at least one viable seed, and “chicks” describes small berries with tiny, degenerated seeds (i.e., seed traces) that often lack an endosperm (Ebadi et al. 1996). Coulure results in very loose clusters, and millerandage leads to highly variable seed numbers and berry sizes, including seedless berries, on the same cluster (Keller 2015).

Grape berry growth

The growth of seeded berries traces a double-sigmoid pattern. Commonly, this curve is divided into three stages. However, according to Coombe and Iland (2004), this division is artificial physiologically: the lag-phase (or stage II) is the slowing part of the first sigmoid curve, ending when ripening begins (second sigmoid curve). Hence, the period required for berry development, approximately hundred days, can be divided into two major growth periods separated by a lag phase (figure 2) (Coombe and McCarthy 2000, Dokoozlian 2000, Coombe 2001, Kennedy 2002, Carbonneau et al. 2007, Ribéreau-Gayon et al. 2012). Development before the lag phase is referred to as the first growth period and the subsequent period of development as the second growth period (Harris et al. 1968). The length of each stage of fruit growth and the final berry size depend on the cultivar but are strongly modified by rootstocks and environmental conditions (Keller 2015).

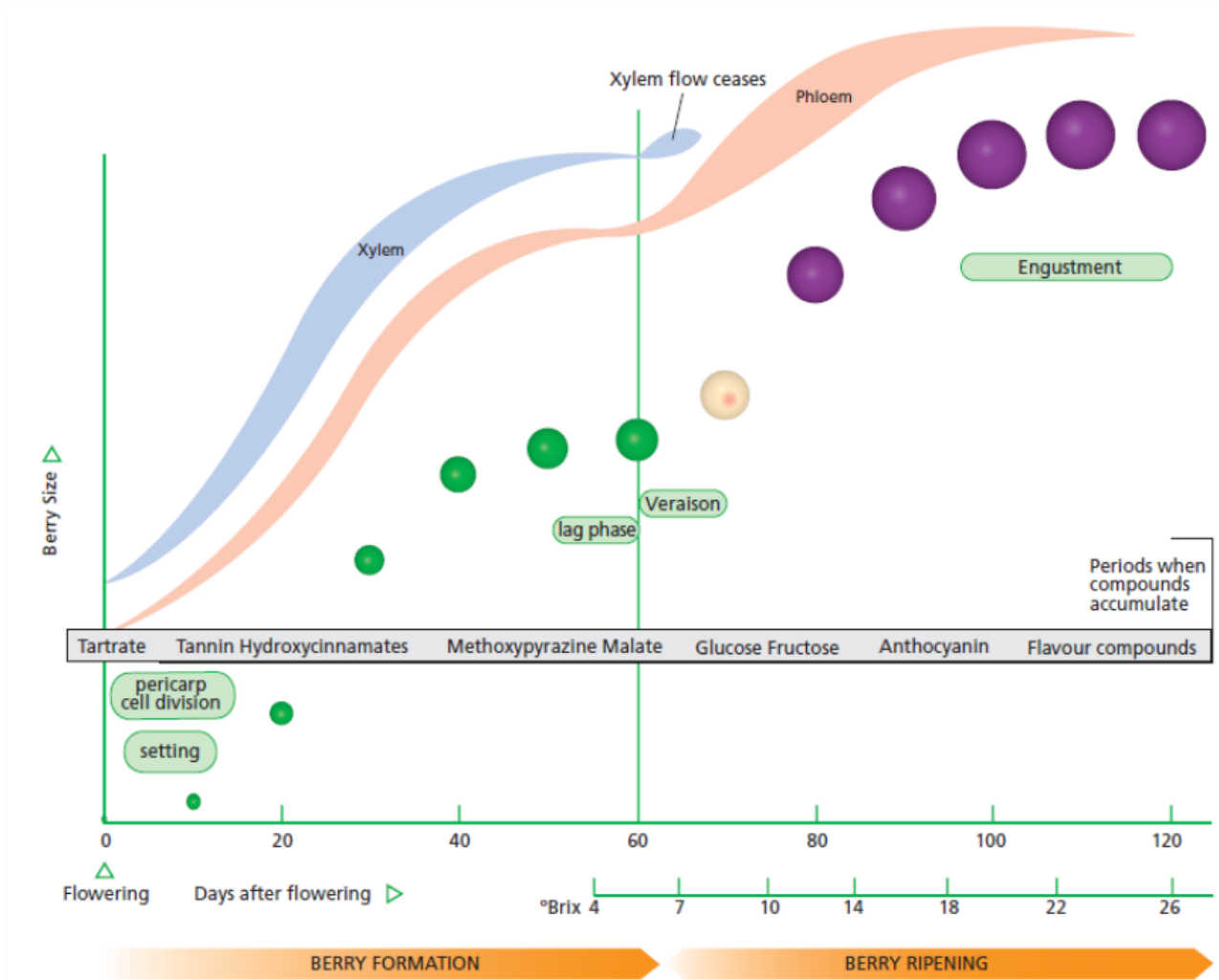


Figure 2 - Diagrammatic representation of the relative size and color of grape berries at 10-day intervals after bloom and of the major changes occurring during berry development. Also shown are the periods when compounds accumulate, the °Brix levels, and flow rate of xylem and phloem
 (Reproduced from Coombe 2001)

First phase: berry formation

The first growth period lasts from flowering to approximately sixty days thereafter. During this phase, the berry is formed and the seed embryos are produced (Kennedy 2002).

This phase is characterized by a very rapid rate of cell division in pericarp tissue, that begins 5 to 10 days post bloom (Pratt 1971), followed by a marked cell enlargement (Coombe and McCarthy 2000). The pericarp expansion during the first two weeks after flowering is associated with a three-fold increase in radial cell number of the mesocarp (forming the flesh) and a seven-fold increase in the hypodermis (forming most of the skin). Expansion of pericarp in the subsequent four weeks is predominated by cell enlargement in the inner mesocarp (Coombe and Iland 2004). The number and size of pericarp cells have been quantified by analysis of the DNA content in Shiraz berries by Ojeda et al. (1999). This confirmed that cell division in flesh cells is most active during the first 14 days after anthesis, especially during the first week. Cell division gradually slows down to reach a plateau, corresponding to lag-phase. By the end of this period the seeds attain nearly their full size (Pratt 1971) and the total number of cells within the berry has been established (Harris et al. 1968).

The lag-phase

The lag-phase starts 35 to 80 days after anthesis (Winkler et al. 1974). It is not always characterized by a total cessation of growth, but often by a markedly decrease in growth rate (Dokoozlian 2000, Ollat et al. 2002). This period usually lasts two to four weeks depending on the cultivar (early or late, seedless or seeded), timing of flowering (early versus late, primary versus secondary clusters), competition between clusters and the vine's environment (Pratt 1971, Coombe 1976, Lavee and Nir 1986). Its duration is important in determining the time of fruit maturity; late-ripening cultivars seem to have a long lag phase (Keller 2015).

During the lag phase, the embryos develop rapidly and generally by 10-15 days before veraison the seeds reach their final size, maximum fresh weight, and maximum tannin content (Ojeda et al. 1999, Ristic and Iland 2005). In addition, the berries lose chlorophyll and soften and the organic acid concentration of the berry also reaches its highest level.

Second phase of growth: berry ripening

The beginning of the ripening period is termed veraison (Winkler et al. 1974). This phenomenon includes several events: berry softening, sugar content increase, malate content decrease, further berry growth and color change in the skin (Coombe and McCarthy 2000). The inception of these events covers about a week (Coombe and Iland 2004), while the whole ripening period lasts 5-10 weeks (Keller 2015). Many others events can be included, as the color change of the seeds from green to yellow and finally brown, due to oxidation of tannins in the parenchyma cells of the outer integument; seeds become hard and desiccated (Ristic and Iland 2005).

Water import via the phloem increases rapidly (water being the solvent for the incoming sugar), while water import through the xylem declines (Keller et al. 2006). Hence, berry sugaring appears to be the earliest event, which occurs together to berry softening (Coombe and Iland 2004), while berry expansion and skin coloring start later. The berry on each bunch do not undergo these changes synchronously: the onset of the ripening typically occurs over a period of 7-10 days within a grape cluster (Winkler et al. 1974). There was no evidence of patterns of onset due to proximity between berries i.e. each berry remained independent (Coombe and Iland 2004). May (2000) has suggested that differences in the timing of development of berries, set in train before flowering, continue through to veraison, thence to harvest.

After the lag phase, pericarp growth results from cell enlargement (volume increase), since cell division ceases one week before the beginning of the stationary phase (Winkler et al. 1974). No further cell division occurs after this moment, so the number of cells in a berry is established in the first weeks after flowering (Dokoozlian 2000). The volume increase is initially very rapid but slows progressively toward fruit maturity; berry size may plateau or decrease due to evaporative water loss during later stages of ripening (Keller 2015). Between the beginning of this phase and harvest, the berry approximately doubles in size (Kennedy 2002).

Hormonal control of grape berry growth and ripening

Regulation of seed and pericarp growth responses are believed to be hormonal (Coombe 1972). Hormones are chemical regulators that act as messengers: their function is to communicate between plant parts and to integrate the responses of one part of the plant with another. The precise mode of action of hormones in fruit is unknown, but they function by stimulating metabolism, cell division, cell enlargement and cell maturation (Davies and Böttcher 2009). Five major groups of

phytohormones have been found in tissues of *Vitis* species: auxins, gibberellins, cytokinins, abscisins and ethylene (Coombe and Hale 1973, Coombe 1976). These endogenous hormones are involved in all aspects of reproductive and vegetative growth of the grape vine. They reach a maximum concentration just before veraison and then decrease sharply along ripening (Coombe 1992).

The growth hormones auxin, cytokinin, and gibberellin produced by the embryos and released into the pericarp reach high concentrations early on and then decrease during the first growth period (Scienza et al. 1978). In addition, import of the germination-inhibiting hormone ABA prevents seed abortion and promotes normal embryo development (Nambara and Marion-Poll 2005). Consequently, the ABA concentration in the berry is high during early development but declines as the berry expands (Davies and Böttcher 2009).

During the lag phase, although the pericarp grows only insignificantly, the concentration of the growth hormone auxin peaks briefly and then declines sharply (Nitsch et al. 1960). The influx of ABA and its production in the berry itself increase toward the end of this phase, suppressing further embryo growth by blocking gibberellin production in the seeds (Pérez et al. 2000). ABA also seems to induce changes in the expression of many genes that ultimately bring about fruit ripening (Gambetta et al. 2010).

Unlike in climacteric fruits, the hormone ethylene does not play a prominent role in grape ripening (Coombe and Hale 1973). ABA acts in a positive feedback loop with sugars, whereby sugars stimulate ABA production and ABA promotes sugar accumulation (Castellarin et al 2007a, 2007b). The concentration of ABA increases rapidly after veraison, peaks during seed maturation, then decreases and is relatively low in mature seeds and berries (Scienza et al. 1978).

Chemical composition of the ripe grape berry

The grape berries physical and chemical composition at harvest is responsible for the fruit quality characteristics and, consequently, the quality attributes of the wine or grape juice produced from the fruit (Keller 2015). As berries ripen, they undergo a multitude of physical and chemical changes, which, in turn, are coordinated by the interplay and cooperation of several genes (Keller 2015 and the references therein). The beginning of grape ripening is recognized by the change of skin color and the sudden softening of the berry. The change in color occurs due to the degradation of the green chlorophylls (Hardie et al. 1996) and due to the simultaneous accumulation of

anthocyanin pigments in dark-skinned cultivars (Keller 2015). Softening, which coincides with the beginning of sugar accumulation (Coombe 1992), occurs due to the gradual disassembly of the mesocarp cell walls and the decline in mesocarp cell turgor during the pre-veraison lag phase of berry growth (Thomas et al. 2008). Cell wall loosening in the pulp seems to be responsible for berry softening, and soon afterward cell wall loosening in both the pulp and the skin enables berry expansion (Keller 2015 *from* Huang and Huang 2001, Huang et al. 2005a). This occurs despite the decline in mesocarp turgor pressure just before veraison (Thomas et al. 2006) due to the accumulation of sugars and other solutes (Keller et al. 2006). Although the berry volume increases during ripening, it cannot do so indefinitely because the skin sets a limit to mesocarp expansion (Matthews et al. 1987). Following a temporary increase at veraison, the extensibility of the skin later decreases. The phenolics may be deposited in the skin cell where they are bounded to cell wall polysaccharides and proteins. This fact stiffens the cell walls and, consequently, limits cell expansion (Keller 2015).

As the grape berry is a fleshy fruit, most of the chemicals in grapes that contribute to wine are in water solution and water is the berry's principle compounds (Ollat et al. 2002, Coombe and Iland 2004). Of the non-water components the largest proportion is made up of the solutes glucose and fructose. The important chemical components of the grape berry are stated by Hulme (1970, 1971) to be sugars, acids, phenolics and flavor compounds. A schematic overview of changes in relative concentration of principal berry metabolites from flowering to senescence is displayed in figure 3.

Water

The rapid growth of the berry during ripening is mostly due to water import and retention by the mesocarp vacuoles. Between flowering and ripening, the amount of water entering the berry is nearly 10 times the volume of water stored in the berry at ripening (Ollat 1997). Water influx into fruits occurs via both the xylem and phloem. Xylem sap is the main source of water for the berry before veraison; it is thought to account for approximately 75% of the total water influx (Ollat et al. 2002). Xylem flow into the berry declines at veraison, and phloem sap concomitantly becomes the primary or only source of berry water (Keller et al. 2006).

The main water pathway controls water relations between vegetative parts and berries (Ollat et al. 2002). During the first growth period, when xylem is the main pathway, the berries are sensitive to water stress (Matthews and Anderson, 1989). After veraison, the sensitivity of berry water status

to soil and plant water status declines: accordingly, whereas pre-veraison berries shrink and expand readily with fluctuating vine water status, post-veraison berries are much less subject to such fluctuations (Greenspan et al. 1996).

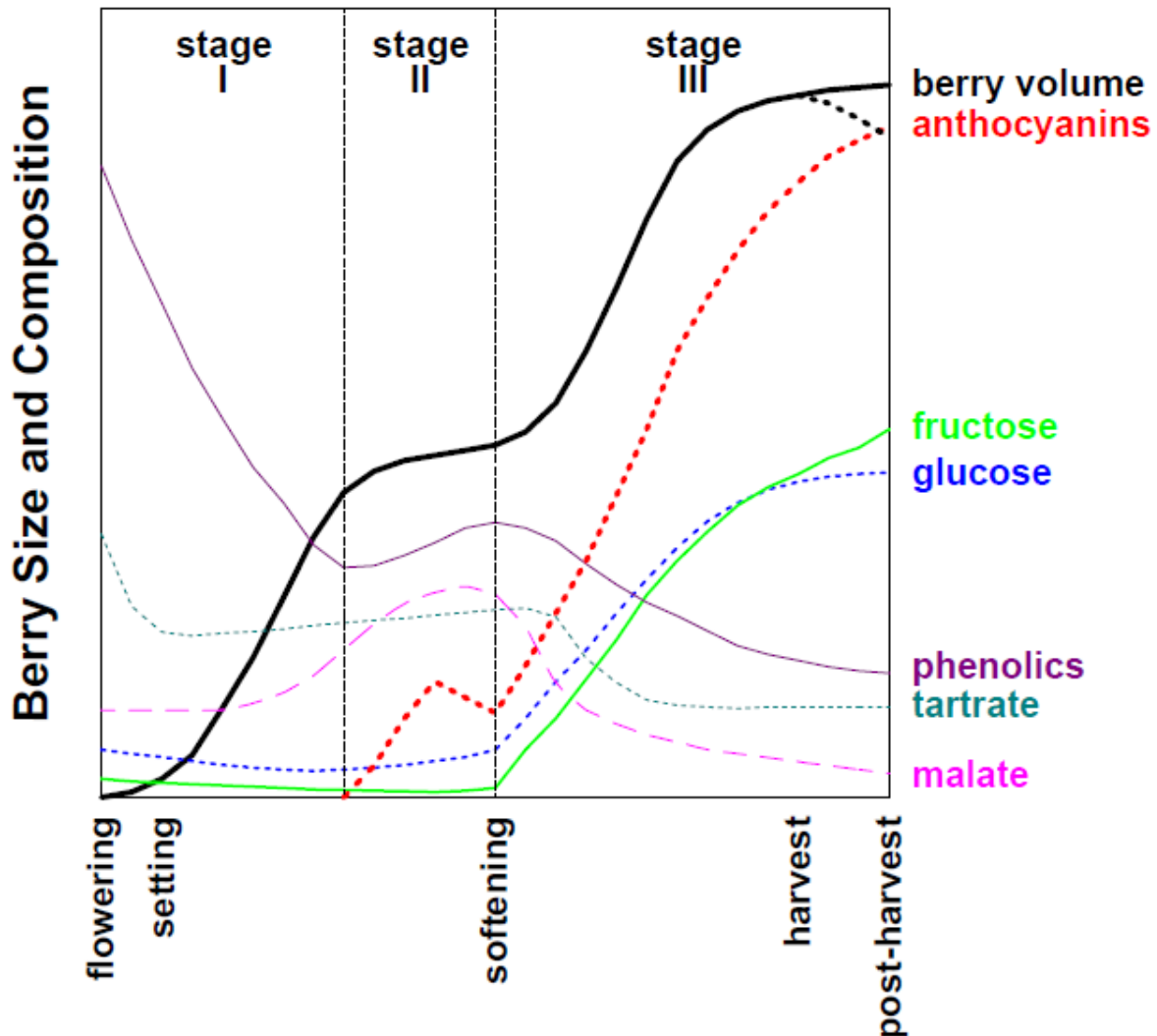


Figure 3 - Developmental changes in grape metabolites.
(Gray 2002 and the references therein)

Sugars

Sugars typically make up more than 90% of the soluble solids in mature berries, with much of the remainder being organic acids (Coombe 1987). In grape berry, 95-99% of these sugars are present in the form of the hexoses glucose and fructose (Dai et al. 2011). The accumulation of hexose sugars in berries is negligible prior to berry softening, but increases rapidly thereafter (figure 3).

Their accumulation in the large vacuoles of mesocarp cells begins a few days before veraison (Coombe 1987), where they are imported by the action of invertase on sucrose, translocated via the phloem from the leaves or from storage tissues (Rebucci et al. 1997). Depending on species and cultivar, the natural sugar content in grape berries reaches a maximum at about 25 °Brix. Environmental factors such as temperature, light, water availability and vine canopy, all impact on the rate and extent of accumulation of glucose and fructose in the berry (Keller 2015).

Acids

The major soluble organic acids in the grape berry are malate and tartrate (Kliewer 1966). Accumulated during pre-veraison period, together these account for 70-90% of the berry's total acids at maturity (Dai et al. 2011). After synthesis inside the berry, which occurs via separate metabolic pathways (Ribéreau-Gayon et al. 2012), the acids are accumulated early in berry development and stored in the vacuoles of the mesocarp and skin cells. However, there is a differential concentration of the acids in various berry tissues (Iland and Coombe 1988).

The course of acid accumulation and degradation is plotted in figure 3. Fruit acidity therefore peaks immediately before veraison. Most of the decrease in acidity occurs early in ripening, slowing around 16-18 °Brix, and often becoming insignificant above 20-21 °Brix (Keller et al. 2012).

After veraison, the amount of malate per berry declines somewhat due to dilution from water import and somewhat due to a re-metabolism of stored malate for respiration. However, malate present in the berry periphery is more readily respired than malate in the core. So, this metabolic activity creates a gradient difference between berry skin and its interior (Martínez-Esteso et al. 2011). Like malate, most tartrate is formed during the pre-veraison period, but, conversely, its amount per berry generally remains fairly constant during berry development (Coombe 1987).

Phenolic compounds

Phenolic compounds attracts much attention as an important contributor to wine style and quality (Lorrain et al. 2011). They are responsible for the color of red grapes and they also contribute to taste and astringency through interactions with salivary proteins (Cheynier et al. 2006). Classically, two groups of phenolic compounds are distinguished: *non flavonoids* and *flavonoids* (Chira et al. 2008). Both groups are additionally divided into several families, with comparable structure features that confer specific properties such as color, aroma and taste.

Non-flavonoids are present in grapes and wines, but with the exception of hydroxycinnamic acids, they are present in low concentrations (Kennedy et al. 2006). These compounds do not present a direct value for organoleptic characteristics of wines, but they are considered being the precursors of volatile phenols, produced by the action of contaminating microorganisms (Ribéreau-Gayon et al. 2012), which have a negative impact on wine quality.

Flavonoids are a group of compounds based on the polyphenolic flavan skeleton, substituted by hydroxyl, methyl, galloyl, glucosyl, and acyl moieties (Downey et al. 2006). They can form complexes with other flavonoids, metal ions and several other molecules. Flavonoids have several physiological roles in plants. Two groups of flavonoids, the anthocyanins and flavanols (tannins), are particularly important to the quality of red wines. Anthocyanins and most tannins are localized in the solid parts of the cluster and are extracted by maceration in the fermenting must. They are highly unstable and undergo various enzymatic and chemical reactions as the wine is made and aged. Because the new compounds formed often exhibit sensory properties different from those of their precursors, these structural modifications change wine quality. In particular, the color change from the purple tint of young red wines to the tawny nuance of older ones is ascribed to reactions of grape anthocyanins with tannins generating new polymeric pigments (Somers 1971). Similarly, the decrease in astringency as wine ages results from polymerization of tannins and/or formation of polymeric pigments (Cheynier et al. 2006).

- Anthocyanins: Anthocyanins are the red grape pigments and hence responsible for red wine color. They are specific to red varieties and localized in berry skins, except in teinturier varieties that have colored flesh. Anthocyanins are glycosylated derivatives of five aglycones or anthocyanidins: cyanidin, peonidin, petunidin, delphinidin, and malvidin. Further diversity results from acylation of the glucose by acetic, *p*-coumaric, and caffeic acids (He et al. 2010). The structures for common anthocyanin in *Vitis vinifera* grapes and wine were determined by Ribéreau-Gayon in 1959, who established that malvidin-3-*O*-glucoside was the major anthocyanin present along with its acylated forms (Ribéreau-Gayon 1959). Work by Ribéreau-Gayon also showed that anthocyanins in *Vitis vinifera* were different in structure than those found in non-*vinifera* species in that they were exclusively present as monoglucosides. Hence, anthocyanin profiles are varietal characteristics and can be used to identify them (Roggero et al. 1988).

- Flavanols: Flavanols exist in grape as monomers and as oligomers and polymers, called proanthocyanidins because they release red anthocyanidins when heated in acidic solutions (Cheynier et al. 2006). Flavan-3-ol monomers (catechins) are responsible for bitterness in wine and may also have some associated astringency. The major flavan-3-ol monomers found in grapes and wine include (+)-catechin, (-)-epicatechin, and (-)-epicatechin- 3-*O*-gallate (Su and Singleton 1969). The chemical structure of the flavan-3-ols, catechin and epicatechin, compared with the known structures of tannin polymers, suggests a precursor product relationship. It is generally agreed that much of the flavan-3-ol monomers originate from seed material, although they are also present in grape skin hypodermal cells (Adams 2006, Kennedy et al. 2006). Catechins, tannins, and anthocyanins are the most concentrated natural antioxidants present in red grape and wine (Mattivi et al. 2002). Proanthocyanidins or tannins are polymers of flavan-3-ols and are the most abundant class of soluble polyphenolics in grape berries. They impart astringency to red wines (Chira et al. 2009, 2011) and are extracted from the hypodermal layers of the skin and the soft parenchyma of the seed between the cuticle and the hard seed coat, as well as, from the peduncle of the grape berry (Ribéreau-Gayon et al. 2012). They are a very diverse set of biomolecules varying in size from dimers and trimers up to oligomers with more than 30 subunits (Adams 2006, Kennedy et al. 2006).

The biosynthesis of soluble phenolic begins with the aromatic amino acid phenylalanine, a product of the shikimate pathway. The first enzyme responsible for the phenolic synthesis is PAL (Phenyl Ammonia Lysase), which converts phenylalanine into cinnamic acid. This compound undergoes a series of transformations resulting in the formation of precursors of several simple phenolics, like phenolic acids, lignin precursors, etc. The incorporation of 3 molecules of malonyl-CoA, produced via the acetate pathway, with the 4-coumaroyl-CoA starts the phenylpropanoid pathway (Dias 2003). These precursors generate complex phenolic compounds, like the flavonoids or the stilbenes, depending on the intervening enzyme, chalcone synthase (CHS) or the stilbene synthase (SS), respectively. Skin anthocyanins appear to behave like typical end products. The other phenolic classes exhibit patterns of accumulation and subsequent decline during ripening, suggesting their degradation and utilization for the biosynthesis of other compounds, or the covalent association with other cellular compounds. In all cases, much more is known about the anabolism than the catabolism of polyphenolics during berry development and ripening. Skin tannins, which are synthesized very early in berry development, change very little on a per berry

basis from veraison to harvest, although their concentration declines along berry growth because of dilution. Qualitative changes, such as the increase of the polymerization degree, can also take place from veraison to harvest (Kennedy et al. 2001). Generally, there is a decline in seed tannins during ripening that accompanies seed browning, possibly due to tannin oxidation (Adams 2006). There are many factors reputed to affect flavonoid biosynthesis in plants, including light, temperature, altitude, soil type, water, nutritional status, microbial interactions, pathogenesis, wounding, defoliation, plant growth regulators, and various developmental processes.

Nutrient availability has a fundamental influence on plant growth (Keller et al. 1998). Both low and excessively high levels of nitrogen fertilizer have been shown to decrease color in grape berries (Delgado et al. 2004). The most likely mechanism for decreasing phenolic content at high nutrient levels is excessive vigor. It is uncertain whether this change is due to the difference in vine vigor or is an indirect effect of changes in canopy architecture resulting in differential bunch exposure effects (Cortell et al. 2005). Soubeyrand et al. (2014) showed that low nitrogen supply caused a significant increase in anthocyanin levels and that nitrogen controls a coordinated regulation of both positive and negative regulators of the flavonoid pathway in grapevine.

Water deficit can result in increased anthocyanin accumulation. Some research suggests that excessive water application reduced tannin content (Kennedy et al. 2000). Water deficit decreased berry size and thus change the ratio of skin mass to total berry mass and therefore anthocyanin and tannin concentration in the berry. Closer investigation of this phenomenon suggested that changes in anthocyanin and tannin concentration did in fact occur with deficit irrigation aside from any effect related to berry size (Roby et al. 2004). However, the authors considered that changes in the structure and development of the skin were responsible rather than any direct effect on flavonoid biosynthesis (Roby and Matthews 2004, Roby et al. 2004).

Finally, many such responses are regulated by plant growth regulators such as abscisic acid, ethylene, cytokinins, gibberellins, and auxins, and the influence of these compounds has been specifically examined with respect to their influence on flavonoid biosynthesis (Downey et al. 2006).

Flavor compounds

Besides sugar accumulation, the major determinants of wine quality are secondary metabolites, which include aromatic compounds. Aroma compounds are distributed in the berry skin and flesh.

These compounds occur widely throughout the plant kingdom, particularly among developing fruit (Coombe 1976). Most of them are produced in the second part of ripening period (Kennedy 2002). Some of these compounds are produced as precursors, and are not volatile until wine has been produced. Nevertheless, they are present in grapes as glycosides. Glycosylation produces stable hydroxylated metabolites that are rapidly conjugated to sugars (Rivière and Cabanne 1987). These more soluble glucose conjugates are stored in cell vacuoles (Ribéreau-Gayon et al. 2012).

Many hundreds of recognized flavor compounds have been isolated from grapes and wines and their number continually increases (Williams et al. 1987). These compounds were linked to wine aroma, because they do not change or undergo minimal changes throughout fermentation. The olfactory impact of these compounds depends on concentration, which range from parts-per-trillion to parts-per-thousand (Park et al. 1991), and the specific properties of the different molecules. Flavor compounds are subject to dynamic variation due to different processes involved in their development: grape metabolism (depending on grape variety and terroir factors), biochemical phenomena happening before fermentation (triggered by maceration or juice extraction), metabolism during fermentation (yeast and bacteria activity), and chemical or enzymatic reactions (during fermentation and aging) (Ribéreau-Gayon et al. 2012).

Environmental factors, in fact, can modify fruit flavor (Williams et al. 1987, Peyrot des Gachons et al. 2005). These effects have a physiological and morphological basis: they may operate by stimulating the production of phytohormones that modify the internal environment of the cell altering its rate of growth and development (Gray 2002).

Origin of variability of grape berry mass and relationship with grape composition

Final berry mass is determined by the number of cell divisions that occur before and after bloom, by the cell expansion degree after bloom and by the possible variation of berry mass by dehydration or hydration just before the harvest (Keller 2015 and the references therein). These phenomena are affected by biotic and abiotic factors (Fernandez et al. 2006, Houel et al. 2011).

Variety is certainly one of the major factors determining the difference in size of the berry as a result of specific genetic characteristics associated with growth and the relative proportion of the components (flesh, seeds and skin) and their relationships (Matthews and Nuzzo 2007, Attia et al. 2010, Barbagallo et al. 2011, Dai et al. 2011). Genetic determines composition and has an influence on the ability of the variety to accumulate compounds, on the way in which photosynthetic

products are distributed within the plant and on their influence on secondary metabolism (Dai et al. 2011, Ferrer et al. 2014). Many other factors impact on berry mass. Some of them are intrinsic to the berry itself. This is the case of seed number per berry (Scienza et al. 1978) and seed mass per berry (Roby and Matthews 2004). Carbon balance of the vine can also impact berry mass (Coombe 1962). Finally, environmental conditions are directly involved in determining the size and the composition of the berries. Among this external factors, vine water status (Matthews and Anderson 1988, van Leeuwen and Seguin 1994, Ojeda et al. 2001, Roby et al. 2004, Chaves et al. 2007, Ferrer et al. 2008, Girona et al. 2009) and vine nitrogen status (Choné et al. 2001a, Trégoat et al. 2002, van Leeuwen et al. 2007) play certainly a key role, affecting berry growth and development and, directly or indirectly, the grape composition (Roby et al. 2004, Roby and Matthews 2004, Walker et al 2005). The impact of factors depends on their intensity and/or the development period at which they act (Ojeda et al. 1999). Hence, because berry mass is the result of the combined effect of all these impacting factors, the final berry mass is a parameter highly variable at all scales (Gray 2002, Dai et al. 2011). This variation is greatest early in the berry developmental cycle and declines as berries resynchronize their growth during the second period of growth (Gray 2002, Pagay and Cheng 2010). This means that the major source of variation are early events (Coombe 1976).

Even when all vineyard management practices are uniform and properly executed, it still extremely difficult to obtain uniform berry diameter and composition under field conditions (Pisciotta et al. 2013). This variability, in fact, may result from parcel heterogeneity, such as soil characteristics, graft combination, plant material quality, node number per shoot, shoot number per cane, bunch number per plant, bunch position, etc. (Di Lorenzo et al. 2007, Hunter et al. 2010, Pisciotta et al. 2013). As a result, variability of berry mass and composition can be observed at different scales: (i) between vines within the vineyards, (ii) between bunches within the vine and (iii) between berries within the bunch (Coombe and Iland 2004, Pagay and Cheng 2010, Dai et al. 2011, Pisciotta et al. 2013). Depending on the considered scale, the hierarchy of these factors may vary. At the parcel scale, grapevine cultivar is likely to be the dominant factor. At intra-parcel scale, the variability may be related to variations in soil characteristics. Finally, at bunch scale, the differences between berries could be related to internal factors (Scienza et al. 1978, Carwthon and Morris 1982, Walker et al. 2005, Roby and Matthews 2004).

Berry seed content

The maximal seed number per berry is four, although the average seed number is less than two (Ollat et al. 2002). Fruit and seed growth and development are two highly dependent phenomena (Coombe and McCarthy 2000). This relationship has been the object of several earlier studies. After bloom, cell division rate and cell expansion degree depend on the number of fertile seeds per berry. Their effect on berry development is primarily related to the growing substances that they issue during the first phase of berry growth (Coombe 1972). These substances are hormones, such as auxins, gibberellins, cytokinins and abscisic acid, which stimulate the division and the expansion of cells (Scienza et al. 1978, Lavee and Nir 1986, Coombe 1992). According to Ojeda et al. (1999) it is more likely that seeds stimulate the cell division, rather than cell expansion. Hence, final berry mass and volume are proportional not only to seed number but also to their fertility (Scienza et al. 1978, Roby and Matthews 2004, Walker et al. 2005, Friend et al 2009). May (2000) showed that the stimulant effect of an individual seed on pericarp development decreases as the number of seed per berry increases. As a result, the relationship between berry volume and berry seed number is not linear but quadratic.

Conversely, the influence of berry seed content on processes linked to the berry maturation, such as accumulation of primary and secondary metabolites, is not so clear. In grape berry, seeds complete their development and maturity at the veraison (Ristic and Iland 2005). At this stage, it is possible to observe a change in hormone levels, especially of auxins and abscisic acid (Davies and Böttcher 2009). At veraison, a phenological variability between berries belonging to the same bunch can be observed. This means that each berry is independent to another and not all berries enter into to the second phase of growth simultaneously. This result could be related to the fact that flowering and/or fertilization of berry are not absolutely synchronized (Friend et al. 2009). Gouthu and Deluc (2015) showed, in fact, that a higher seed number delay veraison and, consequently, maturity. As a result also the final concentration of sugar in berry is reduced. This could be related to the higher concentration of auxins observed in berries containing a higher number of seed, which limit the sugar accumulation (Sundberg et al. 2009). Conversely, higher level of ABA, stimulating the sugar transport into the berry (Castellarin et al. 2007a), were found into the berries containing a lower seed number. However, different observations were made by Carwthon and Morris (1982), who found a lack relationship between berry seed number and auxin and abscisic acid concentration.

Vine water status

The influence of environmental factors on the composition and quality of wines is known in viticulture under the concept of terroir. Soil, as one of the major environmental factors and terroir parameters, clearly influences the vine-fruit-wine continuum. It is possible that in some wine-producing regions, fruit and wine quality are primarily controlled by factors that regulate vine water status (Reynard 2011).

Plant water transport from soil to atmosphere can be divided in four steps: soil to root, root to shoot xylem, shoot to leaf through the petiole and leaf to atmosphere through stomata. Vine water status depends on water potential in soil layers close to the root system, canopy size and evaporative demand. Water deficits occurs when transpiration exceeds the ability of the roots to supply water to the transpiring leaves (Choné et al. 2001a, 2001b).

Water is essential source for growth and performance of the vine. It is considered as one of the major environmental factors limiting growth of plant organs (Chaves et al. 2010) and water deficit represents the most frequent form of environmental stress (van Leeuwen et al. 2007).

Under natural conditions, vine water status depends on climatic variables, such as rainfall and reference evapotranspiration, and soil variables (Soil Water Holding Capacity, SWHC) (van Leeuwen et al. 2004). The amount of rainfall varies from region to region and from season to season. Moreover, water availability depends not only on how *much* rainfall a vineyard receives but also on *when* the rain falls and how rapidly it evaporates (Keller 2005). In a given season, within a limited zone, weather conditions can be considered as homogeneous. However, water availability can vary on court distances (e.g. within a parcel), according to variation of soil characteristics (Corteel et al. 2005).

SWHC and hence the amount of water available for the vine is highly variable among vineyard soils because it depends on soil texture, but also on the proportion of stones and rooting depth. Soil water is stored in the porosity of the soil. The state of the water in the soil depends on the size of the pores. In large pores (> 10 µm in diameter), water cannot be held in the soil and drains out of the soil by gravity, unless an impermeable layer provokes water logging. Hence, sand holds little water, but a large proportion of the water is readily available for plant use. In very small pores (< 0.2 µm in diameter), the water is so firmly held by the soil that plants are not able to extract it. Hence, clay holds a lot of water, but the majority of it is held in very small pores and cannot be extracted by plant roots. In pores between 0.2 µm and 10 µm in diameter, water is held by the soil

but can be extracted by plant roots. Hence, silt holds a relatively large amount of water and the majority of this water can be used by the plants (van Leeuwen 2010).

Variation in soil moisture due to differences in water holding capacity and effective root zone have a pronounced impact on vine performance both between and within vineyards (Hall et al. 2002, Lamb et al. 2004).

In grapevine, stomatal conductance and photosynthesis can be reduced by soil water deficit. Under moderate water deficits, in fact, stomatal guard cells responds to leaf water potential in order to restrict the water loss. This limits also the carbon assimilation (Chaves et al. 2003). As a consequence, growth and development of the shoots, leaves and fruits are affected (Bravdo et al. 1985). However, the vegetative growth seems to be more sensitive to water stress than fruit growth (Williams et al. 1994). Several previous studies reported that water stress enhanced earliness of phenological stages and shoot growth cessation, which increased the proportion of carbohydrates available for fruit ripening (van Leeuwen and Seguin 1994, Trégoat et al. 2002, Koundouras et al. 2006). The effects of a limited water condition vary according to its duration and intensity and in function of the phenological phase at which water deficit occurs (van Leeuwen and Seguin 1994, Ojeda et al. 2002, Deloire et al. 2004).

Reproductive development is most sensitive to water stress from meiosis to fruit set (Keller 2015). Water deficit, typically reduces yield, particularly if the deficit occurs early in the growing season (Williams and Matthews 1990). Limited water supply during the period of berry cell division and cell expansion restricts berry enlargement, which limits berry size (Williams and Matthews 1990, Roby and Matthews 2004, Shellie 2014). Whereas cell division in young grape berries is relatively insensitive to water deficit, cell expansion responds readily to changes in water supply (Ojeda et al. 2001). The limitation in berry size imposed by early-season stress is irreversible, it seems likely that it involves changes in the composition of skin cell walls (Ojeda et al. 2001). Yield reductions due to drought stress can still be severe after fruit set, whereas after veraison berries seem to become increasingly insensitive to water deficit. Thus, the same extent of water deficit occurring during the pre-veraison phase of berry growth normally reduces berry size much more than if it occurs after veraison (Hardie and Considine 1976, Matthews and Anderson 1989, Williams and Matthews 1990, McCarthy 1997). Applying more water later in the growing season cannot compensate the decrease in berry size due to early-season deficit (Ojeda et al. 2001, 2002). Despite the fears of many winemakers, therefore, drip or flood irrigation close to harvest is ineffective in

increasing berry size and “diluting” fruit composition (Keller et al. 2006). The same cannot be said for rainfall or overhead irrigation, however, because uptake of water through the berry skin may induce berry cracking (Keller 2015). Nevertheless, post-veraison water deficit can lead to berry shrinkage by dehydration (Keller et al. 2006).

Mild water deficits were shown to have a positive impact on wine quality in red varieties. Seguin (1975) showed that grape composition was positively influenced by a regulation of the water uptake condition of the vines. Similarly, van Leeuwen et al. (2009) showed that vintage quality in Bordeaux is related to the water balance: the drier the year, the better the overall quality of the wine produced.

Vine water status is known to influence fruit composition through an indirect effect on berry size, and therefore the ratio of skin to pulp, which increases in the smaller berries of vines subjected to water deficits (Bravdo et al. 1985, Kennedy et al. 2002).

Moderate water deficit promotes sugar accumulation as a result of inhibiting lateral shoot growth, which induces a reallocation of carbohydrates to fruits (van Leeuwen and Seguin 1994, Trégoat et al. 2002, Koundouras et al. 2006). Sugar accumulation is also stimulated by an increase of the berry acid abscisic (ABA) concentration (Castellarin et al. 2007b, Deluc et al. 2009). However the impact of vine water status on sugar concentration seems to be variety-dependent (Gaudillère et al. 2002). While no significant relationships were observed in Merlot, Castellarin et al. (2007a, b) found a significant increase of sugar content in Cabernet Sauvignon berries produced under water deficits.

The effect of water deficit on titratable acidity is also unclear. Several studies, did not observe changes in titratable acidity in the must from moderately water-stressed vines (Matthews and Anderson 1989, Esteban et al. 1999). Other authors report a reduction of titratable acidity due to deficit irrigation as compared with full irrigation (Shellie 2006). Many previous studies report that reduced water supply to the vines limited berry malic acid concentration (Duteau et al. 1981, van Leeuwen et al. 1994, Choné et al. 2001a, Trégoat et al. 2002, Koundouras et al. 2006). This is due to increased malate breakdown in vines with low water status (Matthews and Anderson, 1989).

Many compounds important for wine quality are located in the skin and in seed endocarp tissues (Ojeda et al. 2002). It is well known that vine water status affects accumulation of polyphenols in these tissues (Matthews and Anderson 1989, Ojeda et al. 2002, Roby and Matthews 2004, Roby et al. 2004).

Water deficit imposed during ripening can increase the skin flavonoid concentration and also alter their composition. The reported increase in skin tannin and anthocyanin that accompanies water deficits seems to result from differences in sensitivity of berry tissues to water deficits, with the exocarp being less affected than the inner mesocarp (Roby et al. 2004).

These effects can be indirect, due to reduced berry growth and to an evaporation of berry water, or direct, due to direct stimulation of phenolic biosynthesis (Roby and Matthews 2004), because of an altered expression of genes coding the enzymes of flavonoids biosynthesis (Castellarin et al. 2007b). While Ojeda et al. (2002) reported that water deficit can alter the concentration in phenolic compounds both indirectly through modifications of berry size and directly by affecting their biosynthesis, Roby et al. (2004) concluded that the effect of whole plant water status on the concentration of skin phenolic compounds is greater than the effect of fruit size on the same variables (Roby et al. 2004). Hence, regulating grapevine water deficit can be a powerful tool to manage the amount of these compounds and improve wine quality (Kennedy et al. 2002).

Vine nitrogen status

Soils vary not only in their capacity to store water but also in the amount and composition of mineral nutrients they contain and in the extent to which these nutrients are available for uptake by the roots. Among all mineral nutrients that vine picks up from the soil, nitrogen is the most in terms of influencing the vine growth, morphology and grape physical and chemical composition (Spayd et al. 1993). This is primarily because nitrogen is a chemical component of many important plant constituents, such as nucleic acid, amino acids, proteins and enzymes. It is an integral part of chlorophyll, responsible for intercepting and capturing sunlight, of hormones used for communication between different plant organs, and of certain secondary metabolites, some of which also contribute to wine flavor (Champagnol 1984).

Nitrogen is present in the soil solution in the form of nitrate (NO_3^-) and ammonium (NH_4^+) ions. Nitrate is the vine roots nitrogen uptake form of choice. It is reduced to ammonium in the roots and metabolized (assimilated) into the amino acids glutamine and glutamic acid (Keller 2015).

Beyond the addition of nitrogen fertilizer, vine nitrogen uptake depends to a large extent on the amount of nitrogen the soil supplies to the vines, through mineralization of soil organic matter. The latter is high when C/N ratio of soil organic matter is low, pH is high, soil temperature is high and soil moisture content is close to field capacity, resulting in high soil microbiological activity.

Soil aeration also stimulates organic matter turnover. Hence, the distribution of both organic matter and available inorganic nitrogen in the soil is extremely heterogeneous. In this way, the nitrogen availability to the vine is soil type-related and can thus be considered as a terroir characteristic (van Leeuwen et al. 2000).

Although the nutrient concentration in a dry soil is usually higher than that in a wet soil, these nutrients are less available in dry soil because the lack of water slows diffusion and mass flow (Marschner 1995). Moreover, mineralization of organic matter and nitrification also slowdown in drying soil. Although this diminishes nutrient uptake and delivery of nutrients to the shoots (Davies et al., 2002), the tissue nutrient concentration may nevertheless increase because water stress curtails carbon assimilation and shoot growth more than nutrient uptake (Keller 2015).

Nitrogen uptake is mainly controlled by the demands of the vine and varies according to growth requirements. Such demand also includes the growing fruit, so that seasonal nitrogen uptake by grapevines varies according to their crop load (Keller 2015).

Because of the rapid shoot growth in spring, vine nitrogen demand is greatest between budbreak and bloom (Peacock et al. 1989). During this period, the vine is mainly dependent on the nitrogen reserves stored in the permanent structure (Keller et al. 1998).

Remobilization from the reserve seems to be independent of soil nitrogen availability. Conversely, poor nitrogen reserve status, due to inadequate refilling in the previous growing season, can restrict early shoot growth and canopy development (Keller 2005). Nitrogen uptake increases progressively through bloom, fruit set, and the first phase of berry growth and may increase further after veraison. When water is not limiting, maximum nitrogen uptake may occur during the warmest period of the growing season. Storage reserves reach a minimum around bloom time or even later, which makes vines vulnerable to deficiency if insufficient nitrogen is available in the soil (Keller 2005).

Grape quality potential for red wine production is correlated to vine nitrogen status, particularly so when water status is not limiting (van Leeuwen 2010).

Mineral nitrogen, as well as water, is one of the most potentially limiting factors for the grape production. Vine nitrogen status affect both yield and grapevine quality. The impact of this factor on enological potential of grape is due to direct and indirect effects (Smart et al. 1990).

Several studies showed that nitrogen strongly impact the shoot growth (i.e. vigor), berry growth and development and biosynthesis of primary and secondary metabolites (Choné et al. 2001a,

Trégoat et al. 2002). Nitrogen contained in chlorophyll and enzymes favor the photosynthesis in the leaves and, as a result, increase the sugar amount available for fruit maturation. However, high nitrogen availability, especially at bloom, favors shoot growth and leaves expansion. Consequently, at maturity, leave number per plant is higher and a competition between growing shoots and maturing berries can occur (Keller et al. 2012). High vigor modifies the microclimate of bunch zone. Temperature, shade and humidity of this zone of canopy have a strong impact on primary and secondary metabolite synthesis (Bell and Henschke 2005).

Insufficient N availability during bloom interferes with flowering and reduces fruit set and cluster initiation in the buds (Spayd et al. 2003, Keller et al. 2001). Low nitrogen status is sometimes associated with berries growing larger (Hilbert et al., 2003). This is probably a consequence of diminished sink number due to poor fruit set. A restriction in crop load at the beginning of berry development tends to result in compensatory growth of the remaining berries (Keller et al. 2008). Other authors found that under limited nitrogen conditions berries are smaller (van Leeuwen and Seguin 1994, Choné et al. 2001a, Trégoat et al. 2002, van Leeuwen et al. 2007). These authors showed also that low vine nitrogen status increases grape sugar concentration.

Sugar is not the only grape component that is affected by vine nitrogen status. Shaded grapes normally have less tartaric and more malic acid, which may result in an increase or decrease of pH. Moreover, phenolic compounds, such as tannins and anthocyanins (red pigments), are reduced along with flavor compounds (Keller 2015). Hence, accumulation of total polyphenols, including anthocyanins, in berries is increased under low nitrogen status (Hilbert et al. 2003, Soubeyrand et al. 2014), whereas it is decreased by excessive nitrogen supply (Keller et al. 1999). In this way, grape quality potential for red wine production is increased by a limited soil nitrogen availability to the vines (van Leeuwen 2010).

Berry size and grape and wine composition

Berry size is a major quality factor in wine production. One of the most widely accepted ideas in winemaking is that large diameter fruit would have a greater solvent to solute ratio as a result of the lower surface to volume ratio compared to smaller fruit (Singleton 1972, Matthews and Anderson 1988, Kennedy 2002). The underlying idea is that the higher concentration of important compounds localized in the skin is favorable for the quality of red wines (Matthews and Anderson 1988). This principle is based primarily on the geometric assumption that grape berry is a sphere.

Hence, according to surface-volume relationship of a sphere, as the berry radius increases, the skin to flesh ratio decreases. (Roby and Matthews 2004, Roby et al. 2004, Walker et al. 2005, Matthews and Nuzzo 2007). Consequently, the concentration of skin solutes would be increasingly diluted with increasing berry size (Matthews and Nuzzo 2007).

In fact, implicit in this believe is the assumption that the proportion of berry tissues remain constant while berry size changes. However, several studies have found that the proportion of skin and flesh did not vary according to the relationship between the surface and volume of a sphere. (Roby and Matthews 2004, Walker et al. 2005, Barbagallo et al. 2011). In other words, the skin does not stretch around a larger flesh but grows with it. Hence, although the relative amount of berry tissues can vary depending on variety and environment (Keller 2015), among berries growing under similar environmental conditions, berry skin and seed tissue development are coordinated with flesh growth (Barbagallo et al. 2011) and, as a result, there may be little variation in the skin to flesh ratio (Matthews 2015). However, some environmental conditions, such as water deficit, alter that general relationship. Roby and Matthews 2004 reported that water deficits inhibit more flesh growth than skin growth, increasing, as a result, the skin-to flesh ratio. This effect can result in higher concentration of skin solutes.

Most grape growers agree also that there is a fixed amount of primary and secondary metabolites in each berry and that the variable in berry size is the water. Consequently, this fixed amount of skin solutes is increasingly diluted by the flesh of ever bigger berries. Effectively, if these assumptions are true, smaller berries will have higher concentrations of solutes. However, Coombe (1987) and Roby and Matthews (2004) showed that the amount of solutes per berry increases linearly with berry size.

The relationship between berry size and grape composition is complex and still far from being fully understood. Actually, there are contrasting conclusions among researchers regarding differences in berry composition when comparing berries strictly on size.

Some authors did not find a significant difference between the composition of small and large berries (Barbagallo et al. 2011). Other studies reported that the sugar concentration is higher in smaller berries (Scienza et al. 1978, Carwthon and Morris 1982). In contrast, Glynn (2003), measuring the sugar content of Cabernet-Sauvignon and Chardonnay berry by berry, did not find a relationship between °Brix and berry size. Similar results were obtained by Walker et al. (2005) on Shiraz berries. Roby et al. 2004 reported that berry sugar content (g/berry) depended on berry

mass, while berry sugar concentration (g/L) did not change with berry mass. Similar relationships were observed by Roby and Matthews (2004), when studying the effect of berry mass on anthocyanins. Anthocyanins per berry were proportional to the size of the berry, while the concentration of anthocyanins decreased with increasing berry size, indicating the possibility of producing different wine styles from berries with different sizes. These results were not confirmed by Ferrer et al. (2014) who reported that total anthocyanin content or concentration was independent of berry size.

Similarly to grape berry composition, there is no consensus among researchers on whether smaller berries make superior wines. Gil et al. (2015) demonstrated that smaller grapes produced wines of deeper colour and that size is inversely correlated with the concentration of phenolics, such as anthocyanins and stilbenes. In contrast, comparing wines from "small" and "large" berries, Walker et al. (2005) came to the conclusion that smaller berries do not produced superior wines. Other researchers found that there is no simple linear relationship between grape composition and wine quality (Johnstone et al. 1995).

The absence of a consensus among researcher could be due to the fact that the final berry composition (physical and chemical) is a result of interactions among factors impacting its growth and development.

Berry composition is dependent on physiological processes other than growth. The way in which berry mass is reduced seems to be more important than the berry mass itself: hence, wine improvement is not due just to berry size, but to changes in vine metabolism provoked primarily by factors like cultural practices or annual weather conditions, which may also impact berry mass (Matthews and Anderson 1988, Roby et al. 2004, Walker et al. 2005, Holt et al. 2008). However, the interaction among factors, under field condition, make difficult the study of berry size impact on grape composition.

OBJECTIVES

Based on previously reported results concerning berry growth and development, factors impacting final berry mass, and the relationships between berry mass and grape and wine composition, the aims of the present works were:

- to investigate on the simultaneous effects of the major factors influencing berry mass and grape composition at different scales: intra-bunch (berry by berry), intra-plant (bunch by bunch), intra-block (plant by plant) and intra-parcel (block by block). For each scale, factors impacting berry mass were hierarchized according to their degree of impact and the effect of berry mass on berry composition was investigated.
- to understand if, at an intra-parcel level, a specific factor directly impacted fruit composition independently of the resultant differences in berry mass (indirect effect).
- to compare the profile of wines made from berries belonging to different size classes (large and small), sorted by a specific machine, the Calibaie[®], and to investigate the possibility for wine producers to obtain two different wines from berries grown on one single parcel.
- to isolate the effects of vine water status on final berry mass and to investigate the interrelationships between berry size at harvest and fresh mass distribution between seed, skin and flesh tissues.

A preliminary study was carried out in the 2012 vintage in a vineyard located in the Saint-Emilion winegrowing area on parcels planted on two different soil types characterized by different Soil Water Holding Capacity (SWHC). The satisfactory results obtained during this first experience allowed the project partners to continue the same research over the 2013 and 2014 seasons. However, the particularly bad weather conditions of the 2013 vintage did not allow to reach the goals that were set. Thus, in order to accomplish a focus on the effect of vine water status on the final berry mass, in 2014, it was decided to carry out simultaneously a study in a commercial vineyard located in western Sicily, where because the semiarid conditions, irrigation is allowed. Two water regimes, Non-Irrigated (NI) and Irrigated (I), were established in a randomized block, and their effect on berry mass was investigated. Supplementary results were obtained in 2015 in both the Saint-Emilion and western Sicily vineyards.

RESULTS

Chapter II

Hierarchy of factors impacting grape berry mass.
Separation of direct and indirect effects on major
berry metabolites

ABSTRACT

Final berry mass, which is a major quality factor in wine production, is the result of the integrated effect of biotic and abiotic factors. Depending on the observation scale, the hierarchy of the impact degree of these factors can vary. These also influence berry composition. Under field conditions, the interactions between these factors make the study of the variability of berry mass and composition difficult. The present work was designed to study the simultaneous effect of the major factors influencing berry mass and composition. The first objective was to hierarchize their impact at an intra-parcel level. The second objective was to distinguish a direct effect of these factors on berry composition from an indirect effect mediated through their impact on berry mass. Vine water and nitrogen status of six blocks, located on a sandy and a gravelly soil, were monitored during two following years. Berries from Cabernet franc were analyzed from veraison to harvest. At each sampling date, fresh berry mass, berry seed mass and number and sugar and malic concentration were recorded. Two statistical approaches were performed to reach our goals. Our results showed that all studied factors significantly impacted the final berry mass, but vine water status represented the most impacting factor on berry mass and composition. However, the interaction between factors, sometimes hid the significant effect of some of them on berry compounds. The order of impact of the considered factors varied among metabolites. Nevertheless, our statistical model series, performed on data collected under field conditions, allowed to prove that all factors had a direct impact on berry composition, independently from the one mediated through their impact on fresh berry mass.

INTRODUCTION

Berry size is a major quality factor in wine production. Most grape grower agree on the assumption that better wines are produced from small berries due to their higher ratio of skin to flesh (Kennedy et al. 2002). The underlying idea is that the higher concentration of important compounds localized in the skin is favorable for the quality of red wines (Matthews and Anderson 1988). Although the influence of berry size on grape composition is complex and still far from being fully understood, their relationship is the subject of many debates. Some authors found a significant relationship between berry mass and grape and wine composition (Melo et al. 2015), whereas other authors didn't find a significant difference between the composition of small and large berries (Barbagallo et al. 2011). These different results may be explained by the fact that small berries can impact wine quality directly by modified skin to flesh ratio. However, there can also be an indirect effect through a limiting factor (e.g. water deficit) impacting simultaneously berry mass and berry composition (Walker et al. 2005). Many studies showed that final berry mass is the result of the integrated effect of biotic and abiotic factors that affect cell number and/or cell volume. In addition to genetic variability (Dai et al. 2011), a factor related to the berry itself is seed number (Walker et al. 2005) or seed mass (Roby and Matthews 2004). Seeds influence the berry cell division and expansion via hormones (Friend et al. 2009). Differences in berry seed content, and consequently in the hormone level (e.g. ABA and auxin), affect the growth of berries and the timing of their ripening (Gouthu and Deluc 2015). Seed number and their fertility depend on the quality of fertilization. Some virus infections can cause either losses of whole parts of inflorescences or poor fruitset. *Grapevine Fan Leaf Virus* (GFLV) has been reported to cause significant reduction in grape yield (Andret-Link et al. 2004). This virus affects the ripening of the berries, which can be irregular on the same bunch (Martelli and Savino 1990).

Environmental conditions are also directly involved in determining the size and composition of the berries. Water deficit is considered as one of the major environmental factors limiting growth of plant organs (Chaves et al. 2007). Vine water status is equally impacted by climate and soil (van Leeuwen et al. 2004). Plant water availability is highly variable inside and among vineyards because Soil Water Holding Capacity (SWHC) varies with soil texture, percentage of stones and rooting depth. Water stress has been shown to inhibit grapevine photosynthesis, plant growth, fruit size and yield (van Leeuwen and Seguin 2006). Positive or negative impacts of limited water uptake conditions depend on the period when it occurs and on its intensity (Deloire et al. 2005).

Water deficit conditions imposed from fruit set to veraison increase the concentration of berry ABA (Deluc et al. 2009), limit cell division and expansion, reducing berry size (Ojeda et al. 2001), and stimulate sugar accumulation (Castellarin et al. 2007b). Sugar accumulation in water deficit conditions is also accelerated by a higher availability of carbohydrates, because of decreased vine vigor and shoot growth (van Leeuwen and Seguin 2006). However, several authors suggest that the impact of water deficit on berry sugar content is cultivar-dependent (Gaudillère et al. 2002, Deluc et al. 2009) and, consequently, not always significant. Berries produced under water deficit conditions are also characterized by lower acid concentration (Esteban et al. 1999), probably due to increased malate breakdown (Matthews and Anderson 1988). Finally, Roby et al. (2004) showed that there are effects of vine water status on fruit composition that arise independently of the resultant differences in fruit size. This result could be the consequence of the impact of water deficit on genes controlling different metabolite pathways (Castellarin et al. 2007b). Among elements the vine picks up from the soil, nitrogen is undoubtedly the one that most impacts on vine growth, vigor and grape composition. Beyond the addition of nitrogen fertilizer, vine nitrogen uptake depends to a large extent on the amount of nitrogen the soil supplies to the vines, through mineralization of soil organic matter. This mineralization is enhanced when soil temperature is high and soil moisture content is close to field capacity (van Leeuwen et al. 2000). Low vine nitrogen status reduces vine vigor, berry mass and increases berry sugar (Choné et al. 2001a, Trégoat et al. 2002). Accumulation of total polyphenols, including anthocyanins, in berries is increased under low nitrogen status, whereas it is decreased by excessive nitrogen supply (Hilbert et al. 2003). Hence, grape quality potential for red wine production is increased by limited soil nitrogen availability to the vines.

Variability of berry mass and composition can be observed at different scales and is the result of the combined effect of all the impacting factors (Dai et al. 2011). Depending on the scale, the hierarchy of these factors may vary. At the parcel scale, grapevine cultivar is likely to be the dominant factor. At intra-parcel scale, the variability may be related to variations in soil characteristics. Finally, at bunch scale, the differences between berries could be related to differences in seed number or mass.

Under field conditions, the interactions between factors impacting berry growth and development make difficult the study of berry size variability. Most studies published on berry mass and composition take into account only one single impacting factor. Though this approach clarify the

specific impact on berry characteristics of each considered factor, it remains descriptive. Hence, it does not lead to a greater understanding of berry mass and composition variability under field conditions, where environmental and plant related factors interact with each other.

The present work was designed to study the simultaneous effect of vine water uptake conditions, vine nitrogen status and berry seed content on berry mass and its composition, in order to hierarchize their impact at an intra-parcel level.

MATERIALS AND METHODS

Location and experimental design

This study was carried out during two consecutive years (2014 and 2015), on two parcels of a commercial vineyard located of the Saint Emilion region, in the South West of France. The selected parcels were dry-farmed and characterized by different soil types. Soils are classified according to the French “*Référentiel Pédologique*” (Baize and Girard, 1995). Inside of each parcel, three experimental blocks were chosen for their different SWHC and water potentials measured during previous years (data not shown). The blocks S5, S6 and S7 were located on a sandy soil (ARENOSOL Redoxique), characterized by a water table within the reach of the roots. For this reason the SWHC of these blocks was high and, because of low spatial variability in soil composition, water availability was similar among them. This homogeneity in soil composition was not observed on the second experimental parcel, where the blocks G1, G7 and G8 were located on a gravelly/sandy soil (PEYROSOL). In G7 and G8 blocks, gravel content was around 25% in the topsoil (0-65 cm) and around 80% in the following soil layers (65-160 cm). Because of this high gravel content, SWHC was low on these two blocks. Conversely, G1 showed a higher SWHC, related to the presence of a layer without gravel at around 120 cm of depth. The experimental design of this study was based on this large range of water availability, possibly impacting on berry mass and juice composition.

Plant material

All experimental blocks were planted with *V. vinifera* L. cv Cabernet franc. Each block was composed by nine adjacent plants, distributed over three rows. In order to minimize the effect of non-environmental factors, special care was taken to achieve maximum uniformity in viticultural conditions. To determine the sanitary status of the plants, samples of young and mature leaves and wood were collected during two seasons from selected vines of each block. Leaf samples of individual vines were analyzed by ELISA test, according to Beuve et al. 2013, to check possible presence of *Grapevine FanLeaf Virus* (GFLV, genus *Nepovirus*), *Arabic Mosaic Virus* (ArMV, genus *Nepovirus*) and *Grapevine LeafRoll associated Virus* (GLRaV-1 and -3, genus *Ampelovirus*). GLRaV-2 detection was performed on samples of pruning wood by RT-PCR method (Beuve et al. 2007, 2013). Result showed that all plants of G1, G7, G8, S5 and S7 were healthy. Conversely, all vines of S6 blocks were infected with viruses involved in fanleaf degeneration

(GFLV and ArMV). Because the impact of these viruses on berry mass and composition was shown in others studies, we decided to not delete this block and to take the presence of these viruses into account during the data analysis.

Vine water status assessment

Stem water potential. Dynamic evolution of vine water status during the growing season was monitored using a pressure chamber (Scholander et al. 1965), equipped with a digital LDC manometer. Several measurements of midday stem water potential (Ψ_{stem}) were carried out with regular intervals from early July until the end of September during both vintages. Ψ_{stem} was estimated in the early afternoon, when the lowest values of the day are recorded. Measurements were carried out on fully expanded leaves from primary stems of different vines. Leaves were enclosed in a reflective plastic envelope for at least one hour before measurement. This time period allowed the water potential in the leaf to reach equilibrium with the water potential in the stem, as transpiration stops in the opaque plastic bag (McCutchan and Shackel 1992). Each measurement was replicated 6 times, on 4 individual vines. This measurement provides an accurate and robust estimation of vine water status (Choné et al. 2001b).

Carbone isotope discrimination. Compared to ^{13}C of ambient CO_2 , ^{12}C isotope is more preferentially used by the enzymes involved in photosynthesis for their production of hexoses (Gaudillère et al. 2002). This process, called ‘isotope discrimination’, is reduced when plants face water deficit conditions, because of stomatal closure. Sugars produced under these conditions contain more ^{13}C compared to those produced when plant water status is not limiting. Therefore, $^{12}\text{C}/^{13}\text{C}$ ratio (so-called $\delta^{13}\text{C}$) measured on products of photosynthesis at ripeness, is an integrative indicator of vine water uptake conditions during grape ripening (van Leeuwen et al. 2001, Gaudillère et al. 2002). In this study, $\delta^{13}\text{C}$ measurements were carried out by mass spectrometry on grape juice extracted from 18 individual berries per block, collected in a random way. The results vary from -20‰ (severe water deficit stress) to -27‰ (no water deficit stress), according to van Leeuwen et al. 2001b.

Vine nitrogen state assessment

In this study, Yeast Assimilable Nitrogen (YAN) content in berry juice was chosen to assess vine nitrogen status from veraison through harvest (van Leeuwen et al. 2000). For each block, the

dynamic evolution of YAN during the ripening period was estimated weekly in grape juice extracted from 200 fresh berries, using Sørensen formol titration method (Ribéreau-Gayon et al. 2012). At harvest, the measurements of YAN content were carried out on grape juice extracted from 54 individual fresh berries per block, using the same method.

Assessment of grape ripening

In order to follow the seasonal dynamics of berry mass and composition, samples of around 400 berries were collected weekly from vines of each experimental block. Berries were counted and weighed to determine mean berry mass. Then they were pressed and the must, after a gentle centrifugation, was analyzed for soluble solids and malic acid, using Fourier Transform Infrared Spectroscopy (FTIR) method (Destrac et al. 2015).

Berry analysis at harvest

In order to determine a highly representative value of berry mass, forty-four day after veraison (DAV), one basal cluster located on a central primary shoot was sampled from nine individual vines per block. Mass of each individual berry was recorded. Berry seed content and the major berry compounds were measured, berry by berry, on a sub-sample of fifty-four berries per block. Berries were selected, according to their differences mass, in order to span the whole range of berry mass variability of the cluster. Sugar concentration, expressed in degree Brix, was measured using a refractometer. Malic acid concentration was estimated by a colorimetric method using a Bran and Luebbe TRAACS 800 autoanalyzer.

Statistical data analysis

Data were analyzed using R software (R development Core Team 2015, version 3.2.3).

Block effect. Effects of blocks on vine water and nitrogen status and on berry characteristics were tested using a one-way ANOVA, considering berries as replicates; Tukey Honest Significant Difference (HSD) test was used as post hoc test for multiple mean comparison.

Factor hierarchy on berry mass and its composition. A balanced dataset was initially created, with all variables characterized by the same number of replicates. This step was necessary because of the large number of observations of some variables. The size of the new dataset was fixed at nine replicates for each variable. A random sample of the specified size was drawn, for each block,

from the original values of each variable. The distribution of the sampled data were then compared to the original ones, by means of graphical tools (boxplots), addressing mean values and variance. With this dataset, the relationships between the berry mass or sugar and acid malic concentrations and the covariates (vine water status, vine nitrogen status and berry seed content), were determined by multiple linear regression analysis. In this model, $\delta^{13}\text{C}$, YAN content and total fresh seed mass per berry were chosen as indicators of vine water status, vine nitrogen status and berry seed content, respectively. Moreover, this analysis was performed on the whole data set, independently from vintage or block. The full model, considering all the interactions among covariates, was reduced by a stepwise procedure. On the final model, an ANOVA type III was performed to compute the contribution of each component to the variance of berry mass and berry composition. The so-called Type III Sum of Squares (SS), does not depend on the order of the model terms (variables), but the individual effect SS do not sum to the total SS. Moreover, type III SS assumes that interaction exists among covariates. Therefore, the incremental contribution of each covariate to the variance of berry mass cannot be calculated as the ratio of the sum of squares due to this covariate to the total sum of squares of the model. As a consequence, in this study, the relative importance of each covariate to explain the variability of berry mass (or berry composition) was calculated as the ratio of the sum of squares among covariates.

Factors impacting on sugar and berry malic acid concentrations. In order to understand if a specific factor directly impacted fruit composition independently of the resultant differences in berry mass (indirect effect), a different model for each factor (vine water status, vine nitrogen status and berry seed content) was considered. In each model sugar or malic acid concentrations were, in turn, the response. Concerning the covariates, berry mass was always the first one, whereas vine water status or vine nitrogen status or berry seed content were, in turn, the second covariate. An ANOVA type I was then considered, because the additive effect of the second covariate with respect to berry mass was tested.

RESULTS

Weather conditions

Temperature and rainfall data were recorded by an automatic weather station based on one of parcels studied. Because the distance between the two experimental parcels is around 500m, the climatic conditions can be assumed as homogenous. The Saint-Emilion area has an oceanic-type climate, with a mean temperature of 17.8 °C and a mean rainfall of 430 mm for the growing season (April-October, station Cheval Blanc, 33330, Saint-Emilion, 1996-2015). Climatic conditions varied during the two vintages studied (figure 1).

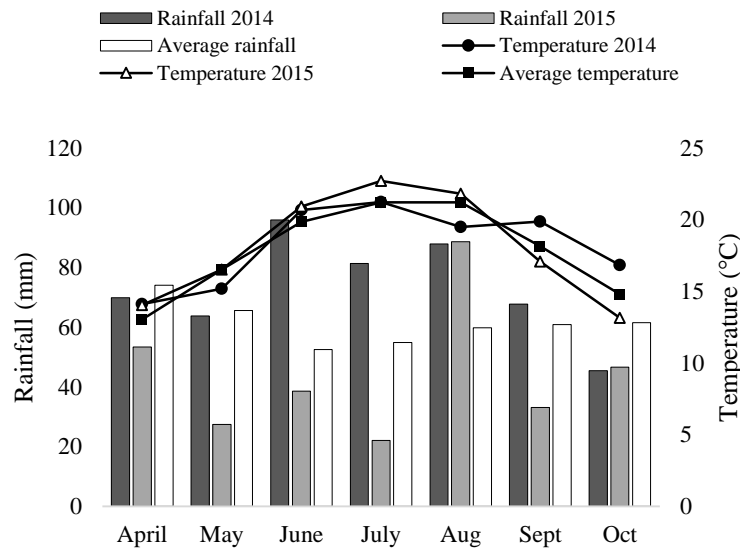


Figure 1 - Seasonal trends (1 April - 31 October) of monthly temperature and rainfall recorded in 2014 and 2015; comparison with average values (1996-2015)

In 2014 the trend of monthly temperatures was close to long-term mean and slightly warmer in September and October. However, growing season rainfall was above than average, except for April, May and October. The year 2015 was warmer than average, except for September and October. Moreover, this vintage was exceptionally dry, especially in the first part of the growing season. Only the month of August was rainy, but actually this phenomenon was mainly related to one rainstorm in the middle of the month.

Vine water status

According to the observed seasonal dynamic of Ψ_{stem} , vine water status varied during the season depending on the climatic conditions of the vintage and this evolution varied from year to year

(Figure 2A and B). Moreover, because vine water status is highly dependent on soil water availability, it varied from one block to another depending on soil characteristics. This explains why vines did not face water deficit on S5, S6 and S7 blocks, planted on a sandy soil with a shallow water table. This was observed during the two experimental seasons (Figure 2A and B).

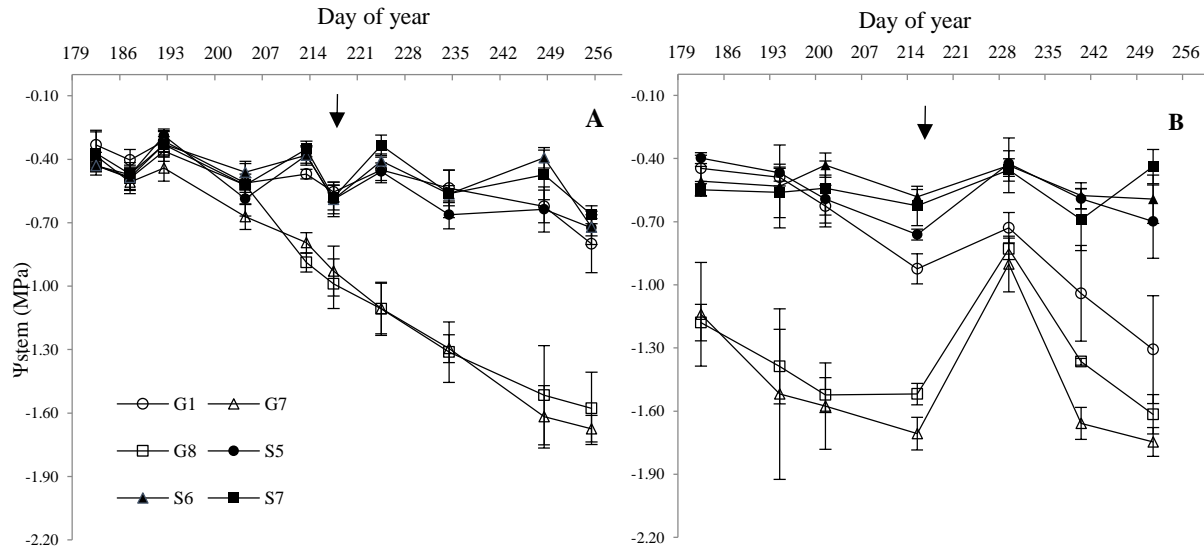


Figure 2 - Seasonal dynamics of stem water potential (Ψ_{stem}) measured in the experimental plots in the summer of 2014 (A) and 2015 (B). Each point represents a mean of four replicates. Arrows indicate 50% veraison.

Vintage effect on seasonal dynamics of Ψ_{stem} was clearly evident on the blocks planted on the gravelly soil. On 1st July 2014 (day of year 182), Ψ_{stem} values were close to -0.4 MPa on the six blocks (figure 2A), showing no limitation in vine water uptake. Significant differences in water status between blocks started to develop from the beginning of August (day of year 216), around veraison (table 1 and figure 2A). Vine water deficit continued to increase during August only on G7 and G8 and, on the final measurement day (day of year 255), Ψ_{stem} values, recorded on these blocks, indicated a severe water deficit. Conversely, no water deficit was recorded on G1 during whole season, showing values similar to those observed on the blocks planted on the sandy soil. This behavior can be attributed to the presence of a layer with a low gravel content at 120cm of depth in this block resulting in a higher SWHC. Seasonal effects on vine water status were also reflected by $\delta^{13}\text{C}$ measurements, measured on grape sugar at ripeness in 2014. Results were consistent with vine water status observed by Ψ_{stem} (table 1). High and significant correlations between two vine water status indicators used in this study were observed (table 2). The consistency between these indicators have already been shown by Gaudillère et al. (2002).

Table 1 - Influence of block on vine water and nitrogen conditions, measured with different physiological indicators, in 2014 and 2015

Block	2014				2015			
	$\Psi_{\text{stem Ver}}^a$ (MPa)	$\Psi_{\text{stem Harv}}^b$ (MPa)	$\delta^{13}\text{C}^c$ (‰)	YAN Harv ^d (mg/L)	$\Psi_{\text{stem Ver}}^a$ (MPa)	$\Psi_{\text{stem Harv}}^b$ (MPa)	$\delta^{13}\text{C}^c$ (‰)	YAN Harv ^d (mg/L)
G1	-0.55 ± 0.04 b ^e	-0.74 ± 0.1 b	-27.31 ± 0.45 a	121 ± 37 c	-0.94 ± 0.07 c	-1.31 ± 0.22 c	-24.81 ± 0.46 b	147 ± 21 c
G7	-1.01 ± 0.11 a	-1.68 ± 0.06 a	-23.43 ± 0.88 c	140 ± 43 d	-1.69 ± 0.07 a	-1.77 ± 0.05 a	-23.19 ± 0.23 d	167 ± 54 c
G8	-0.99 ± 0.11 a	-1.61 ± 0.15 a	-24.85 ± 0.77 b	101 ± 30 b	-1.55 ± 0.04 b	-1.57 ± 0.01 b	-23.93 ± 1.04 c	118 ± 12 b
S5	-0.57 ± 0.06 b	-0.69 ± 0.06 b	-26.85 ± 0.76 a	64 ± 25 a	-0.77 ± 0.02 d	-0.75 ± 0.12 d	-26.78 ± 0.34 a	92 ± 8 ab
S6	-0.6 ± 0.07 b	-0.71 ± 0.04 b	-26.73 ± 0.21 a	66 ± 22 a	-0.6 ± 0.03 e	-0.62 ± 0.11 d	-26.98 ± 0.17 a	86 ± 4 ab
S7	-0.57 ± 0.05 b	-0.67 ± 0.02 b	-27.12 ± 0.35 a	58 ± 13 a	-0.65 ± 0.07 e	-0.42 ± 0.08 e	-27.44 ± 0.2 a	69 ± 3 a

^aStem water potential at veraison, ^bStem water potential at harvest, ^cCarbon isotope discrimination, ^dYeast Assimilable Nitrogen at harvest
The difference between blocks was tested with a one-way ANOVA.

^eDifferent letters in the same column denote statistically significant differences between blocks at P<0.05 by Tukey's HSD test.

Table 2 - Correlations between indicators of vine water and nitrogen status used during the two years of experimentation.

	$\Psi_{\text{stem Ver}}$ (MPa)	$\Psi_{\text{stem Harv}}$ (MPa)	$\delta^{13}\text{C}$ (‰)	YAN Harv (mg L ⁻¹)
$\Psi_{\text{stem Ver}}$ (MPa) ^a	1			
$\Psi_{\text{stem Harv}}$ (MPa) ^b	0.67 ***	1		
$\delta^{13}\text{C}$ (‰) ^c	0.67 ***	0.81 ***	1	
YAN Harv (mg/L) ^d	0.36 ***	0.50 ***	0.41 ***	1

^aStem water potential at veraison, ^bStem water potential at harvest, ^cCarbon isotope discrimination, ^dYeast Assimilable Nitrogen at harvest

^eSignificance. codes: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 'ns' '1'

^fr² and p – value were computed using a linear regression.

In 2015, which was an exceptionally dry vintage, Ψ_{stem} values recorded on G7 and G8, were significantly different, compared to other blocks, starting right from the first measurement day (day of year 182), when water deficit was moderate to severe on these blocks (figure 2B and table 1). Compared to 2014, vines on these blocks were subject to water deficit before veraison, during first stages of berry development. Moreover, water deficit was always more intense on G7 compared to G8. These water deficits continued to increase slightly until veraison, but disappeared after rainfall event in the middle of August (day of year 229). Moderate to severe water deficits reappeared at the end of the same month. At harvest, Ψ_{stem} values were significantly different between parcels and among blocks in each parcel (table 1). At the beginning of the 2015 season, vines on block G1 did not face water deficit and Ψ_{stem} values were similar to those recorded on the blocks S5, S6 and S7 (figure 2B). However, Ψ_{stem} values of G1 became progressively more negative over the season. At the beginning of August (day of year 215), measured Ψ_{stem} values were intermediate compared to the remaining blocks, indicating a weak water deficit. Vine water deficit continued to increase during the following months and, on the final measurement day (day of year 251) a significant but moderate water deficit appeared on this block (figure 2A and table 1). $\delta^{13}\text{C}$ values, measured in grape sugars at ripeness, indicated mild water deficit on G1 and no water deficit on the S5, S6 and S7 blocks (table 1). They also confirmed the water stress observed on G7 and G8. Water deficit during grape ripening was slightly more intense on G7 compared to G8. Because climatic conditions are homogenous among the blocks for a given vintage, observed differences in vine water status reflect SWHC in relation to soil texture, gravel content, rooting depth and possible access to a shallow water table.

Vine nitrogen status

In this study, no nitrogen fertilizer was added in 2014 and 2015, neither during the preceding years. Consequently, vine nitrogen status was related to soil organic matter content and its mineralization rate which depends on soil temperature, soil aeration and water availability (van Leeuwen and Friant 2011). YAN values followed a similar tendency in 2014 and 2015 (figure 3A and B). A regular increasing must YAN content was observed on the six experimental blocks during the ripening period. YAN level was clearly dependent on soil characteristics. Differences between the soil types were noticeable during the growing season, for both vintages, and this was particularly obvious in 2014. Soil type has been reported to have an influence on vine nitrogen status (Choné

et al. 2001a, Peyrot des Gachons et al. 2005). Vine nitrogen status was not significantly different among blocks S5, S6 and S7 (sandy soil), neither in 2014 nor in 2015. However, these blocks showed significantly lower nitrogen content in must when compared to blocks planted on the gravelly soil, indicating a nitrogen deficit of the vines (Table 3). G7 block showed higher values of YAN during both years, indicating moderate to high vine nitrogen status. Slightly lower YAN were observed in G1 and G8 during the two experimental years. In these blocks, vine nitrogen status was moderate.

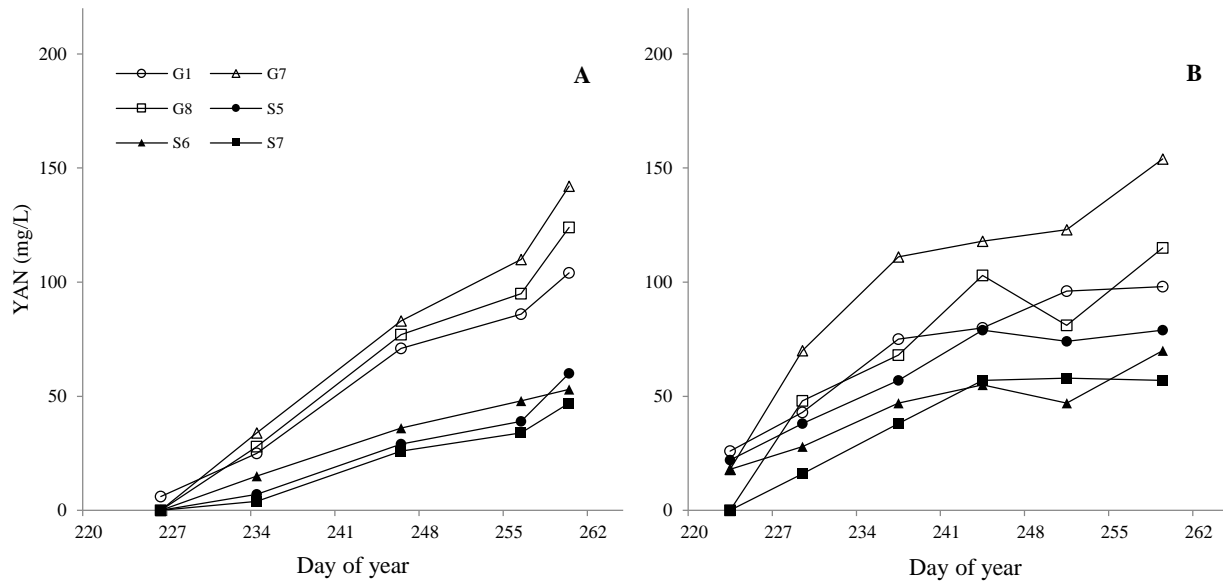


Figure 3 - Seasonal dynamics of Yeast Assimilable Nitrogen (YAN) measured in the experimental blocks in the summer of 2014 (A) and 2015 (B), from veraison through ripeness.

Combined vine water and nitrogen status

The combination of soil type and climatic conditions of the two vintages resulted in a wide range of combination in vine water and nitrogen status (table 3). On blocks S5, S6 and S7, vine water status was high and vine nitrogen status was low in both vintages. Vine water and nitrogen status were high on block G1 in 2014, while on this block moderate water deficit was associated to high nitrogen status in 2015. On blocks G7 and G8 vines faced moderate to severe water deficit in both vintages, associated to non-limiting nitrogen conditions. Between these blocks, G7 had slightly higher nitrogen status and lower water status compared to G8.

Table 3 – Vine water and nitrogen status on the six experimental blocks in 2014 and 2015

Block	2014		2015	
	Water deficit	Nitrogen deficit	Water deficit	Nitrogen deficit
G1	No water deficit	No nitrogen deficit	Moderate to weak	No nitrogen deficit
G7	Moderate to severe	No nitrogen deficit	Severe	No nitrogen deficit
G8	Moderate to severe	No nitrogen deficit	Severe	No nitrogen deficit
S5	No water deficit	Moderate to severe	No water deficit	Moderate to severe
S6	No water deficit	Moderate to severe	No water deficit	Moderate to severe
S7	No water deficit	Moderate to severe	No water deficit	Moderate to severe

Threshold for vine water and nitrogen deficit class definition are those proposed in van Leeuwen *et al* (2009) and adapted from van Leeuwen and Friant (2011) respectively.

Berry seed content

Table 4 summarizes the effect of block characteristics on berry seed content during the two years of experimentation. Berry seed content is expressed as the percentage of berries with respectively 1, 2, 3 or 4 seeds, as average of seed number per berry and as total seed mass per berry. Most berries contain one or two seeds. This was observed on all blocks and for both years. However, the distribution of berries into seed number classes varied slightly among vintages and blocks. The most striking difference is a lower number per berry on block S6 (most berries with one seed). This result could be related to health status of the plants in this block, affected by GFLV. The impact of plant health status on average seed number per berry was significant (p -value < 0.01). A vintage effect was observed when berry seed content was expressed as total seed mass per berry. The values are lower in 2015 compared to 2014. No significantly differences are observed between blocks in 2014. In 2015 berries from G7 block showed a lower berry seed mass compared to berries from S5.

Table 4 - Effect of block on seed content per berry

	2014							2015						
	G1	G7	G8	S5	S6	S7	G1	G7	G8	S5	S6	S7		
Seed number														
Berries with 1 seed (%)	44	26	31	48	59	41	38	50	43	45	69	63		
Berries with 2 seed (%)	37	50	45	37	31	31	40	33	46	36	30	33		
Berries with 3 seed (%)	17	22	22	15	8	24	21	17	9	19	1	4		
Berries with 4 seed (%)	2	2	2	0	2	4	1	0	2	0	0	0		
Average seed number per berry	1.76 ab	2.00 b	1.94 ab	1.67 ab	1.52 a	1.91 ab	1.83 c	1.67 ac	1.70 ac	1.74 bc	1.33 a	1.41ab		
Total seed mass per berry (g)	0.079 a ^a	0.086 a	0.086 a	0.071 a	0.071 a	0.074 a	0.059 ab	0.052 a	0.065 ab	0.068 b	0.055 ab	0.057 ab		

The difference between blocks was tested with a one-way ANOVA.

^aDifferent letters in the same row denote statistically significant differences between blocks at P<0.05 by Tukey Honest Significant Difference (HSD) test.

Fresh berry mass during ripening and at harvest

Fresh berry mass followed a similar trend from veraison through harvest during 2014 and 2015 (figure 4A and B). However, a vintage effect can be observed.

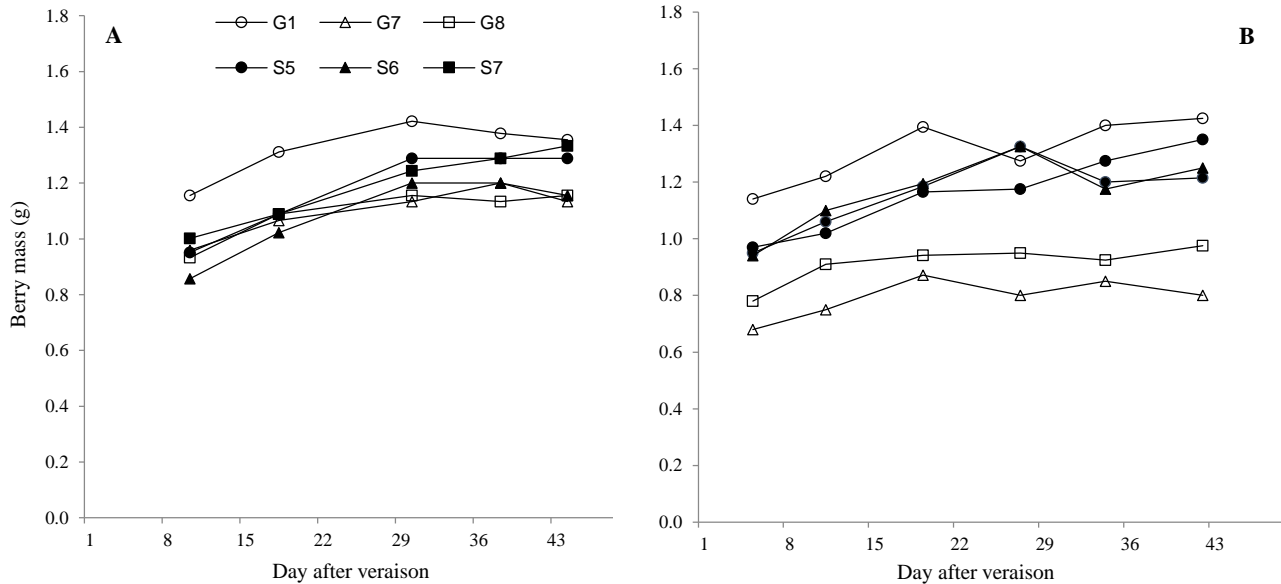


Figure 4 - Seasonal dynamics of fresh berry mass of each experimental block for 2014 (A) and 2015 (B).

In 2014, berry mass increases until the thirtieth day after veraison and then reaches a plateau. In 2015, the increase of berry mass was erratic. Differences among blocks were more obvious in 2015 than in 2014. The weather conditions of 2015, a warm and dry vintage, increased probably the impact of soil characteristics (water and nitrogen availability) on berry mass. G1 berries showed higher mass compared to others blocks, in particular during the first part of the ripening period, for both experimental years. However, the differences were never significant compared to S5 and S7. In 2014, berry mass was high on block G1 and differences at harvest were significant with blocks G7, G8 and S6 (table 5). In 2015, G1 produced also heavier berries compared to G7 and G8. In this vintage, berry mass at harvest was particularly low on G8 and even lower on G7. In 2014, lowest berry mass was recorded on block S6, although only the difference with blocks G1, S5 and S7 was significant.

Berry composition through ripening and at harvest

Figure 5 shows the evolution of the major parameters of berry composition measured in 2014 and 2015.

Table 5 - Effect of block on berry mass, sugar and malic acid measured just prior to harvest in 2014 and 2015

2014						
	G1	G7	G8	S5	S6	S7
Berry mass (g)	1.31 ± 0.14 c ^a	1.10 ± 0.08 ab	1.11 ± 0.10 ab	1.19 ± 0.11 bc	1.00 ± 0.15 a	1.20 ± 0.6 bc
Sugars (Brix)	22.1 ± 0.9 bc	22.0 ± 0.7 bc	22.3 ± 1.5 bc	22.5 ± 1 c	21.7 ± 1.1 bc	20.9 ± 1.2 a
Malic acid (g/L)	3.23 ± 0.9 b	3.04 ± 0.7 ab	2.75 ± 0.8 a	2.7 ± 0.5 a	2.91 ± 0.6 ab	2.75 ± 0.5 a
2015						
	G1	G7	G8	S5	S6	S7
Berry mass (g)	1.29 ± 0.10 c	0.79 ± 0.10 a	0.98 ± 0.14 b	1.29 ± 0.12 c	1.13 ± 0.12 bc	1.23 ± 0.13 c
Sugars (Brix)	21.8 ± 1.02 b	20.7 ± 0.87 a	21.2 ± 1.35 ab	21.1 ± 0.85 a	20.7 ± 0.82 a	20.7 ± 0.95 a
Malic acid (g/L)	1.05 ± 0.2 a	0.91 ± 0.3 a	0.88 ± 0.37 a	0.96 ± 0.32 a	1.35 ± 0.4 b	1.64 ± 0.6 c

The difference between blocks was tested with a one-way ANOVA.

^aDifferent letters in the same row denote statistically significant differences between blocks at P<0.05 by Tukey Honest Significant Difference (HSD) test.

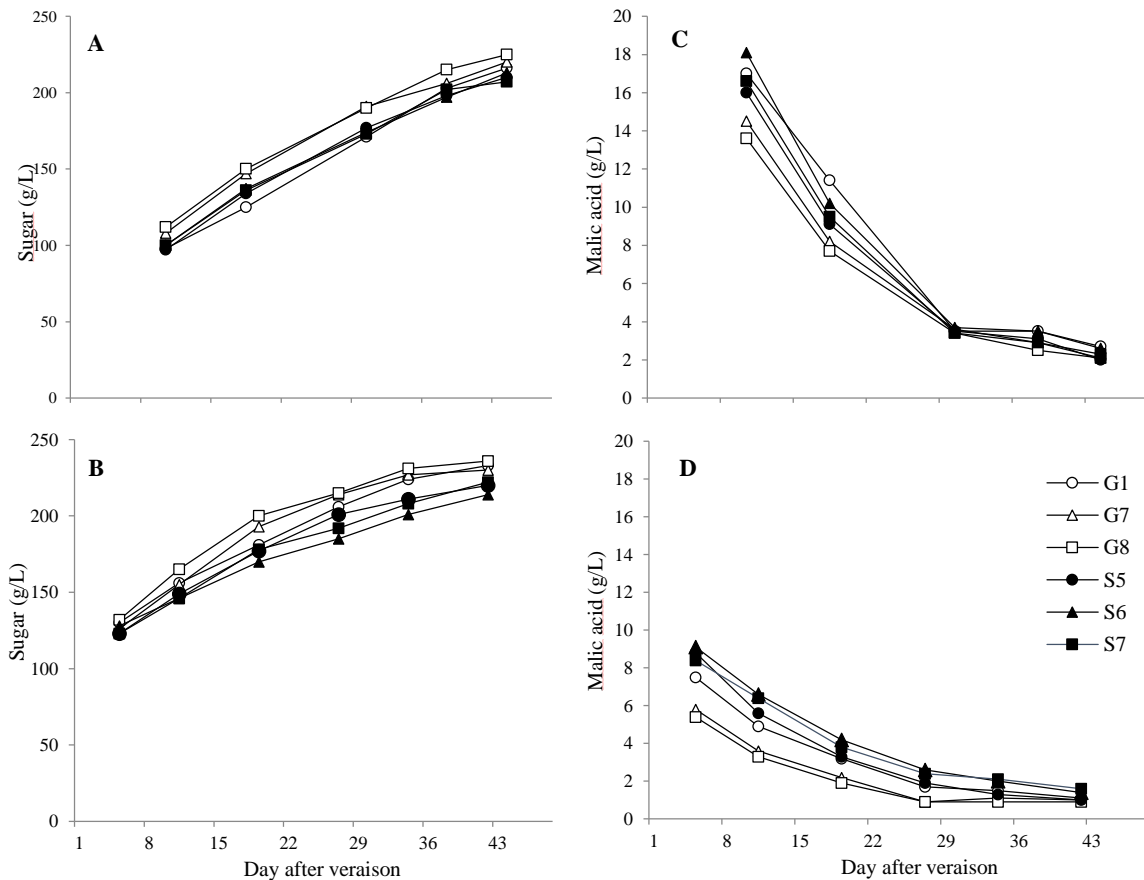


Figure 5 - Seasonal dynamics of sugar (A and B) and malate (C and D) concentration for the season 2014 and 2015, respectively, measured on berry juice for each experimental block.

Like for fresh berry mass, a block effect on sugar and malic acid concentration was observed during both years. However these differences decreased during berry ripening. From veraison to maturity, must from G7 and G8 contained the highest sugar and the lowest malic acid concentrations at most sampling dates. This was observed in 2014 and 2015. In 2014, the dynamics of sugar accumulation and malic acid degradation in block G1 was similar to those observed on S5, S6 and S7. The behavior of G1 was closer to blocks G7 and G8 in 2015.

Some significant differences were observed at harvest between blocks (table 5). In 2014, S7 berries were low in sugar and G1 berries high in malic acid. In 2015, G1 berries were high in sugar, whereas S6 and S7 berries were high in malic acid. A clear vintage effect was noted for berry composition. Sugar concentration was higher in 2014, and malic acid concentration was markedly lower in 2015.

The effect of water, nitrogen and berry seed content on fresh berry mass

Effects of vine water and nitrogen status and berry seed content (independent variables) on fresh berry mass (dependent variable) were tested by a multiple linear regression analysis. Excluding one by one the non-significant covariates on berry mass from the full model (stepwise procedure), we obtained the following final model: fresh berry mass = vine water status + vine nitrogen status + berry seed content + vine water and nitrogen status interaction. So, fresh berry mass was significantly and simultaneously influenced by vine water status, vine nitrogen status and berry seed content (table 6).

Table 6 - Effects of vine water and nitrogen status and berry seed content on fresh berry mass

	Sum Sq	Df	F value	Pr (>F)	Signif.
Vine water status	6.9284	1	310.0228	< 2.2e-16	****a
Vine nitrogen status	0.3144	1	14.067	0.00029	***
Berry seed content	0.0881	1	3.9412	0.04975	*
Water : nitrogen interaction	0.3337	1	14.9315	0.000194	***
Residuals	2.3242	104			

Results were obtained from an Anova type III performed on the following model: berry fresh mass = vine water status + vine nitrogen status + berry seed content + vine water and nitrogen status interaction.

a Significance. codes: '****' 0.001 '***' 0.01 '*' 0.05 '.' 0.1 'ns' 1

Moreover, a significant interaction between vine water and nitrogen status on berry mass was also observed. However, the extent to which these factors influenced the berry mass varied. As in this study vine water and vine nitrogen status were significantly correlated, the contribution of each component to the variance of the berry mass was calculated using an Anova type III performed on

the final model. As previously described, the relative importance of each covariate was calculated as the ratio of the sum of squares due to this component to the sum of squares due to another component (e.g. vine water status vs berry seed content = $6.9284/0.0881 = 78.64$). Results showed that the incremental impact of vine water status on fresh berry mass was around 80 times and around 22 times more important than the impact of berry seed content and vine nitrogen content, respectively. The impact of vine nitrogen status, in turn, was around four times bigger than the impact of berry seed content.

The effect of water, nitrogen and berry seed content on sugar and malic acid concentration

The same approach was used to assess the influence of different covariates on berry sugar and malic acid concentration. However, in addition to the previously considered covariates (vine water and nitrogen status and berry seed content), we also included the berry mass in the statistical analysis, given its possible impact on berry composition. According to an ANOVA type III, berry mass, vine water status and berry seed content significantly impacted on berry sugar concentration (table 7). Conversely, vine nitrogen status was not significant. Several interactions between considered covariates were observed. All factors significantly influenced the malic acid concentration of berries, except berry mass (table 8).

Table 7 - Effects of berry mass, vine water and nitrogen status and berry seed content on berry sugar concentration

	Sum Sq	Df	F value	Pr(>F)	Signif.
Berry mass	81.52	1	61.9145	3.931e-12	****
Vine water status	479.58	1	364.2234	2.2e-16	***
Vine nitrogen status	2.17	1	1.6468	0.20231	ns
Berry seed content	10.52	1	7.9869	0.00657	**
Residuals	134.30	102			

Results were obtained from an Anova type III. ^aSignificance. codes: '****' 0.001 '***' 0.01 '**' 0.05 '.' 0.1 'ns' 1

Table 8 - Effects of berry mass, vine water and nitrogen status and berry seed content on berry malate concentration

	Sum Sq	Df	F value	Pr(>F)	Signif.
Berry mass	1.891	1	2.2783	0.1343211	ns
Vine water status	13.085	1	15.7629	0.0001346	***
Vine nitrogen status	11.896	1	14.3301	0.0002605	***
Berry seed content	8.165	1	9.8354	0.0022426	**
Residuals	83.844	101			

Results were obtained from an Anova type III. ^aSignificance. codes: '****' 0.001 '***' 0.01 '**' 0.05 '.' 0.1 'ns' 1

Results reported in tables 7 and 8, allowed hierarchizing the impact of covariates on sugar and malic acid concentration. As previously done for berry mass, the relative importance of each covariates on sugar and malic acid concentration was calculated as the ratio of the sum of squares due to this component to the sum of squares due to another component. Our results showed, once again, that vine water status was the most impacting factor among those studied. This was true for both sugar and malic acid concentration. The incremental impact of vine water status on sugar concentration was around five times and around 45 times more important than those of berry mass and berry seed content, respectively. The impact of berry mass was around eight times bigger than berry seed content. Concerning malic acid, the incremental impact of vine water status and vine nitrogen status was similar and was almost one time and a half greater than berry seed content. So, the hierarchy of factors differed between the metabolites considered. Considering the sugar concentration, vine water status outweighed, by far, the other factors. For malic acid, the different considered factors contributed more equally and many interactions among covariates were observed (data not shown).

Is the relationship between berry mass and berry composition direct or indirect?

The significance of the additive effect (or direct effect) of vine water status, vine nitrogen status and berry seed content on berry sugar and malic acid concentration were tested with an Anova type I. The results reported in tables 9 and 10 are derive from three different models where each of these covariates were introduced respectively as a second covariate after berry mass. Table 9 summarizes the effect of the considered factor on berry sugar concentration. The impact of berry mass on sugar was significant (data not reported in the table). Nevertheless, the effect of each second covariate is still significant. Therefore, we can consider that vine water status, vine nitrogen status and berry seed content have both a significant indirect effect (through berry mass) and a significant direct effect on berry sugar concentration (independent of berry mass).

Table 9 - Direct effect of vine water and nitrogen status and berry seed content on berry sugar concentration

	Sum Sq	Df	F value	Pr(>F)	Signif.
Vine water status	993	1	705.57	< 2.2e-16	****a
Vine nitrogen status	227	1	61.451	3.935e-12	***
Berry seed content	147	1	43.375	1.837e-9	***

Results were obtained from an Anova type I. ^a Significance codes: '****' 0.001 '***' 0.01 '**' 0.05 '.' 0.1 'ns' 1

Table 10 reports the effects of the considered covariates on berry malic acid concentration. In this case, berry mass presented a borderline effect (data not shown). Therefore, the effects of vine water and nitrogen status and berry seed content can be considered exclusively of direct type. While vine nitrogen status and berry seed content present a significant direct effect on malic acid, vine water status is only significant at 10%.

Table 10 - Direct effect of vine water and nitrogen status and berry seed content on berry malate concentration

	Sum Sq	Df	F value	Pr(>F)	Signif.
Vine water status	2.445	1	2.9454	0.0891891	. ^a
Vine nitrogen status	5.240	1	6.3116	0.0135782	*
Berry seed content	9.551	1	11.5047	0.0009922	***

Results were obtained from an Anova type I. ^a Significance codes: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 'ns' 1

DISCUSSION

In this study, conducted under field conditions, the effect of vine water status, vine nitrogen status and berry seed content on berry mass and two major berry compounds were studied simultaneously. Significant influences on berry mass, sugar and malic acid concentration caused by additional factors, not initially considered, were observed during the two experimental years. Due to the large number of impacting covariates and their interactions, it was difficult to isolate the influence of each of them. Most of previous works examined the effect of a single factors on berry size and/or berry composition, e.g vine water status (Ojeda et al. 2001, Roby and Matthews 2004, Chaves et al. 2007, Santesteban et al. 2011), vine nitrogen status (Hilbert et al. 2003) or berry seed content (Walker et al. 2005, Friend et al. 2009). Moreover, in most cases, these studies were conducted under controlled experimental conditions. Only a few studies, done under field conditions, accounted for more than one terroir factor simultaneously (Choné et al. 2001a, van Leeuwen et al. 2004, Barbagallo et al. 2011). However, with the aid of appropriate statistical models, the present study allowed us to graduate the contribution of vine water status, vine nitrogen status and berry seed content to the variance of berry mass, sugar and malic acid concentration and to hierarchize these factors according of the level of their impact.

In this study, experimental blocks were neither irrigated nor fertilized. Hence, water and nitrogen availability to the vines were exclusively dependent on variability in environmental conditions. Among these, soil can be considered as a key factor (van Leeuwen and Seguin 2006). Its impact on vine water and nitrogen uptake was clearly shown in this study, as it has been observed previously in other studies conducted in Bordeaux area (Choné et al. 2001a, van Leeuwen et al. 2004). Vine water status exhibited a significant variation at the intra-parcel level, because it is related to variations in soil composition. Vines of blocks located on a sandy soil, characterized by a water table accessible to the roots, did not face any water deficit during the two experimental years. On the gravelly soil, vine water status was variable at the block level, in relation to variations in rooting depth and gravel content. Only two of the three blocks located on the gravelly soil, faced severe water deficit during the two years. However, the period when vine water deficit occurred varied among vintages. In contrast, the third block (G1) located on gravelly soil showed a similar stem water potential pattern as the blocks on the sandy soil, during the whole 2014 season and at beginning of the 2015 season. These results were obtained by two physiological indicators used in

this study to estimate vine water status, which were highly correlated. According to van Leeuwen et al. (2004), our results confirmed that the intensity and the timing of a vine water deficit stress depend not only on SWHC, but also on weather conditions of the year, well known as the “*vintage effect*” (van Leeuwen and Seguin 2006). In 2014, the spatial heterogeneity of vine water status was particularly evident at the end of summer, when significant water restriction occurred. The 2015 vintage, which was drier and warmer than 2014, not only highlight the differences among blocks, but also induced earlier water deficit stress. In 2015, vine water status of the G1 plant was significantly different compared to sandy soil blocks. Moreover, the weather characteristics of this vintage submitted the vines planted on G7 and G8 blocks to early severe water stress, during the first phase of berry development. Vintage effect on vine nitrogen status was smaller compared to vine water status. YAN levels were similar in both vintages. However, they were highly variable among experimental parcels. Vine nitrogen availability is related to the soil type and to the soil depth (van Leeuwen and Seguin 2006). Without addition of nitrogen fertilizer, which was the case in the present work, vine nitrogen status depends on soil organic matter content and its mineralization rate (Choné et al. 2001a). The latter increases with soil temperature increase and soil aeration. In this study, the gravelly soil is a warm and well aerated soil, favoring the turn-over of organic matter. The sandy soil is cooler and less well aerated, due to water logging in the spring. These differences explain higher vine nitrogen status on the gravelly soil compared to the sandy soil in our experiment. Similar results were obtained by Peyrot des Gachons et al. (2005). It also explains the significant relationship between vine water and nitrogen status, tested on the complete dataset. However, as the nitrogen availability was estimated by a physiological indicator, a dilution effect, related to vine vigor and berry size, could not be excluded.

Seasonal dynamics of berry growth reflected primarily vine water and nitrogen status of the different blocks. Their influence was highlighted by a vintage effect. In 2015 the differences of berry fresh mass among blocks was bigger and occurred early in the season, due to the impact of the specific climatic conditions of this year on vine water availability. The combined effect of vine water and nitrogen status on fresh berry mass was particularly highlighted by results obtained on the G1 block. As previously mentioned, this block showed an “intermediate” profile compared to remaining blocks. It was characterized by an unlimited nitrogen supply during both experimental years and by a high water availability, which was limited however at the end of the 2015 growing season. Berries produced under these conditions were bigger compared to those produced on the

other blocks. In contrast, berries produced under limited water and/or nitrogen conditions, were smaller. However, the impact of a limited water availability seemed to be stronger compared to the impact of a limited nitrogen status. Berries produced on the sandy soil (limitation in nitrogen, not water), were smaller than G1 berries, but bigger than G7 and G8 berries, where vines were subjected to severe water deficit. The exception observed on S6 berries was related to the health status of the vines on this block. S6 vines were affected by GFLV and, despite unlimited water availability, they showed a similar size compared to those grown on G7 and G8. This results confirmed those reported in previous studies (Martelli and Savino 1990), where a decrease of berry mass was observed on vines affected by GFLV. According to May (2004), severe incidence of the fanleaf virus is generally accompanied by poor setting. Our results support this observation. When the berries of each block were divided in four different classes, according to their seed number, most of them belonged to the first two classes (one seed and two seeds). However, the proportion of berries in each of these two classes varied among blocks. The different behavior of S6 block was clearly highlighted, where the size of first class was much higher than that of all other blocks.

Differences in berry size among blocks, are the result of the combined impact of several factors. The major objective of this work was to create a hierarchy among these factors. To achieve this goal we implemented a multiple regression, which allowed us to quantify the contribution of each covariate to the variance of berry mass. Results of an Anova type III confirmed that vine water availability, vine nitrogen status and berry seed content impacted significantly the final berry size. Moreover, as expected, an interaction between vine water and nitrogen status was found. At the intra-parcel scale, which was the observation level in this study, berry seed content was the less impacting factor among those considered. In contrast, vine water status was the most important factor. Its impact on fresh berry mass was much greater compared to the effect of nitrogen availability. In this study, berry sugar and malate concentration were considered as major compounds representing berry composition. We investigated the relations between these compounds and vine water status, vine nitrogen status and berry seed content. Difference among blocks was not so large, likely because of interaction between impacting factors. However, observing the accumulation and degradation curves of these compounds during the ripening period, it is still possible to observe that berries produced under water deficit conditions were characterized by higher sugar levels and lower malate levels. Similar results were found in Bordeaux (France) by Trégoat et al. (2002). Conversely, berries produced under unlimited water

supply conditions contained less sugar levels and more malic acid, which was already observed by Storchi et al. (2005), in Sangiovese grapevines. The combined effect of the soil and climatic conditions (vintage effect) created an interesting set of conditions of water and nitrogen availability and influenced the patterns of accumulation and degradation of sugars and malate, respectively. Observing these curves, the behavior of G1 was particularly interesting, which can be related to its vine water status during both years. In this block, located on gravelly soil, vine water status was similar to S5, S6 and S7 in 2014, but not in 2015, when G1 vines faced moderated water deficit.

Although the relationship between berry size and grape composition is still a subject of debate, a large number of studies showed a significant impact of berry mass on berry composition. In this study, statistical models applied to the whole data set, allowed us to (i) hierarchize the factors which influenced berry sugar and malate concentration, according to their impact degree and (ii) to separate their direct effect from that mediated through their impact on berry size (i.e. indirect effect). In our second model, we considered fresh berry mass as a covariate, which was added to those previously considered. Not all the covariates did significantly affect sugar and malate concentration simultaneously, but vine water status was, once again, the most important factor. Moreover, the overall effect of the considered covariates on malic acid is consistently lower than their effect on sugar. As a consequence, the correlations and interactions, present in the model, play an important role. The order of impact of the studied covariates, within each hierarchy created by the models, changed among compounds. Except for berry seed content, which had a significant impact in all cases, berry fresh mass presented a borderline effect (not significant) when related to malic acid concentration. Hence, because all remaining covariates significantly affected berry malate, we can conclude that their impact is exclusively direct and not mediated through berry mass. This was not the case for berry sugar where, in contrast, fresh berry mass had a large significant influence. Apparently, berry sugar level did not change with vine nitrogen status. Indeed, the effect of vine nitrogen status on sugar concentration was hidden by the effect of fresh berry mass and by the interaction among others covariates. This hypothesis was confirmed by the results obtained with a model chain where, through a multiple linear regression applying an Anova type I, the effect of each covariate was separated from that of fresh berry mass. Results obtained with this approach showed that all considered factors in this study, including vine nitrogen status, affected the berry sugar concentration.

CONCLUSION

The aims of the present work were to investigate on the impact of the major factors influencing fresh berry mass and berry composition, in order to (i) create a hierarchy of these factors, according to their impact degree, and (ii) to separate their possible direct effect on berry composition from a possible indirect by mediated through their impact on fresh berry mass. The simultaneous effects of vine water status, vine nitrogen status and berry seed content were studied at an intra-parcel level. In addition, the impact of plant health status with regard to virus diseases on berry mass was also considered in this study. Fresh berry mass at harvest was mainly related to vine water and nitrogen status of the different blocks. Water and nitrogen availability were highly dependent on the combined action of soil type and climatic conditions of the year (vintage effect). All studied factors significantly impacted the final fresh berry mass. However, vine water status represented the most impacting factor. In contrast, berry seed content was the less impacting factor among those considered at this scale. Vine water status was also the most impacting factor on berry sugar and malic acid concentration. However, the simultaneous action of impacting factors and their correlation and/or interaction, sometimes hid their real significant effect on berry composition. Hence, not all the remaining considered covariates (including fresh berry mass) significantly affected the concentration of these two major compounds. Consequently, the order of impact of the factors varied among metabolites. Nevertheless, our statistical model series, implemented on data collected under field conditions allowed to prove that all factors had a direct impact on berry composition, independently from the one mediated through their impact on fresh berry mass. So, the statistical approaches used in the present work allowed to clarify the importance of each factor responsible of variability in berry mass and berry composition, under field conditions, where various factors act simultaneously. No indirect effect of berry mass was shown on malic acid, but its concentration is directly impacted by berry seed content and vine nitrogen status; the effect of vine water status is only marginally significant (p-value 10%). In contrast, grape sugar concentration is mainly driven by vine water status, while vine nitrogen status and berry seed content also have a significant direct effect.

In this study, for the first time, factors impacting grape berry mass have been clearly hierarchized in field conditions. Our statistical approaches also allowed to separate the direct effect of these factors on major berry compounds from a possible indirect effect through their influence on fresh berry mass.

Chapter III

Hierarchy of factors impacting on berry mass and its consequences on grape composition at intra-bunch and intra-plant scale

ABSTRACT

Final berry mass is the result of the integrated effect of biotic and abiotic factors, which can be intrinsic or extrinsic to berry. Fruit growth is positively correlated with seed content. Environmental factors can also play a key role on berry mass. Together with nitrogen supply, vine water status is one of the most important factors affecting grapevine yield and quality parameters. Their availability depend mainly on pedological conditions. Consequently, these factors are highly variable inside and among vineyard parcels. In grapevine, variation in berry mass occurs within a bunch, within a vine, within and between vineyards. Depending on the considered scale, the hierarchy of factors may vary. Among various practices implemented in viticulture, some involve postharvest sorting in order to select berries according to their size. This practice is based on the belief that smaller berries would be better for wine quality. Nevertheless, there are contrasting conclusions regarding differences in berry composition when comparing berries strictly on size. Post-harvest berry sorting appears to be a promising approach to reduce variability of berry size. However, the ability of a sorting machine to produce wines of variable composition from a single vineyard depends on the scale at which berry mass variability occurs and the relations between berry mass and berry composition at this particular scale. The aims of this study were to hierarchize the factors impacting berry mass according to their degree of influence and to investigate the influence of berry size on grape composition at intra-bunch and intra-plant level. The work was carried out over two seasons, on six experimental blocks located in the Saint-Emilion region, planted with Cabernet franc on two soil types: a sandy soil and a gravelly soil. Variability of berry mass and impacting factors and berry mass effect on grape composition were studied within a bunch, (berry by berry), and within a plant (bunch by bunch). The results of this work indicate that within a bunch and within a plant, berry seed number is the driving factor of the berry mass variability, while berry mass was never related to $\delta^{13}\text{C}$, nor to YAN at these two scales. All berries of a bunch and all bunches of a plant are submitted to similar water and nitrogen uptake conditions. When the variability of berry mass is driven by the variability of seed number, the berry mass effect on grape composition seems to be only marginal. When the variability of berry mass is driven by external factors the berry mass effect on grape composition is more obvious. Small and large berries, produced from a single parcel with homogenous water and/or nitrogen conditions, tend to have similar enological profiles. Small and large berries from a parcel with heterogeneous soil conditions may have different enological profiles.

INTRODUCTION

Variability is an intrinsic property of all biological systems and may occur at different levels (Dai et al. 2011). In grapevine, variation in berry mass occurs between berries within the bunch, between bunches within the vine, between vines within the vineyard and between vineyards (Gray 2002). Consequently, it is extremely difficult to obtain uniform berry diameter under field conditions, even when all vineyard management practices are properly executed (Pisciotta et al. 2013).

Final berry mass is the result of the integrated effect of biotic and abiotic factors that affect cell number and/or cell volume, which are determined by cell division and cell expansion, respectively (Fernandez et al. 2006). Many factors influencing berry size are intrinsic, being related to the individual berry itself, such as seed number (Scienza et al. 1978) and seed weight (Roby and Matthews 2004). Carbon balance of the vine can also impact berry mass (Coombe 1962). Environmental factors such as plant water status (Matthews and Anderson 1988, Williams and Matthews 1990, van Leeuwen and Seguin 1994, Ojeda et al. 2001, Roby et al. 2004, Chaves et al. 2007, Ferrer et al. 2008, Girona et al. 2009) and vine nitrogen status (Choné et al. 2001a, Trégoat et al. 2002, van Leeuwen et al. 2007) can play a key role. The impact depends on their intensity or the development period at which they act (Ojeda et al. 1999). However, some external factors, such as vine water status, have been shown to homogeneously inhibit berry growth in all berries, indicating that internal factors influence differences among individual berry growth (Shellie 2010). Hence, depending on the considered scale, the hierarchy of these factors may vary. At the parcel scale, grapevine cultivar is likely to be the dominant factor. At intra-parcel scale, the variability may be related to variations in soil characteristics. Finally, at bunch scale, the differences between berries could be related to internal factors (Scienza et al. 1978, Carwthon and Morris 1982, Walker et al. 2005, Roby and Matthews 2004).

The mass of grape berries in the bunch may vary by a magnitude two and the coefficient of variance can reach, at this scale, a maximum of 25-30% (Friend et al. 2009, Shellie 2010). Fruit growth is closely and positively correlated with seed content (Walker et al. 2005), which affect the cell proliferation and expansion in pericarp through the production of hormones (Friend et al. 2009). Seed number and their fertility depend on the quality of fertilization (Gillaspy et al. 1993). Some virus infections can cause either losses of whole parts of inflorescences or poor fruitset. Grapevine Fan Leaf Virus (GFLV) has been reported to cause significant reduction in grape yield (Andret-

Link et al. 2004). This virus affects the ripening of the berries, which can be irregular on the same bunch (Martelli and Savino 1990). It has also been reported that fruit quality can be affected by GFLV due to a decrease in sugar content, titratable acids and total anthocyanin content in berries (Cretazzo et al. 2009, Andret-Link et al. 2004). Among environmental factors, water deficit is a major one limiting growth of plant organs (Chaves et al. 2007). Roby and Matthews (2004) manipulated Cabernet Sauvignon vineyard irrigation in order to have different berry sizes: irrigated vines produced larger berries compared to deficit irrigated vines. Similar results were obtained by Santesteban and Royo (2006) in Tempranillo. However, berry size changes in response to water deficit, mostly when low water supply occurs during the first stages of development (Ojeda et al. 2001, 2002), as a result of an increase in abscisic acid (ABA), which limits cell division and expansion (Castellarin et al. 2007). Water availability depends equally on climate and soil characteristics (van Leeuwen et al. 2004). Consequently, vine water status is highly variable inside and among vineyards because Soil Water Holding Capacity (SWHC) varies with soil texture, percentage of stones and rooting depth (van Leeuwen and Darriet 2016). However, the variability of water status within a bunch or within a plant, was not yet investigated.

Among elements the vine picks up from the soil, nitrogen is undoubtedly the one that most impacts on vine growth, vigor and grape composition (Spayd et al. 1993). Beyond the addition of nitrogen fertilizer, vine nitrogen availability depends to a large extent on the amount of soil organic matter and its mineralization rate. This latter depends on pedological conditions, such as soil temperature, soil humidity and aeration (van Leeuwen and Friant 2011). Hence, it is enhanced when soil temperature is high and soil moisture content is close to field capacity (van Leeuwen et al. 2000). Together with water supply, vine nitrogen status is one of the most important factors affecting grapevine yield and quality parameters. Its effect on enological potential of grapevine is due to direct and indirect effects (Smart et al. 1990). Nitrogen plays also an important role on the final berry size. Several authors showed that high vine nitrogen uptake corresponds to a decrease of berry mass and berry number per bunch, while the bunch number per vine increases. This fact could be linked to excessive vegetative development and consequently competition with berry development (Keller et al. 1998). Other authors reported that high vine nitrogen uptake increases final berry mass (Choné et al. 2001a, Trégoat et al. 2002), while low vine nitrogen status reduces vine vigor, berry mass and increases berry sugar concentration (van Leeuwen et al. 2007). Finally, accumulation of anthocyanins in berries increases under low nitrogen status (Hilbert et al. 2003,

Soubeyrand et al. 2014), while it is decreased by excessive nitrogen supply (Keller et al. 1999). Hence, grape quality potential for red wine production is increased by limited soil nitrogen availability to the vines.

Berry size is also affected by carbon availability during early stages of development (Coombe 1962). The carbon pool that supports berry growth and development is mainly translocated from the leaves. As a strong competition between the different parts of the vines has been shown and as bunches are weak sink until ripening commences, a decrease in leaf area just after fruitset can induce a reduction in berry growth (Ollat et al. 2002 and the references therein).

Under field conditions, the interactions between factors impacting berry growth and development make difficult the management of berry size variability. Among various practices that grape-growers implement to optimize grape quality attributes, some involve postharvest sorting in order to select berries according to size. This practice is based on the belief that smaller berries would be better for wine quality due to their higher surface:volume ratio (Singleton 1972, Matthews and Anderson 1988). However, this concept has gained acceptance based primarily on intuition rather than on scientific evidence (Matthews and Nuzzo 2007). In fact, implicit in this belief is the assumption that the proportion of berry tissues remain constant while berry size changes. So, if the berry is considered a sphere, then the berry surface:volume ratio would decrease when the berry size increases according to the ratio $3/\text{radius}$ (Barbagallo et al. 2011). However, Roby and Matthews (2004) found that the proportion of skin and flesh did not vary according to the relationship between the surface and volume of a sphere. In other words, the skin doesn't stretch around a larger flesh but grows with it. Consequently, among berries growing under similar environmental conditions, berry skin and seed tissue development are coordinated with flesh growth (Barbagallo et al. 2011).

Nevertheless, there are contrasting conclusions regarding differences in berry composition when comparing berries strictly on size. Roby et al. (2004) found a proportional increase of all berry solutes, such as sugars, skin tannin, seed tannin and anthocyanin (expressed in content per berry), with berry size. Under similar cultural conditions Barbagallo et al. (2011) found that the skin total anthocyanin content (mg/berry) changed positively with increasing berry mass. Conversely, Poni and Libelli (2008) found that berry size had no effect on grape and wine composition.

Several studies show that berry sugar content (g/berry) depends on berry mass, while berry sugar concentration (g/L) does not change with berry mass (Roby et al. 2004, Ferrer et al. 2014). Similar

relationships were observed by Roby and Matthews (2004), when studying the effect of berry mass on anthocyanins. Anthocyanin per berry was proportional to the size of the berry, while the concentration of anthocyanins decreased with increasing berry size, indicating the possibility of producing different wine styles from berries with different sizes. These results were not confirmed by Ferrer et al. (2014) who reported that total anthocyanin content or concentration was independent of berry size.

Some authors concluded that the way in which berry mass is reduced is more important than the berry mass itself and highlighted that wine improvement is not due just to berry size, but to changes in vine metabolism provoked primarily by factors like cultural practices or annual weather conditions, which may also impacted berry mass (Matthews and Anderson 1988, Roby et al. 2004, Walker et al. 2005, Holt et al. 2008). Post-harvest berry sorting appears to be a promising technological approach to reduce variability of grape composition introduced by intra-vine and intra-bunch variations. These practices are becoming increasingly popular and machines are even sold to automatically sort berries based on size (Wong et al. 2016). However, the ability of these machine to produce wines of variable composition from a single vineyard block depends on the scale (intra-bunch, intra-vine, intra-block) at which berry mass variability occurs and the relations between berry mass and berry composition at this particular scale.

The aim of this study was to investigate on the origin of berry mass variability at two different scales (bunch and plant). For each scale, factors impacting berry mass were hierarchized and the effect of berry mass on berry composition was investigated.

MATERIALS AND METHODS

Location, experimental design and plant material

This work was carried out over the 2014 and 2015 seasons, in a commercial vineyard of the Saint Emilion region (44°55'37.42''N; 0°08'59.71''O; 5m; 33330 Vignonet, Aquitaine, France). Two dry-farmed parcels, planted on two different soil types, were selected. Within each parcel, three experimental blocks were chosen, according to their soil conditions observed during previous years. All experimental blocks were planted with *V. vinifera* L. cv Cabernet franc. Soils are classified according to the French “Référentiel Pédologique” (Baize and Girard 1995). The first parcel is planted on a sandy soil (ARENOSOL Redoxique), characterized by a water table within the reach of the roots. For this reason, the three experimental blocks (S5, S6 and S7) chosen within this highly homogenous parcel, are characterized by a similarly high Soil Water Holding Capacity (SWHC). The second parcel is located on a gravelly/sandy soil (PEYROSOL). However, the soil is less homogeneous compared to the first soil. The three experimental blocks (G1, G7 and G8) showed different behavior in term of water and nitrogen availability (which are highly dependent on soil characteristics). G7 and G8 blocks were characterized by a low SWHC, due to the high gravel content (approximately 80%) of soil layers present between 65 and 160 cm in depth. Conversely, G1 showed a higher SWHC, related to the presence of a layer without gravel around 120 cm in depth. The experimental design of this study was based on this large range of water availability, possibly impacting on berry mass and juice composition. In order to determine the health status of the plants, samples of young and mature leaves and wood were collected from each experimental vine. Samples of individual vine were analyzed to check possible presence of *Grapevine FanLeaf Virus* (GFLV, genus *Nepovirus*), *Arabic Mosaic Virus* (ArMV, genus *Nepovirus*), *Grapevine LeafRoll associated Virus* (GLRaV-1-2 and -3, genus *Ampelovirus*). Virus detection was performed by ELISA test and RT-PCR method (Beuve et al. 2007, 2013). All experimental vines were healthy, except for S6 vines. All plants of this block were affected by viruses involved in fanleaf degeneration (GFLV and ArMV). Nevertheless, given the possible impact of this factor on berry mass, we decided to not delete this block, but to take into account the presence of these viruses during the data analysis. Given the goals of this study, two different approaches were performed. Berry mass variability, variability of impacting factors and berry mass effect on grape composition were studied at two different scales: (i) within a bunch, berry by berry,

and (ii) within a plant (bunch by bunch). For this reason, the following paragraph is divided in two sections: bunch level and plant level.

Bunch level

This study was carried out during the two experimental years on all experimental blocks. Inside each block, six plants with a similar architecture were chosen. All measurement were carried out on the basal bunch of the central shoot. According to Carbonneau et al. (1991) this was the most appropriate bunch to represent the plant.

Berry mass variability. At maturity (44 day after veraison), berry mass variability within a bunch was quantified by recording the mass of each individual berry of each experimental bunch. Three plants per block were chosen to carry out this observation. Plants were the same for the two experimental years, except on S6 block.

Berry composition. Berry physical and chemical composition was analysed on 18 berries per bunch of the remaining three plants per block. These plants varied over the two experimental years.

In order to take into account the possible effect of berry position on berry characteristic (Pisciotta et al. 2013), berries were collected in different parts of bunches, according to their differences in mass. Nevertheless, the effect of berry position is not considered in this study. Mass of each individual berry was recorded. Then the three berry tissues were separated and their mass was recorded. Seed number per berry was also recorded. Finally, berry flesh was pressed and the must, after a gentle centrifugation, was analysed. Sugar concentration, expressed in degree °Brix, was measured using a refractometer. Malic acid concentration was estimated by a colorimetric method (Bran and Luebbe TRAACS 800 autoanalyzer, 22844 Norderstedt, Germany). Finally, total anthocyanins of the berry skin were analysed. Each skin sample contained the whole berry skin. In this study we chose to express the anthocyanins as concentration (mg/g berry). Dried skins were extracted according to Acevedo de la Cruz et al. (2012). Extracts were analyzed using an UltiMate 3000 UHPLC system (Thermo Electron SAS, Waltham, MA USA). The compounds were quantified by their peak area with Chromeleon software, version 7.1 (Thermo Electron SAS, Waltham, MA USA). Malvidin-3-O-glucoside was used as external standard for all the quantified anthocyanins at 520 nm.

Berry water and nitrogen status. Carbon isotope discrimination ($\delta^{13}\text{C}$) was chosen in this study as indicator of berry water status. ^{12}C isotope of ambient CO_2 is preferentially used by the enzymes involved in photosynthesis compared to ^{13}C (Farquhar et al. 1989). This process (called “carbon isotope discrimination” or $\delta^{13}\text{C}$) is reduced under water deficit stress conditions, because of stomatal closure. Consequently, hexoses produced under these conditions contain more ^{13}C . So the $^{12}\text{C}/^{13}\text{C}$ ratio measured on products of photosynthesis at ripeness, is an integrative indicator of vine water uptake conditions during grape ripening (van Leeuwen et al. 2001b, Gaudillère et al. 2002). Moreover, according to Santesteban et al. (2016) $\delta^{13}\text{C}$ is a useful tool for modelling variations in yield and berry mass at different scales. In order to investigate on the possible variation of $\delta^{13}\text{C}$ value within a bunch, from one berry to another, in this study, $\delta^{13}\text{C}$ measurements were carried out by mass spectrometry on grape juice extracted from 6 individual berries per bunch, collected in a random way. The results vary from -20‰ (severe water deficit stress) to -27‰ (no water deficit stress, van Leeuwen et al. 2009). The same approach (berry by berry) was used to measure and test the variability of Yeast Assimilable Nitrogen (YAN) within a bunch. YAN, chosen as indicator of berry nitrogen status, was analyzed on the same berries where the previous parameter had been measured. After extraction of berry juice, YAN was measured by the determination of α -amino nitrogen and ammonia content, using enzymatic methods (BIOSENTEC, 31320 Auzeville-Tolosane, FRANCE). The results vary from <50 mg N/L, corresponding to severe nitrogen deficit stress, to >250 mg N/L, corresponding to high nitrogen availability (van Leeuwen and Friant 2011).

Plant level

This study was carried out only in 2015 and only in three experimental blocks. The three blocks were chosen according to their different water availability observed during the previous experimental years. G8, G1 and S7 blocks were characterized by three different SWHC, which was low, medium and high, respectively. Within each block, three plants were chosen. At harvest, each individual bunch of each experimental plant was collected, recording its exact position inside the plant (cane, shoot position on the cane, bunch position on the shoot). The total mass of each bunch was recorded. Following, all berries of each cluster were separated and, one by one, they were weighted and their seed content was recorded. Berry mass and seed number of each bunch are expressed as the mean of all values recorded berry by berry. All berries of each bunch were

pressed and the must, after a gentle centrifugation, was analyzed for soluble solids, total acidity, pH and malic acid, using Fourier Transform Infrared spectrometer (FTIR, WineScan FOSS®, FRANCE, 92000 Nanterre; Destrac et al. 2015). Finally, a part of juice, extracted from the whole bunch, was analyzed by mass spectrometry, in order to obtain the $\delta^{13}\text{C}$ value.

Statistical data analysis

Data were analyzed using R software (R development Core Team 2015, version 3.2.3).

Variability of factors impacting berry mass. In this work, the variability of all variables (dependent and independent) was studied. Variability was quantified by the coefficient of variation (cv), which is standard deviation as a percentage of the mean (Zar 1999). The lower the cv, the more uniform is the population. (Coombe and Iland 2004, Gray and Coombe 2009).

Hierarchy of factors impacting berry mass. The statistical approach to hierarchize the factors impacting on berry mass according to their degree of influence did not change among the observation scales. In order to fit the best model, the relationships between the berry mass and the covariates (water status, nitrogen status and berry seed content), were determined by multiple linear regression analysis. The full model, considering all the interactions among covariates, was reduced by a stepwise procedure. On the final model, an ANOVA was performed to compute the contribution of each component to the variance of berry mass and berry composition.

Berry mass effect on berry composition. The relationships between berry mass and berry compounds were performed by a partial least squares regression analysis. The distribution of points was observed and, when possible, a linear regression was performed.

RESULTS

Berry mass variability and consequences on grape composition: a study at bunch level

Berry mass variability

The berry mass variability inside a single bunch was studied on a total of 18 bunches, belonging to 18 different plants of six different blocks. Results of this investigation, conducted berry by berry, are resumed in figure 1, where the coefficient of variation (cv) values, used to quantify the berry mass variability, are reported. Over two years, variability of berry mass for each bunch was similar among bunches of the same block, while the degree of variability of berry mass was less constant among blocks. In 2014, S6 block showed a mean cv value significantly higher compared to other blocks and also a bigger number of outliers (figure 1A). However, this was not observed in 2015 (figure 1B). In the latter, the higher cv values were recorded on G7 and G8 bunches. Their mean cv values were significantly higher compared to other blocks. Some significant differences of berry mass were observed among bunches within a given block, but these were lower than differences recorded between blocks, reflecting the soil conditions. This result was particularly obvious in 2015, which was a warm and dry vintage.

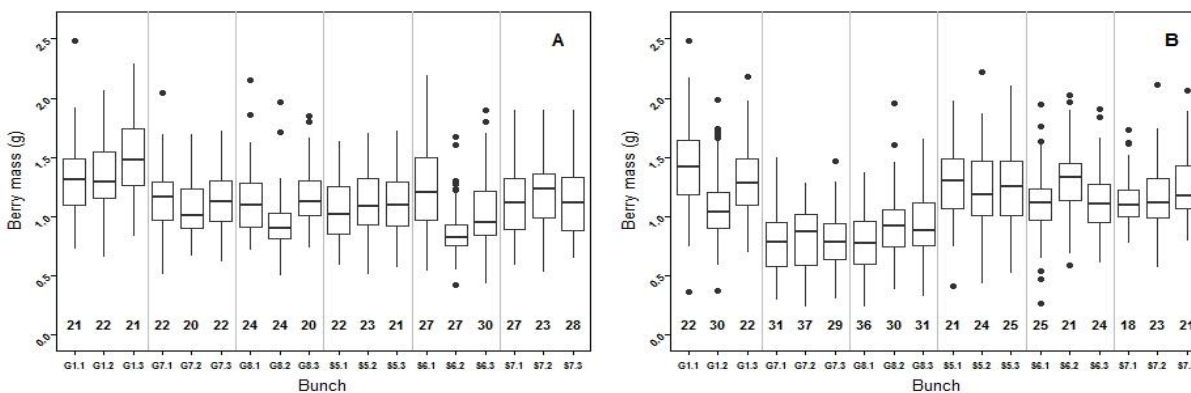


Figure 1 - Berry mass variability at a bunch level in 2014 (A) and 2015 (B). Values reported under each boxplot correspond to coefficient of variation of berry mass

Variability of factors impacting on berry size inside a bunch

Each factor possibly impacting on berry mass was measured on several berries in each experimental bunch.

Figure 2 shows the variability of $\delta^{13}\text{C}$ inside a bunch, but also allowed to compare the variability among bunches belonging to the same block or to different blocks. Berries from a single bunch show similar $\delta^{13}\text{C}$ values, which vary from one bunch to another of the same blocks and among different blocks. Hence, these results show that the variability inside each bunch is lower compared to variability inside a block, which, in turn, in most of cases, is lower than variability among blocks. A small variability of $\delta^{13}\text{C}$ was observed at bunch level, expressed by cv (table 1). However, $\delta^{13}\text{C}$ was not related to any of the berry parameters measured in this study such as berry position inside bunch, berry mass or berry volume (data not shown).

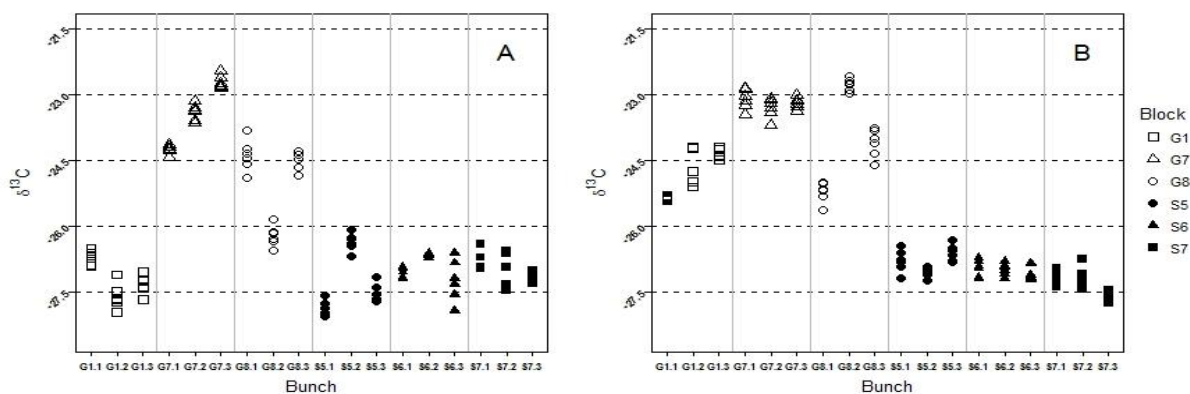


Figure 2 - Carbon isotope discrimination ($\delta^{13}\text{C}$) variability at bunch level in 2014 (A) and 2015 (B).

Hence, considering these results, we conclude that all berries belonging to the same bunch are characterized by the same water status, which is related to the SWHC of the block and plant root system development. According to values reported by van Leeuwen et al. (2009), in 2014 all berries from G1, S5, S6 and S7 blocks and G8.2 berries, did not face any deficit water stress. Weak water deficit stress characterized berries from G7.1, G8.1 and G8.3 bunches, while berries from G7.2 and G7.3 bunches were submitted to moderate water deficit stress (figure 2A). Despite the fact that experimental plants on each block varied over the two years, similar trends were observed in 2015 compared to 2014, excepted for G1. The particular behavior of this block is the result of the combination of soil and vintage effects, which represents two of most important factors of the terroir effect.

Table 1 - Variability of factors impacting on berry mass inside a bunch, measured in 2014 and 2015, on 18 different bunches.

	Coefficient of variation (%)																	
	G1.1	G1.2	G1.3	G7.1	G7.2	G7.3	G8.1	G8.2	G8.3	S5.1	S5.2	S5.3	S6.1	S6.2	S6.3	S7.1	S7.2	S7.3
$\delta^{13}\text{C}$	0.6	1.0	0.8	0.5	0.8	0.7	1.5	0.9	0.8	0.6	0.8	0.8	0.4	0.2	1.8	0.9	1.5	0.4
2014 YAN	18	8	10	21	15	15	11	13	8	5	11	16	11	12	26	4	19	8
Berry seed content	36	40	49	37	34	29	31	43	38	39	39	38	47	41	42	47	46	43
$\delta^{13}\text{C}$	0.2	1.5	0.5	1.0	1.0	0.6	0.9	0.6	1.3	1.0	0.4	0.7	0.7	0.5	0.6	0.5	0.9	0.4
2015 YAN	3	22	7	4	15	19	8	10	14	8	4	9	13	5	17	12	14	7
Berry seed content	45	51	52	40	43	37	37	36	42	34	34	34	39	36	29	40	39	36

Each value was calculated considering each berry of each bunch as a replicate. Vines, on which bunches were sampled, varied in the two experimentation years.

YAN variability inside a bunch was analyzed in a similar way. Results of this study show that YAN variability was small in all bunches over the two years, except for G1.1, G7.1, G7.2, G7.3 and S6.3 in 2014 (figure 3A). In order to understand if this variability depended on any of the measured berry characteristics, YAN values were plotted against berry mass, berry position, berry volume and berry $\delta^{13}\text{C}$. Because within each bunch none of these correlations were significant (data not shown), we presumed that these singular cases of higher variability at bunch level could be related to a lack of analytical precision in YAN measurement. Comparing the two experimental years (figure 3A and 3B), in 2014 a bigger variability among plants at intra- and inter-block level was observed. Nevertheless, in this vintage, most of berries were characterized by a low to very low nitrogen status (van Leeuwen and Friant, 2011), except for berries from G1.1 and G7 bunches. Despite the large variability characterizing these bunches, most of their berries showed moderate to high nitrogen status, especially berries from G1.1 and G7.3 bunches. In 2015, all berries showed lower values of YAN compared to 2014 (figure 3B). Most berries did show a moderate to low nitrogen status. YAN values were particularly low in berries from the sandy soil and from G8.3. Despite the YAN variability among blocks being lesser than the one observed with $\delta^{13}\text{C}$, YAN values measured berry by berry reflected the real nitrogen availability of block, depending on soil characteristics (data not shown).

In this study, berry seed content was been measured as seed number per berry and seed mass per berry. Despite the individual seed mass decreasing while seed number per berry increases, in this study seed number and seed mass per berry were highly correlated (data not shown). Hence, in order to represent the berry seed content variability in the best possible way, we choose to express this factor as seed mass per berry. Data recorded are resumed in figure 4 and table 1. In 2015, the total seed mass per berry was lower than in 2014. Nevertheless, the trend of cv values seems to be similar in the two years. Compared to the previous factors, berry seed content shows high cv values when studied at bunch level (table 1). Conversely, these values are similar among plant belonging to the same block and among blocks. This was observed in both experimental years.

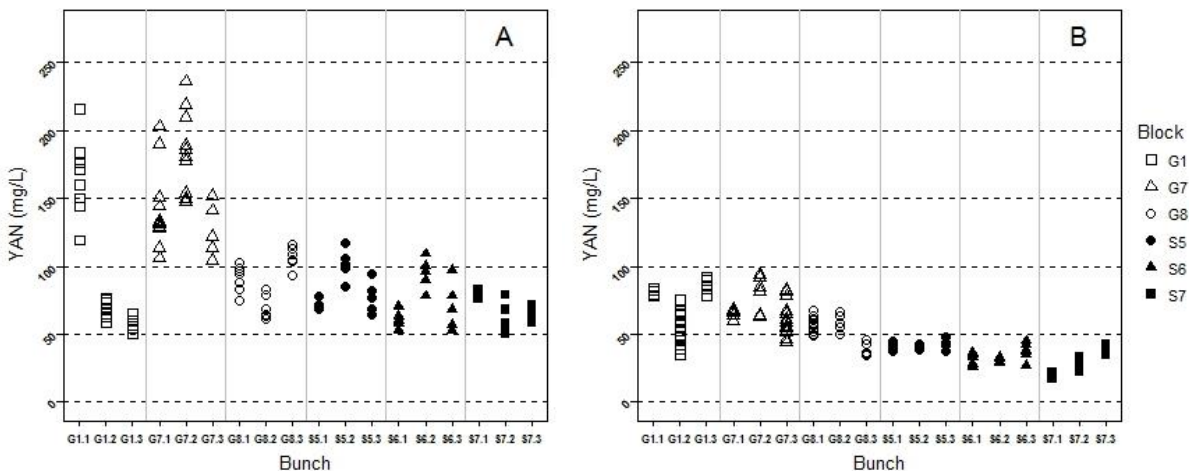


Figure 3 - Yeast Assimilable Nitrogen (YAN) variability at bunch level in 2014 (A) and 2015 (B).

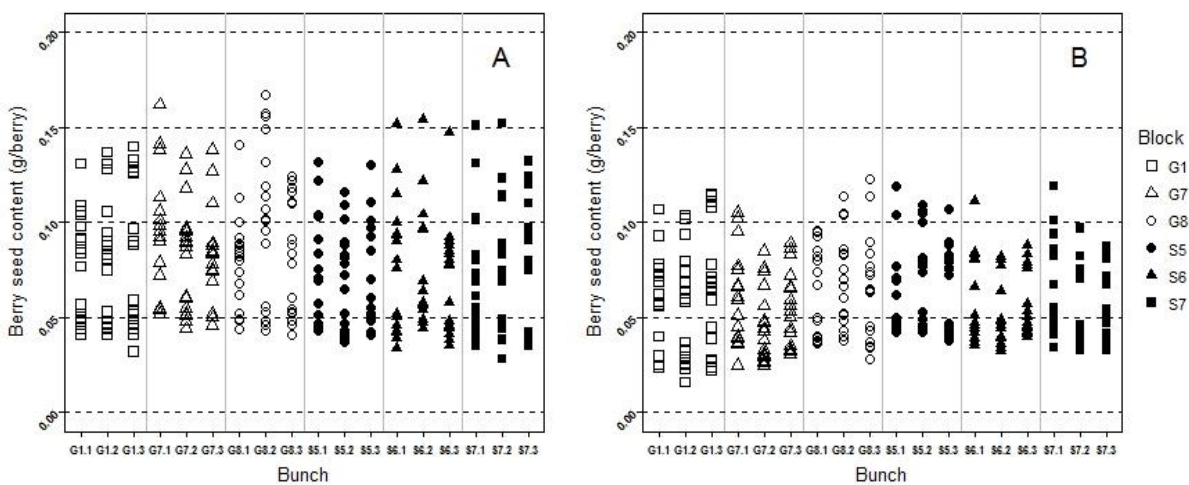


Figure 4 - Berry seed content variability at bunch level in 2014 (A) and 2015 (B).

Hierarchization of factors impacting berry mass inside a bunch

The relationships between each considered factor and berry mass were studied for each of the 18 experimental bunches, by a linear regression where each point corresponded to a berry.

Results obtained by each linear regression between $\delta^{13}\text{C}$ and berry mass didn't show any consistence. Similar results were obtained when possible relationships between YAN and berry mass were investigated. These results were observed in both years of experimentation (data not shown). The absence of a coherent and significant relationship between berry mass and $\delta^{13}\text{C}$ and YAN reflected mainly the low variability of the two factors at bunch level. This result supports our first hypothesis, that all berries of an individual bunch are submitted to the same water and nitrogen status, independently from berry mass. Conversely, high and significant relationships were found between berry seed content and berry mass, for each bunch and in the two experimental years. Results of linear regressions are reported in table 2. Hence, the high cv values of berry mass and berry seed content could indicate that the variability of the dependent variable reflects the variability of the independent variable.

In order to compute the contribution of each component ($\delta^{13}\text{C}$, YAN and berry seed content) to the variance of berry mass (dependent variable) a multiple linear regression analysis (ANOVA) was performed. Results of this analysis (table 3) are consistent with previous results. They confirm that, at bunch level, nor $\delta^{13}\text{C}$ nor YAN have a significant effect on berry mass variability. On the other hand, berry seed content is highly impacting berry mass. Its effect is highly significant and explain the high percentage of variance of berry mass. These results were obtained in 2014 and confirmed in 2015.

Table 2 - Relationship between berry seed content and berry mass inside a bunch.

	G1.1	G1.2	G1.3	G7.1	G7.2	G7.3	G8.1	G8.2	G8.3	S5.1	S5.2	S5.3	S6.1	S6.2	S6.3	S7.1	S7.2	S7.3
2014	0.86	0.94	0.76	0.83	0.77	0.83	0.52	0.85	0.37	0.72	0.59	0.85	0.88	0.69	0.83	0.41	0.77	0.88
2015	0.83	0.77	0.88	0.79	0.88	0.79	0.76	0.83	0.79	0.72	0.81	0.76	0.85	0.85	0.77	0.69	0.71	0.85

Relationship between the two variables was tested by a linear regression. Reported values corresponded to R². Each value was calculated considering each berry of each bunch as a replicate. Number of replicates varied from one bunch to another. Vines on which bunches were sampled varied over the two experimentation years.

Table 3 - Effect of berry water and nitrogen status and berry seed content on berry mass inside a bunch

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Berry seed content	1	5.255	5.5255	179.628	<2e-16 ***
Berry water status	1	0.000	0.000	0.002	ns
Berry nitrogen status	1	0.011	0.011	0.383	ns
Residuals	86	2.516	0.029		
Berry seed content	1	2.6338	2.6338	102.551	1.67e-13 ***
Berry water status	1	0.0589	0.0589	2.294	0.136 ns
Berry nitrogen status	1	0.717	0.0717	2.291	0.101 ns
Residuals	48	1.2328	0.0257		

Results were obtained from an Anova type performed on the following model: berry fresh mass = berry seed content + berry water status + berry nitrogen status. ^a Significance codes: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 'ns' 1

Relationship between berry mass and berry compounds inside a bunch

The effect of berry mass on sugar, malic acid and anthocyanin concentration was studied by linear regressions, over the two years of experimentation, for each experimental bunch. The results of this analysis are resumed in table 4. Except for some singular cases, most linear regressions showed low coefficient of determination (R^2) values and not all relationships were significant. The relationships between berry mass and malic acid concentration did not show consistent results. Berry mass was never related to malic acid, except some cases observed in 2014, where smaller berries contained less malic acid. The relationships between berry mass and sugar concentration varied over the two years. While berry mass almost never showed significant correlations with sugar concentration in 2015, some consistent and significant relationships were observed in 2014. In these bunches, small berries were characterized by a slightly higher sugar concentration level. The effect of berry mass on anthocyanin concentration, expressed as mg/g of berry, was not always significant. However, all significant relationships were negative, indicating that anthocyanin concentration increases when berry mass decreases. This fact was particularly obvious in 2015 on all three G7 bunches. Correlations were much lower on S5, S6 and S7 blocks, compared to G1, G7 and G8 blocks.

Finally, when the relationships between berry mass and berry compounds were studied from a global point of view (independently from berry origin), some consistent trends could be observed. In 2014, the relationships between berry mass and sugar and anthocyanin concentrations were negatives and significant, despite low coefficients of determination ($R^2 = 0.20$ and 0.21 , respectively). Hence, most of the small berries were characterized by higher sugar and anthocyanins concentration levels. In contrast, none of remaining relationships showed consistent results (data not shown). In 2015, only the relationship between berry mass and anthocyanin concentration was significant. This correlation showed a negative slope, indicating that smaller berries were characterized by higher anthocyanins concentration levels ($R^2 = 0.44$).

Table 4 - Relationship between berry mass and concentration of the major berry compounds, observed in 2014 and 2015, studied inside each experimental bunch.

	G1.1	G1.2	G1.3	G7.1	G7.2	G7.3	G8.1	G8.2	G8.3	S5.1	S5.2	S5.3	S6.1	S6.2	S6.3	S7.1	S7.2	S7.3
2014																		
Sugar	-0.36*	-0.31*	-0.35*	-0.25*	-0.31*	-0.06	-0.45*	-0.52*	-0.45*	-0.50*	-0.36*	-0.38*	-0.11	-0.27*	-0.58*	-0.15	-0.16	-0.40*
Malate	0.05	0.21*	0.25*	0.18	0.05	0.00	0.01	0.18	0.34*	0.08	0.14	0.00	0.28*	0.06	0.09	0.00	0.23*	0.08
Anthocyanin	-0.24	-0.40*	-0.07	-0.32*	-0.35*	-0.56*	-0.37*	-0.59*	-0.74*	-0.58*	-0.28	-0.55*	-0.36*	-0.35*	0.00	-0.53*	-0.04	-0.03
2015																		
Sugar	-0.38*	0.00	0.00	-0.15	-0.08	0.02	-0.12	-0.18	-0.01	0.00	-0.16	-0.18	-0.13	-0.02	-0.23*	0.00	0.00	-0.07
Malate	0.13	0.10	0.00	0.12	-0.02	0.00	-0.08	0.00	-0.01	0.02	-0.01	0.03	0.46	0.10	0.03	-0.02	0.19	0.22
Anthocyanin	-0.18	-0.23*	-0.45*	-0.62*	-0.81*	-0.71*	-0.64*	-0.46*	-0.46*	-0.08	-0.40*	-0.10	-0.01	-0.02	-0.15	-0.53*	-0.20	-0.69*

Relationships between the variables were tested by a linear regression. Reported values corresponded to R². * indicates the significance of the relationship. Each value was calculated on 18 berries per bunch. Vines on which bunches were sampled varied over the two experimentation years. Sugar, malate and anthocyanin concentration are expressed as BRIX, g/L and mg/g of berry, respectively.

Berry mass variability and consequences on grape composition: a study at plant level

Berry mass variability

Intra-vine berry mass variability was studied in 2015 on nine different vines, comparing mean berry mass of each bunch belonging to the same plant. Results of this investigation are resumed in figure 5, where the coefficient of variation (cv) values recorded for each plant are reported. Intra-vine (i.e. bunch by bunch) berry mass variability is smaller compared to the one observed at the bunch level, but it varied among plants. The intra-plant variability was not linked to the position of the bunch inside the plant (data not shown). Once again, we observed that the block effect was the most important. Differences among blocks were bigger than differences among plants of the same block.

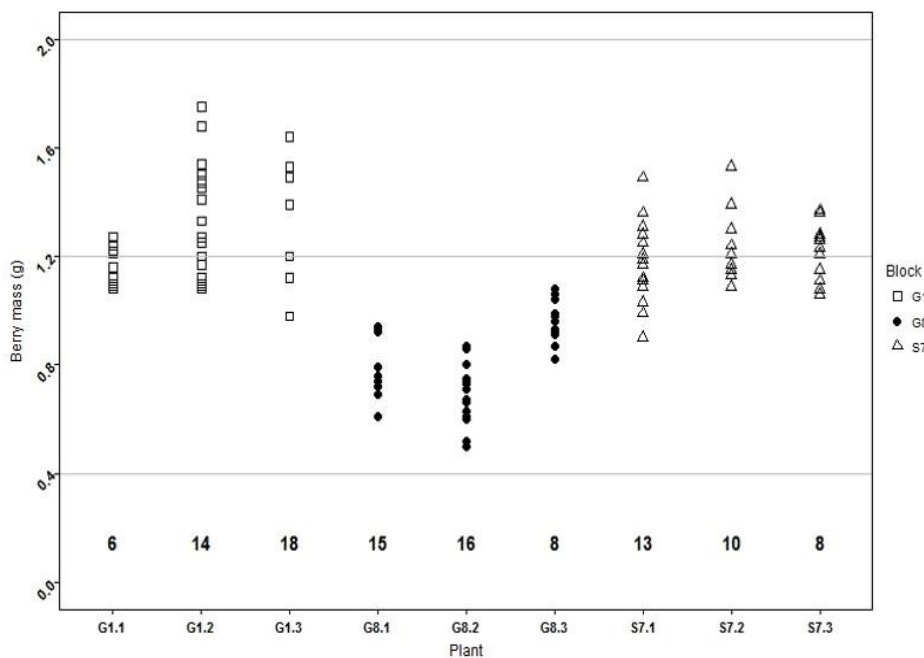


Figure 5 - Intra-vine berry mass variability.

Values reported in the figure represent coefficient of variation of berry mass.

Variability of factors impacting on berry size inside a plant

Each factor possibly impacting on berry mass was measured on each individual bunch of nine experimental vines. Variability of $\delta^{13}\text{C}$ is resumed in table 5, where cv values are reported. Because a small intra-plant variability of $\delta^{13}\text{C}$ among bunches was observed, we investigated a possible effect of bunch position. A multiple regression model was performed. No consistent cane effect,

nor shoot effect, nor effect of the position of the bunch on the shoot were observed. The $\delta^{13}\text{C}$ variability among bunches of a single plant needs further investigation. Despite the observed variability, the cv values are low, indicating that bunches from the same plant are submitted to similar water uptake conditions. Moreover, small differences among vines planted on the same block were observed, excepted for G1 vines. Water status measured at bunch level reflected the SWHC of the block where vines were planted. The water availability varied among blocks, as expected. So, all bunches from S7 block did not face any water deficit. Intermediate values, corresponding to a weak water deficit were recorded on most of the G1.1 and G1.2 bunches. Conversely, a bigger intra-plant variability was observed on G1.3, where half of bunches did face a moderate water deficit. All bunches from G8 block also faced moderate water deficit.

Table 5 - Variability of $\delta^{13}\text{C}$, YAN and berry seed content at plant level

		G1.1	G1.2	G1.3	G8.1	G8.2	G8.3	S7.1	S7.2	S7.3
$\delta^{13}\text{C}$	Mean	-25.52	-24.95	-24.20	-23.22	-23.41	-24.14	-26.93	-27.15	-27.22
	SD	0.33	0.54	0.54	0.46	0.26	0.19	0.62	0.47	0.29
	cv	1	2	2	2	1	1	2	2	1
YAN	Mean	132	215	174	171	157	138	114	136	105
	SD	32	33	24	23	31	16	15	38	9
	cv	24	15	14	14	20	12	13	28	8
Berry seed content	Mean	1.50	1.62	1.67	1.65	1.61	1.76	1.40	1.55	1.54
	SD	0.13	0.14	0.18	0.16	0.20	0.13	0.16	0.14	0.12
	cv	9	9	11	10	12	7	12	9	8

Values reported in table are obtained considering each bunch of the same plant as a replicate.

$\delta^{13}\text{C}$, Yan and berry seed content are expressed as ‰, mg/L and mean of seed number per berry, respectively. Standard Deviation (SD) and Coefficient of Variation (cv) are also reported.

The same approach was used to investigate YAN variability at an intra-plant level. However, at this scale higher cv values were observed compared to $\delta^{13}\text{C}$ variability. Nevertheless, once again, no consistent tendencies emerged when the effect of bunch position (cane and shoot on the plant) was plotted against YAN levels. Hence, the underlying drivers of YAN variability among bunches of a single plant need further investigation. Among the investigated vines, G1.1, G8.2 and S7.2 showed bigger cv values. Part of their bunches was moderately low in nitrogen, while another part was low in nitrogen. Smaller ranges of YAN values were observed in the remaining vines. All bunches of G1.2, G1.3 and G8.1 showed YAN value indicating no nitrogen deficit (van Leeuwen and Friant 2011). Conversely, as expected, lower YAN values were recorded on S7.1 and S7.3,

reflecting the nitrogen availability of the blocks, observed in previous studies conducted on the same experimental site.

Finally, the cv values of berry seed content are also calculated for each bunch, in order to observe the intra-plant variability. Value reported in table 5 are the mean of the seed number recorded for each berry of the bunch. So, the range of mean values is small (1.40 to 1.76) and the intra-vine variability is smaller than the intra-bunch variability. Nevertheless, berry seed content varied inside each experimental vine. Once again, the possible effect of the position of the bunch was investigated. No cane, nor shoot effect were observed (data not shown). Moreover, differences of berry seed content observed among bunches were statistically not significant.

Hierarchization factors impacting on berry mass at the intra-plant level

In order to understand the effect of each factor potentially impacting berry mass, several partial least squares regression analyses were performed (plant by plant). Berry mass was plotted against each factor to investigate whether the relationship between independent and dependent variables was linear or not. Figure 6 shows the relationship between berry mass and $\delta^{13}\text{C}$. Average berry mass and $\delta^{13}\text{C}$ were recorded for nine individual grapevines. Inside each grapevine, berry mass and $\delta^{13}\text{C}$ measured at bunch level were not related. When all the data was pooled together, a significant relationship could be established: berry mass was lower in vines which faced water deficit. However, the coefficient of determination is low ($R^2 = 0.27$) because two wines (G1.2 and G1.3) produced heavy berries despite water deficit conditions.

No consistent relationship was established between YAN and berry mass, nor among bunches of individual vine, nor when the results of nine vines were pooled together (figure 7).

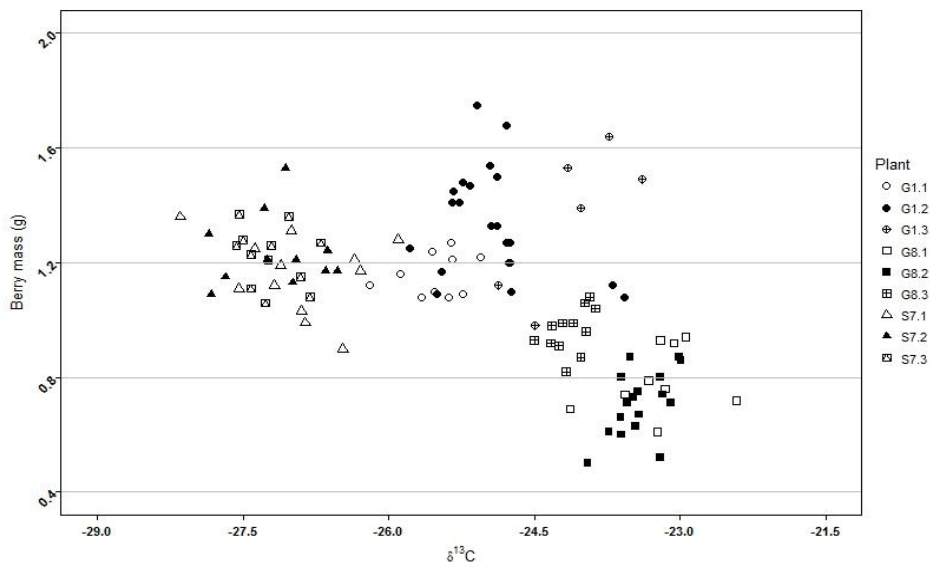


Figure 6 - Relationship between $\delta^{13}\text{C}$ and berry mass
 Average $\delta^{13}\text{C}$ and average berry mass were measured for each bunch of 9 individual grapevines (2015)

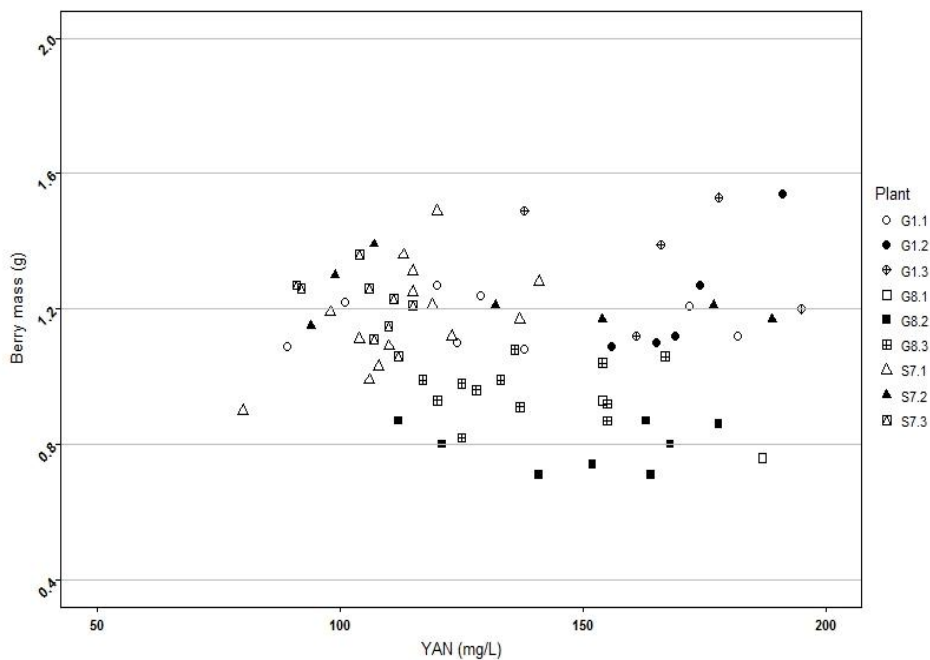


Figure 7 - Relationship between YAN and berry mass.
 Average YAN and average berry mass were measured for each bunch of 9 individual grapevines (2015)

Figure 8 shows the relationship between average berry seed number and berry mass recorded for nine individual grapevines. This relationship was statically significant for each plant, except S7.3 and G1.1. All the significant relationships were positive and linear. G8.3, S7.1 and G1.2 showed the highest R^2 values (0.83, 0.69 and 0.65, respectively). However, these high correlation values do not seem to be linked to other investigated variables. When all the data was pooled together, a significant relationship was established. However, the R^2 value of this global linear regression was low. This fact was related to the high berry mass variability among block. All bunches belonging to the G8 block showed low average berry mass. Hence, we divided our dataset in two parts: we considered on a hand only bunches from G8 block and on the other hand bunches from the two remaining blocks. In this way we obtained two significant relationship characterized by higher coefficient of determination values (R^2 of G8= 0.44, R^2 of G1+S7= 0.33).

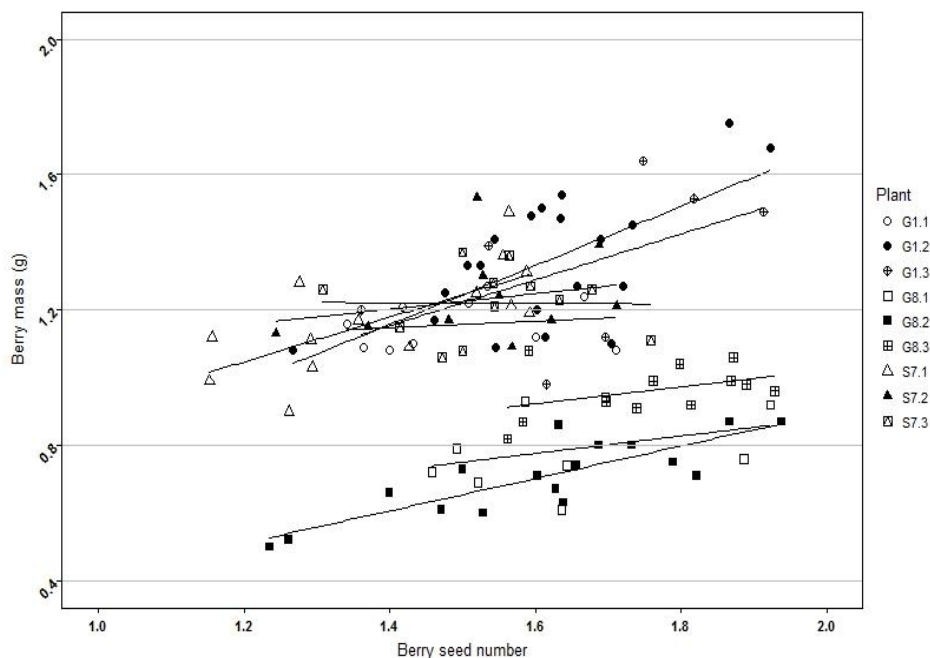


Figure 8 - Relationship between berry seed content and berry mass. Average berry seed content and average berry mass were measured for each bunch of 9 individual grapevines (2015)

The results obtained by these correlations were confirmed by a multiple regression model, performed in order to hierarchize the impact of the three factors on berry mass, within a plant (table 6). As expected, berry seed mass is the driving factor impacting berry mass at bunch level.

Nitrogen status, nor water status had a significant effect, although the nitrogen status was significant at 10% level.

Table 6 - Effect of bunch water and nitrogen status and berry seed content on berry mass within a plant

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Berry seed content	1	0.2554	0.2554	18.307	5.7e-05	***
Nitrogen status	1	0.0509	0.05087	3.647	0.0602	ns
Water status	1	0.0003	0.00030	0.022	0.8834	ns
Residuals	72	1.0045	0.02395			

Results were obtained from an Anova type performed on the following model: berry fresh mass = berry seed content + bunch nitrogen status + bunch water status.

^a Significance. codes: '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 'ns' 1

Intra- and inter-vine relationships between berry mass and berry compounds

Berry sugar concentration, total acidity, pH and berry malic acid concentration show much less variability among bunches of a single vine compared to variability among vines (figure 9-12). Pooling all data together, berry sugar concentration and pH decreases with berry mass (R^2 respectively 0.15 and 0.37, figures 9 and 10). Despite the R^2 values being low, these relationships were highly significant. These tendencies are driven by inter-vine variability in berry mass. No consistent tendencies are visible when plotting berry mass against sugar concentration or pH at the intra-vine level.

Total acidity and berry malic acid concentration increase with berry mass (R^2 respectively 0.24 and 0.26, figures 11 and 12). Despite R^2 values being low, these relationships were highly significant. Similarly to sugar and pH, no consistent tendencies are shown when berry mass is plotted against total acidity or berry malic concentration at the intra-vine level.

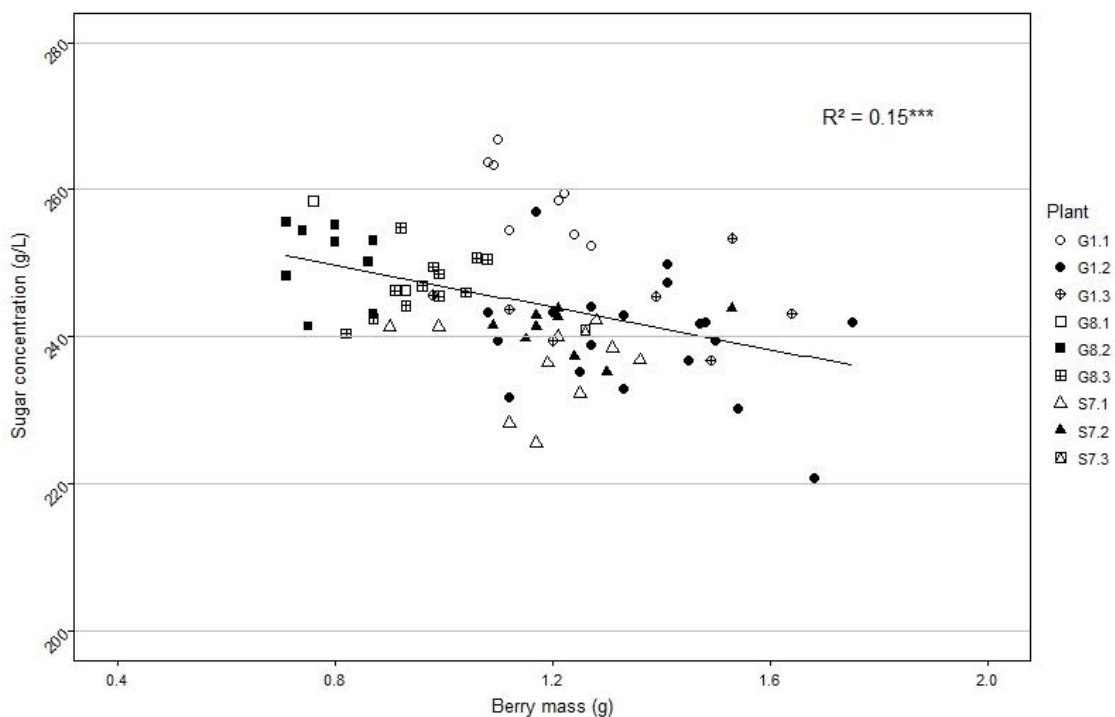


Figure 9 - Relationship between average berry mass and sugar concentration measured for each bunch of 9 individual grapevines in 2015

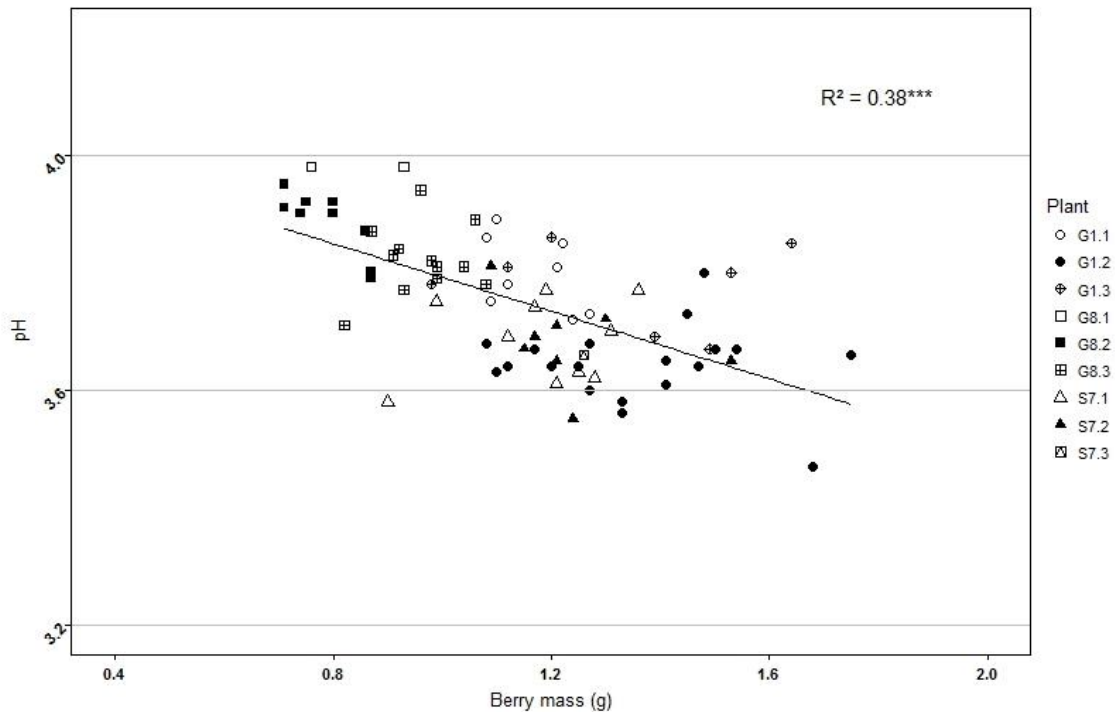


Figure 10 - Relationship between average berry mass and pH measured for each bunch of 9 individual grapevines in 2015

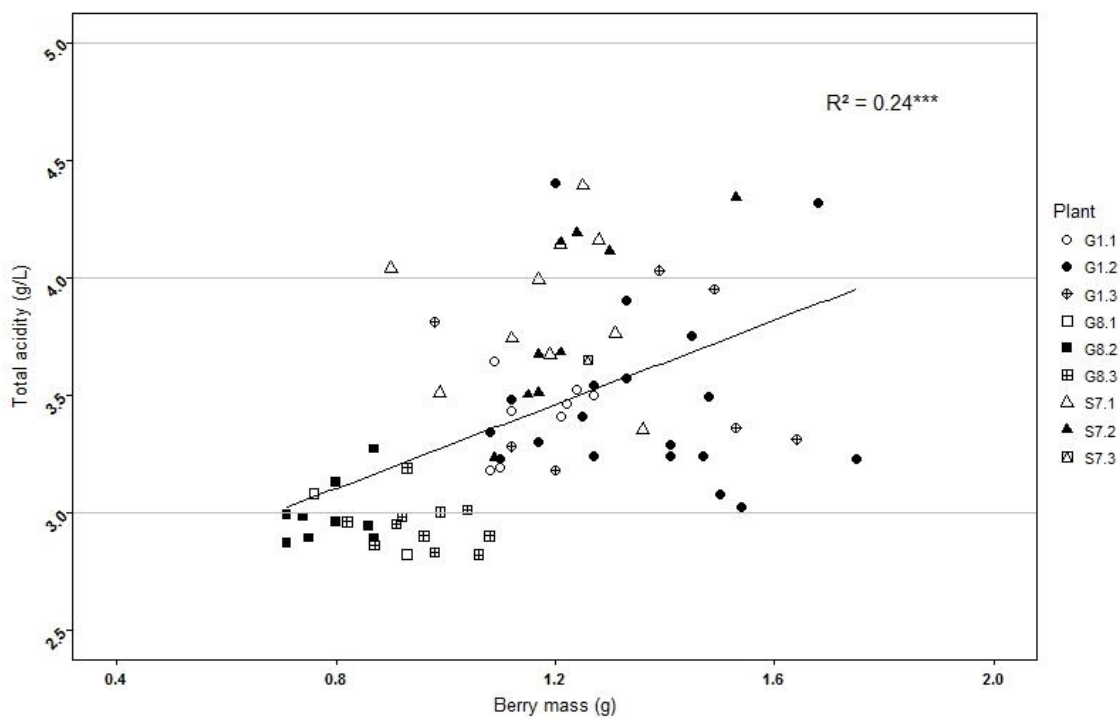


Figure 11 - Relationship between average berry mass and total acidity measured for each bunch of 9 individual grapevines in 2015

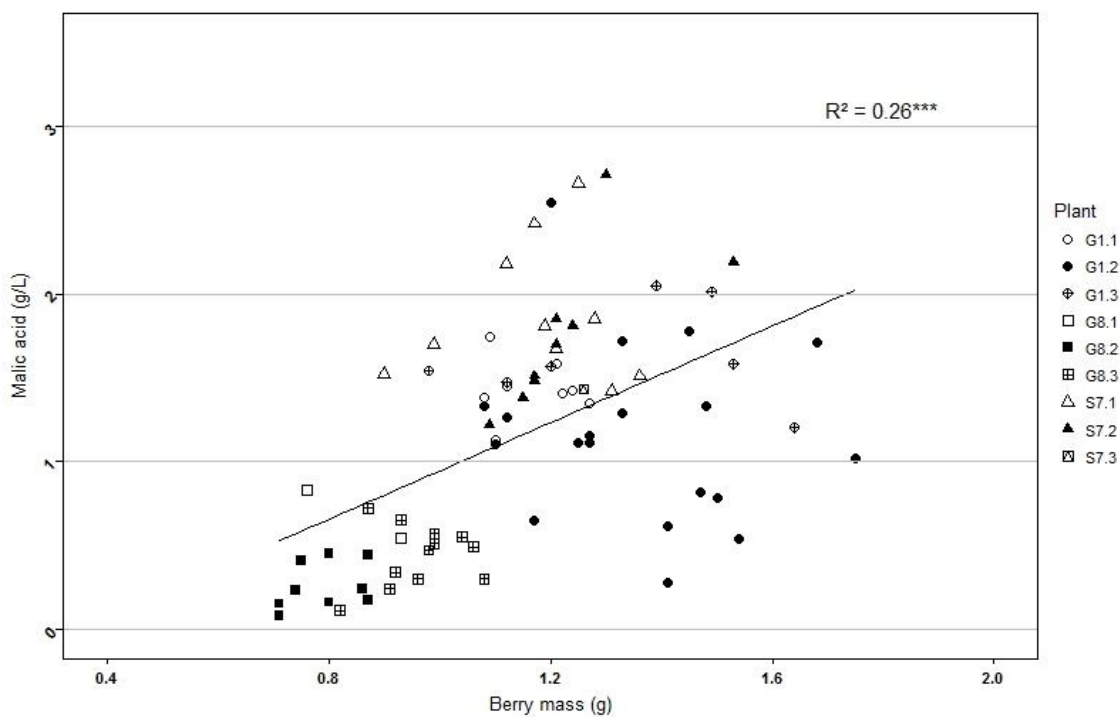


Figure 12 - Relationship between average berry mass and malic acid measured for each bunch of 9 individual grapevines in 2015

DISCUSSION

The objectives of this work were to hierarchize the factors impacting on berry mass according to their degree of influence and to investigate the influence of berry size on grape composition at intra-bunch and intra-plant level. Several previous studies showed that the final berry size depend on many factors (environmental factors and factors related to the berry itself), which can affect cell number and/or cell volume. Among these factors, vine water and nitrogen status (Matthews and Anderson 1988, van Leeuwen and Seguin 1994, Ojeda et al. 2001, Choné et al. 2001a, Trégoat et al. 2002, Roby et al. 2004, Chaves et al. 2007, van Leeuwen et al. 2007) and berry seed content (Scienza et al. 1978, Roby and Matthews 2004) play a key role. Availability of carbon can also possibly impact berry mass (Coombe 1962). However, in our experimental conditions, leaf area to fruit mass ratio per plant was high and not likely to be a limiting factor for berry growth. Hence, vine carbon source was not considered in this study.

The observations of the present work were carried out at two different scales: within a bunch (berry by berry) and within a plant (bunch by bunch). In order to reach our goals, for each observation scale, we started measuring berry mass variability, quantified by the coefficient of variation (cv).

In this study in most cases the cv of berry mass within a bunch varied from ~21 to 30%, which is consistent with Shellie (2010). However, some bunches showed higher cv values. This was often the case for bunches characterized by low berry mass values. This was the case in S6 in 2014, probably related to the health status of the wines, which were affected by *GFLV*, or G7 and G8 bunches in 2015, where vines were submitted to early water deficit stress. Differences of berry mass was bigger among blocks than within a block, reflecting different level of variability of pedological conditions. Moreover, the inter-block differences were bigger in 2015, which was a warm and dry vintage. Hence, we observed an integrated effect of terroir factors on berry mass.

In order to fit the best model, necessary to hierarchize the different factors according to their level of impact, the variability of each of them within a bunch was observed. Relationships between berry seed content and berry mass, berry by berry, were previously investigated in several works (Scienza et al. 1978, Carwthon and Morris 1982, Roby and Matthews 2004, Walker et al. 2005, Friend et al. 2009). In contrast, no studies have been carried out in order to investigate $\delta^{13}\text{C}$ (indicator of water status) and YAN (indicator of nitrogen status) variability at bunch level. In this study, possible variability of these two factors was measured at this scale. Concerning the $\delta^{13}\text{C}$,

results show that the variability within each bunch is lesser compared to variability inside a vine (between bunches), which, in turn, is often lesser than variability among vines inside a block and among blocks. Moreover, the small variability observed within a bunch was not related to berry position, nor to berry mass, nor to berry volume. Linear regressions between $\delta^{13}\text{C}$ and berry mass at bunch level did not show any consistence. Hence, we conclude that all berries belonging to the same bunch are subjected to the same water uptake conditions, which reflects mainly the SWHC of the block and the rooting depth of the studied vine. Differences among blocks showed similar trends over the two years, but they were bigger in 2015, as a result of more contrasting weather conditions characterizing this vintage.

Similar results were observed when YAN variability within a bunch was measured. Results of this study show that, over the two years, YAN variability within a bunch was small in most cases. Because none of the correlations between YAN and berry mass, berry position, berry volume and berry $\delta^{13}\text{C}$ was significant, we suppose that the singular cases of high cv values could be linked to measurement errors. Each sample was obtained with the total amount of berry juice from one single berry. Hence, it was not always possible to replicate analyses, in particular for small berries. Cv values were higher in 2014 compared to 2015. YAN variability among blocks was lower compared to $\delta^{13}\text{C}$ variability. However, YAN values measured berry by berry reflected the real nitrogen availability of the block, depending on soil characteristics. As observed for $\delta^{13}\text{C}$, no consistent results were obtained when the possible relationship between YAN and berry mass was investigated. Hence, these observations support our hypothesis, that all berries of an individual bunch are submitted at the same water and nitrogen uptake conditions.

In this study, berry mass within a bunch was closely correlated with berry seed content, which is consistent with earlier studies (Ollat et al. 2002, Roby and Matthews 2004, Walker et al. 2005). Among considered factors, intra-bunch berry seed content shows high cv values, which are similar among plants from the same block or from different blocks. They did not change in magnitude over the two experimental years. These high cv values seem to be linked to the cv of berry mass observed within each bunch. Hence, this result supports the hypothesis that, within a bunch, berry mass variability is mainly related to seed content. Confirmation was obtained by the multiple regression model, performed in order to identify the most important factor impacting berry mass within a bunch. Results of this analysis confirm that, at this level, berry seed content is the most important determinant of berry mass, while nor $\delta^{13}\text{C}$ nor YAN have a significant effect on berry

mass variability. Although water and nitrogen conditions are known to affect berry growth (Matthews and Anderson 1988, Williams and Matthews 1990, Ojeda et al. 2001, Choné et al. 2001a, Trégoat et al. 2002, Chaves et al. 2007, van Leeuwen et al. 2007, Ferrer et al. 2008, Girona et al. 2009), berry size in a cluster seems to be homogeneously influenced by these factors. This hypothesis confirms the results obtained by Shellie (2010) on Merlot, who demonstrated that berry growth in a cluster was homogeneously inhibited by water stress.

An important goal of this study was to investigate the effect of berry mass on grape composition, depending on the factors responsible of the berry mass variability. Relationships between berry mass and sugar, malic acid and anthocyanin concentration were studied by linear regressions performed on data obtained on berries from each experimental bunch. Our results show that most of the linear regressions were low or not significant. At bunch scale, berry mass was rarely related to malic acid, which was consistent with Wong et al. 2016. Relationships between berry mass and sugar concentration varied over the two years. While berry mass did not show significant correlations with sugar concentration in 2015, low negative relationships were observed in 2014 in some bunches. In these bunches small berries were characterized by slightly higher sugar concentration levels. Similar results were obtained by Melo et al. 2015. The relationships between berry mass and anthocyanin concentration varied among bunches. However, all significant relationships were negative, indicating that anthocyanin concentration (expressed in mg/g of berry) increases when berry mass decreases. This fact was particularly obvious in 2015 on all three G7 bunches, where vine faced severe early water deficit stress. These results seem to confirm the results obtained in other studies, which showed that the way in which berry size is reduced is more important than the berry size itself with regard to grape composition (Roby and Matthews 2004, Roby et al. 2004, Walker et al. 2005, Matthews and Nuzzo, 2007).

Nevertheless, when the relationships between berry mass and berry compounds are studied at a global level (independently from berry origin), our results show similar trends obtained in previous studies (Melo et al. 2015, Wong et al. 2016). In 2014, small berries were characterized by higher sugar and anthocyanins levels. Correlations between berry mass and anthocyanin concentration were negative and significant in 2015 as well, characterized by a higher value of R^2 compared to 2014.

A similar approach was performed at plant level. This study was carried out in 2015 on three blocks, characterized by different SWHC. Nitrogen availability was also variable. Within each plant, the origins and the consequences of berry mass variability were studied bunch by bunch. Berry mass variability within a plant was lower than the one observed within a bunch, which is consistent with Pagay and Cheng (2010). Bigger difference of berry mass were observed between blocks, in relation to variation in water and nitrogen offer from the soil. Bunches from blocks with low SWHC were characterized by lower mean values of berry mass, confirming the impact of water uptake conditions observed in previous studies (van Leeuwen and Seguin 1994, Santesteban and Royo 2006, Chaves et al. 2007). Reynolds and Wardle (1997) showed the existence of intra-vine variations of berry mass and composition related to the position of the bunch. Results obtained in our experimental conditions do not confirm this relationship. Variation in berry mass between bunches within a plant can be linked to the competition between the different parts of the vine for the carbon source (Coombe 1962). However, in our experimental conditions, leaf area to fruit weight ratio was consistently over 1m²/kg (data not shown). In these conditions carbon balance is unlikely to impact on berry mass.

Variability of impacting factors, studied at plant level, varied compared to bunch level. While $\delta^{13}\text{C}$ and YAN cv values increase, berry seed content cv values decrease. Berry seed content shows low variability among bunches from one single plant and among different plants. In contrast, YAN and $\delta^{13}\text{C}$ level varied between bunches of the same vine, and between bunches of different plants. Nevertheless, the inter-block variability of $\delta^{13}\text{C}$ and YAN is higher compared to that observed at the intra-block and the intra-plant scale. Because an intra-plant variability of $\delta^{13}\text{C}$ and YAN was shown among bunches, we investigated a possible effect of bunch position, by three multiple regression models, one for each factor. No consistent cane effect, nor shoot effect, nor effect of the position of the bunch on the shoot was evidenced. Hence, the YAN and $\delta^{13}\text{C}$ variability among bunches of a single plant need further investigation.

In order to understand the effect of each impacting factor on berry mass, several regressions were performed, plant by plant. Within a plant, berry mass was never related to $\delta^{13}\text{C}$. None of studied relationships were significant. However, a global relationship between these variables when several vines are taken into consideration allowed to obtain a significant *p-value*. The relationships between vine water uptake conditions and berry mass observed in previous studies was confirmed (van Leeuwen and Seguin 1994). However, the R² value was not so high, because affected by the

particularly behavior of G1.2 and G1.3 vines, which showed high berry mass values, despite restricting water uptake conditions. This fact could be linked to the higher nitrogen status characterizing these plants. Under field conditions, the interactions between factors impacting berry growth and development make the study of berry size variability challenging.

No consistent results were observed either when berry mass was plotted against YAN level bunch by bunch, within a plant, nor when the results of nine vines were pooled together. In contrast, significant relationships were observed between berry seed number and berry mass. However, due to the low variability among bunches within a plant, the R^2 values were lower than those observed inside a bunch.

The results obtained by these correlations were confirmed by a multiple regression models, performed in order to hierarchize the degree of impact of the three factors on berry mass, within a plant. Despite a lesser variability and lower level of relationships with berry mass, berry seed content is still the driving factor of berry mass variability within a plant. Nitrogen status, nor water status had a significant effect, although the nitrogen status can be considered as significant at 10% level of significance. The absence of a significant effect of nitrogen and water status within a plant supports the hypothesis that all bunches of a vine are submitted to the same water and nitrogen uptake conditions.

The study of relationships between berry mass and major berry compounds at plant scale did not show consistent results. Despite the observation of some variability of bunch composition, sugar concentration, total acidity, pH and malic acid concentration are not considerably affected by berry mass at plant scale. Nevertheless, when the global distribution of points measured on several grapevines is plotted, a statistically significant trend appears between each quality parameter and berry mass. In this study, bunches characterized by a small mean value of berry mass showed higher values of sugar concentration and pH and lower values of total acidity and malic acid concentration. We observed that when the variability of berry mass is driven by the variability of seed number, it does not affect the berry composition (or only marginally so). In contrast, when these relationships are observed in a global way (among vines), which also takes into account other impacting factors, like water and nitrogen status, berry mass has an impact on major quality parameters.

CONCLUSIONS

Intra-bunch berry mass variability is approximately similar among bunches from the same block but is higher compared to the one observed at the intra-plant level (bunch by bunch). Bigger differences of berry mass have been observed between blocks in relation to variation in water and nitrogen availability of the soil.

$\delta^{13}\text{C}$ and YAN variability, measured berry by berry, changes with the observation scale, increasing from intra-bunch to intra-parcel level, while intermediate values are been observed at intra-block level. Within a bunch, nor $\delta^{13}\text{C}$, nor YAN affect berry mass. As a result, we conclude that all berries belonging to the same bunch are subjected to the same water and nitrogen uptake conditions, which reflect mainly the vine water and nitrogen status, related to the soil conditions of the block, and secondly the rooting depth of the studied vine. An integrated vintage effect was also observed. Conversely, intra-bunch berry seed content shows higher cv values, which are similar among plants from the same block or from different blocks and over the studied vintages. Confirming the results of previous studies, berry mass within a bunch was closely correlated with berry seed content. Thus, within a bunch, among factors considered in this study, berry seed content is the most important determinant of berry mass, while nor $\delta^{13}\text{C}$ nor YAN have a significant effect on berry size variability, affecting homogenously all berries of the same bunch. Average berry seed content is similar among bunches from one single plant, while average YAN and $\delta^{13}\text{C}$ values varied between bunches of the same vine. As a result, the intra-plant $\delta^{13}\text{C}$ and YAN variability and the intra-plant berry seed content variability are, respectively, higher and slower, than the ones observed at intra-bunch level. Nevertheless, the inter-block variability of $\delta^{13}\text{C}$ and YAN is higher compared to all other scales, reflecting the soil water and nitrogen availability of each block. Within a plant, berry mass was never related to $\delta^{13}\text{C}$, nor to YAN. In contrast, average berry mass of a bunch is significantly affected by the average berry seed number. Thus berry seed number is the driving factor of the berry mass variability also within a plant. Because the absence of a significant effect of nitrogen and water status on berry mass at this scale, we suppose that all bunches of a plant are submitted to the same water and nitrogen uptake conditions. Nevertheless, the $\delta^{13}\text{C}$ and YAN variability observed among bunches of a single plant, which is not related to the bunch position, needs further investigation.

At bunch scale, the relationships between berry mass and sugar and malic acid concentration varied over the two years. Berry mass was rarely related to malic acid, while the number of significant

relationships with sugar concentration was higher. Nevertheless, these relationships have been observed only in one year of experimentation, where small berries showed slight higher sugar levels. Among compounds measured berry by berry within a bunch, anthocyanin concentration shows the larger number of significant correlations and the higher R^2 values. Over the two years, the anthocyanin concentration increases when berry mass decreases. This fact was particularly obvious in 2015 on bunches belonging to vines subjected to severe early water deficit stress. When all berries were pooled together, relationships between berry mass and sugar and anthocyanin concentration show that smaller berries are characterized by higher sugar and anthocyanin levels. These results seem to confirm that a part of the berry size effect on grape composition is an indirect effect, related to the factors responsible for berry size variability. In other words, the way in which berry size is reduced is more important than the berry size itself with regard to grape composition. At the intra-vine level, the influence of berry mass on sugar, total acidity, pH and malic acid concentration is not consistent. Conversely, consistent trends between each quality parameter and berry mass appear when global relationships are performed (independently from the bunch origin). At small mean values of berry mass correspond high values of sugar concentration and pH and low values of total acidity and malic acid concentration.

The results of this work indicate that when the variability of berry mass is driven by the variability of seed number (intra-bunch or intra-plant level), the berry mass effect on grape composition seems to be only marginal. When this relationship is studied in a global way (intra- and inter-parcel level), which takes into account also of the other external impacting factors, like water and nitrogen status, berry mass has a bigger impact on major quality parameters. Thus, when the variability of berry mass is driven by external factors, varying at large scales and from one vintage to another, the berry mass effect on grape composition is more obvious. This would mean that, for a given vintage, small and large berries, produced from a single parcel with homogenous water and/or nitrogen conditions, tend to have similar enological profiles. In contrast, in the parcels where the spatial variability of water and/or nitrogen availability is high, small berries are likely to have significant compositional differences compared to large berries. This is more likely to happen in a dry vintage.

Chapter IV
The impact of berry mass on
wine quality

ABSTRACT

The grape berry is a fruit extremely rich in secondary metabolites, affecting wine colour, taste and flavour. Berry size has always been considered as a major quality factor in wine production. Wines produced from smaller berries are supposed to be higher in quality. This fact is based on the assumption that berry is a sphere. Hence, as its radius increases, the skin to flesh ratio decreases. Consequently, the concentration of skin solutes would be increasingly diluted with increasing berry size. However, several studies showed that the amount of skin increases in proportion to the berry size. The study of the differences in berry composition when comparing berries strictly on size leads to contrasting conclusions among researchers. Final berry size is highly variable at multiple levels. This variation is an expression of the integrated effect of many impacting factors. In order to reduce this variability and to optimize wine quality attributes, Amos Industrie developed Calibaie[®], a machine able to sort berries according to their size. Similarly to grape berry composition, there is no consensus among researchers on whether smaller berries make superior wines. This result could be due to the fact that berry composition is dependent on physiological processes other than growth.

The aim of this study was to compare the profile of wines made from berries belonging to different size classes (large and small), sorted by Calibaie[®]. The work was carried out over two seasons, on four experimental parcels located in the Saint-Emilion region, planted with Cabernet franc and Merlot on two soil types: a sandy soil and a gravelly soil. At harvest, physical analyses of berries were performed. Following, berries mechanically harvested, and sorted in “small” and “large” categories by Calibaie[®], were vinified separately. Grape juice and wine were analyzed before and after fermentations, respectively. Wine phenolic and aroma profiles were investigated after several months of aging. A sensory analysis was also performed.

Berry tissue masses increase proportionally with berry growth. Thus, little variation in skin to flesh ratio were observed when plotted against berry size. Nevertheless, grape juice and wine composition seemed to be impacted by berry size. Grape juice extracted from small berry show higher sugar and lower malic acid concentration. Most of the wines produced from small berries showed higher flavanol, anthocyanin and lactone levels. The absence of a linear relationship between berry size, wine quality and sensory attributes can be the result of the fact that considered impacting factors both influence berry size and berry composition and their effect on berry composition can be direct, or indirect, mediated through berry size.

INTRODUCTION

The grape berry is a non-climacteric fruit extremely rich in secondary metabolites, such as anthocyanins, carotenoids, norisoprenoids, tannins, terpenes, and other volatile organic compounds. These metabolites are highly important in wine production as they affect wine quality by determining its colour, taste and flavor (Lund et al. 2008).

Beyond sugar accumulation, the major determinants a grape quality are the secondary metabolites. In red grape varieties, phenolic compounds are probably the most obvious important compounds (Kennedy 2002, Chira et al. 2011). They are responsible for the taste sensations such as bitterness (Robichaud and Noble 1990), tactile sensations such as astringency (Arnold et al. 1980), and visual sensations such as colour saturation and hue (Somers 1978). Grape phenolic substances are not distributed homogeneously in the berry (Gil et al. 2015). Anthocyanins, directly related to red wine color (Glories 1984), accumulate in grape skins (and in the mesocarp of the teinturier cultivars) as glycosylated monomers of malvidin, cyanidin, petunidin, peonidin, delphinidin, and pelargonidin (Cheynier 2006). Proanthocyanidins, also referred to as condensed tannins, occur in seeds, skins, and stem/rachis as oligomers and polymers of four flavan-3-ol subunits: (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, and (-)-epicatechin-3-O-gallate (Souquet et al. 1996). They are thought to make an important contribution to colour stabilization by combining with the anthocyanins (Mazza and Francis 1995). Additionally, condensed tannins are responsible of astringency perception (Kennedy et al. 2006, Chira et al. 2009).

Concerning the flavor of grapes and must it is well accepted that grape maturation give the grape particular flavor associated with specific chemical compounds. In short, from veraison to maturity a complex and deep modification of the volatile component of grapes and musts takes place, impacting terpenoids, norisoprenoids, aromatic and aliphatic alcohols and carbonyls as well as methoxypyrazines (Dunlevy et al. 2009). Moreover, it has been shown that the accumulation of free and glycosylated aroma compounds and precursors during grape ripening is compound dependent (Yuan and Qian 2016). For example, β -Damascenone, a C13 norisoprenoid described as having a “fruity flowery”, “stewed apple” aroma arises from carotenoid degradation during grape ripening (Razungles et al. 1993). Predominantly present in grapes as glycosidically bound precursors, those compounds could be released in wine by enzyme and acid hydrolysis (Sefton et al. 2011).

Grape berry is composed by three major types of tissue: flesh, skin, and seed (Kennedy 2002), which differ considerably in composition, and accordingly contribute differently to overall wine composition (Gonzalez-Barreiro et al. 2015). Due to this fact, berry size has always been considered as a factor of quality assessment of grape berries used in wine production. Final berry size is highly variable at multiple levels: (i) between vines within the vineyards, (ii) between bunches within the vine and (iii) between berries within the bunch (Coombe and Iland 2004, Pagay and Cheng 2010, Dai et al. 2011, Pisciotta et al. 2013). This variation is an expression of the integrated effect of many biotic and abiotic factors (Fernandez et al. 2006, Dai et al. 2011). It may result from parcel heterogeneity, due to spatial variability of soil characteristics, graft combination, plant material quality, node number per shoot, shoot number per cane, bunch number per plant, bunch position, etc. (Di Lorenzo et al. 2007, Pisciotta et al. 2013). Variation in berry size (and hence diameter) is greatest in early berry developmental stages and declines as berries resynchronize their expansion during the second period of growth (Gray 2002, Pagay and Cheng 2010). This observation implies that the source of variation impacts berry size and mass at an early stage (Coombe 1976). Even when all vineyard management practices are uniform and properly executed, it still extremely difficult to obtain uniform berry diameter and composition under field conditions (Pisciotta et al. 2013).

Different berry sizes, as well as the ripeness level at which grapes are harvested, can affect mass:volume ratios at harvest as well as during maceration and alcoholic fermentation (Barbagallo et al. 2011, Guidoni and Hunter 2012). Hence, in order to optimize wine quality attributes, in recent years several companies have developed berry sorting machines, permitting the reduction of variability in berry physical and chemical composition, induced by intra-vine and intra-bunch variations in berry mass. In 2011, Amos Industrie (21200 BEAUNE, France) developed Calibaie® a machine able to sort berries post-harvest according to their size. In fact, one of the most widely accepted ideas in winemaking is that wines produced from smaller berries are higher in quality (Singleton 1972, Matthews and Anderson 1988, Kennedy 2002). However, this principle is based primarily on intuitions, assumptions and traditional beliefs, rather than on scientific evidence (Roby and Matthews 2004, Roby et al. 2004, Walker et al. 2005, Matthews and Nuzzo 2007). Matthews (2015) refers to the “*BBB myth*” (Big Bad Berry), affirming that “large diameter fruit would have a greater solvent to solute ratio as a result of the lower surface to volume ratio

compared to smaller fruit” is a widespread belief. However, in previously published researches, Matthews was a believer himself in this myth (Matthews and Anderson 1989).

The *BBB myth* is based on the geometric assumption that grape berry is a sphere. Hence, according to surface-volume relationship of a sphere, as the berry radius increases, the skin to flesh ratio decreases. Consequently, the concentration of skin solutes would be increasingly diluted with increasing berry size (Matthews and Nuzzo 2007). Implicit in this assumption is that the amount of skin tissue remains fixed as the berry grows. However, several studies have shown that the amount of skin increases approximately in proportion to the size of the berry (Roby and Matthews 2004, Walker et al. 2005, Barbagallo et al. 2011). Thus, although the relative amount of berry tissues can vary depending on variety and environment (Keller 2015), there may be little variation in the skin to flesh ratio among fruit developing under the same conditions (Matthews 2015). However, some environmental conditions, such as water deficit, alter that general relationship. Several studies reported that water deficits inhibit more flesh growth than skin growth, increasing, as a result, the skin-to flesh ratio (Roby and Matthews 2004, Bucchetti et al. 2011). This effect can result in higher concentration of skin solutes. Finally, the Matthews *BBB myth* assumes also that there is a fixed amount of primary and secondary metabolites in each berry and that the variable in berry size is the water. Consequently, this fixed amount of skin solutes is increasingly diluted by the flesh of ever bigger berries. Effectively, if these assumptions are true, smaller berries will have higher concentrations of solutes.

The study of the differences in berry composition when comparing berries strictly on size leads to contrasting conclusions among researchers, because the interactions between factors impacting berry growth and development under field condition. Some early studies reported that the sugar concentration is higher in smaller berries (Scienza et al. 1978, Carwthon and Morris 1982). In contrast, Glynn (2003), measuring the sugar content of Cabernet-Sauvignon and Chardonnay berry by berry, did not find a relationship between °Brix and berry size. Similar results were obtained by Walker et al. (2005) on Shiraz berries, while Roby et al. (2004) showed that, in Cabernet-Sauvignon, °Brix decreased with increasing berry size.

The relationship between berry size and total amount of solutes per berry seems to be clearer. Coombe (1987) and Roby and Matthews (2004) showed that the amount of sugar per berry increases linearly with berry size. Few studies addressed the question of whether smaller berries

are less acidic. In one of these, carried out on Shiraz berries separated into size categories, the acid concentration of grape was not related to size (Walker et al. 2005).

Similarly to grape berry composition, there is no consensus among researchers on whether smaller berries make superior wines. Gil et al. (2015) demonstrated that smaller grapes produced wines of deeper colour and that size is inversely correlated with the concentration of phenolics, such as anthocyanins and stilbenes. In contrast, comparing wines from "small" and "large" berries, Walker et al. (2005) came to the conclusion that smaller berries do not produced superior wines. Other researchers found that there is no simple linear relationship between grape composition and wine quality (Hunter et al. 1991, Johnstone et al. 1995). This result could be due to the fact that berry composition is dependent on physiological processes other than growth (Matthews 2015).

It's often assumed by grape growers and wine consumers that the quality of a red wine increases with its aging potential. The latter is mainly related to the quantity and quality of phenolic compounds. Hence, if it's true that small berries increase the concentration of major secondary metabolites accumulated in the skin, then they could be defined as "*better*" to make a high quality red wine. However, it is important to define quality. In the "*Quality Handbook*" (1999), Dr M. Juran defines the quality as "*fitness to use*". So, fitting this sentence to quality concept in viticulture, it would mean that it not possible to define grape quality without referring to the wine profile that will be produced.

The aim of this study was to compare the profile of wines made from berries belonging to different size classes (large and small), sorted by a specific machine, the Calibaie®. The final objective was to investigate the possibility for wine producers to obtain two different wines, in relation to their production goals, from berries grown on one single parcel and separated on size categories by Calibaie® machine.

MATERIAL AND METHODS

Experimental vineyards and plant material

A preliminary study was carried out in the 2012 growing season in order to compare the profile of wines made from berries belonging to two size categories sorted out by Calibaie machine. The satisfactory results obtained during this first experience allowed the project partners to continue to perform the same research over the two following years (2013-2014). However, the particularly bad weather conditions of the 2013 vintage did not allow to reach the goals that were set. For this reason, we decided to carry out some supplementary analyses in the 2015 vintage. During each experimental season, the study was carried out on four parcels of a commercial vineyard located in the Saint Emilion region (44°55'37.42''N; 0°08'59.71''O; 5m; 33330 Vignonet, Aquitaine, France). The selected parcels were dry-farmed and planted on two different soil types. The soils are classified according to the French “*Référentiel Pédologique*” (Baize and Girard, 1995). The first one, is a sandy soil (ARENOSOL Redoxique), characterized by a water table within the reach of the roots and, as a result, by a high Soil Water Holding Capacity (SWHC). This soil is particularly homogenous within each parcel. Conversely, this homogeneity was not observed on the second soil, which is a gravelly/sandy soil (PEYROSOL), characterized by a low SWHC due to its high gravel content. On each soil, two of most important varieties of the Bordeaux region were planted: *Vitis vinifera* L. cv Cabernet franc and *Vitis vinifera* L. Merlot. In the present work, parcel are coded as MNG, MNS, CFG and CFS (MN= Merlot noir, G: gravelly soil; S: sandy soil). Vines from MNS and CFG are grafted onto 101-14 MGt rootstock, while CFS and MNG are grafted onto 196-17C and 3309C rootstock, respectively. Despite the possible impact of the rootstock on berry size, in the present work this factor was not considered, because all comparisons have been performed at an intra-parcel level, rather than at an inter-parcel level.

Physical analysis of grape berry

In order to investigate relationships between berry size and berry tissue masses at harvest of the 2014 and 2015 seasons, a sample of 300 berries, mechanically harvested, was randomly collected, before the berry sorting in two different berry size categories by Calibaie®. Each berry was weighed and the diameter was recorded. Then, skin was removed from berry by first making a small cut with a razor blade, following carefully peeling it from the berry. Skin was weighed, while peeled berries were sliced in halves and seeds were then carefully separated from berry flesh,

counted and weighted. In this study, flesh mass was determined as difference between total berry fresh mass and skin and seeds masses.

The theoretical berry volume (v), derived from the diameter measurement, and the theoretical surface (S) of the skin was calculated considering that the berries were perfect spheres. Following, skin surface:berry volume ratio was determined.

Grape sorting

Merlot and Cabernet franc berries, mechanically harvested on the four experimental parcels, were sorted according to their diameter (hence volume) by a specific machine (Calibaie®, Amos Industrie, 21200 BEAUNE, France). Calibaie consists of a chain-driven roller table with parallel rollers which rotate towards the front of the machine. Carried by the moving rollers, the berries are separated and spread out across the width of the machine. They continue to roll until they reach a gap wide enough to let them through without being compressed or crushed. Grapes larger than the size set are carried to the end of the roller table and fall into a tray, pump or lift. It is possible to adjust the machine according to the desired separation in berry size. In this study we chose to adjust the machine in order to obtain a similar amount of small and large berry per time unit. After sorting, from each size category (small and large), as well as from unsorted berries (control), 30 kg of grapevine were randomly sampled and distributed in three groups of 10 kg each. A sample of 200 berries was collected from each batch in order to measure the physical parameters of berries, such as mass and diameter.

Micro-scale winemaking procedure and fermentation

Following separation, berries were manually crushed, 50 mg/hL of SO₂ was added and following they were placed in 10-L tanks. A sample of juice was collected (50mL), centrifuged and the supernatant was analyzed with Fourier-Transform Infrared spectrometer (FTIR, WineScan FOSS®, FRANCE, 92000 Nanterre; Destrac et al. 2015), proving results for malic acid (g/L), total acidity (TA, g/L), pH and reducing sugars (RS, g/L). The following day, juices were inoculated with 20g/hL of selected yeast (*Sacharomyces cerevisiae*, ZYMAFLORE FX10, Laffort, 33072 Bordeaux, France). During fermentation at room temperature of 26 °C, density of the juice and temperature were recorded daily, after a mechanical punch-down of the cap. Fermentation was considered as completed when the residual sugar of all samples was below 9 g/L. At that stage,

samples were pressed with a pneumatic press (Bellot SA, 33170 Gradignan, France) at 2 atm for 2 minutes. Five liters of free-run wines for each sample were finally bottled. A sample of 50 mL of wine was collected for the standard analysis, using WineScan FOSS[®]. Wines were inoculated with 1g/hL of selected bacteria (*Oenococcus oeni*, LACTOENOS[®] SB3 Direct, Laffort, 33072 Bordeaux, France). During malolactic fermentation at a temperature of 26 °C, wines were analyzed once a week. At the end of this second fermentation, a sample of each wine was analyzed for standard parameters and then wines were finally bottled. After 12 months of aging, the three replicates of each berry size category from each experimental parcel were assembled in order to create the most representative sample for each treatment.

Wine phenol analysis

In 2014, wines were analyzed at the end of malolactic fermentation, while in 2015 the wine phenol analysis were carried out after 12 months of aging.

Monomeric and dimeric flavan-3-ols concentration of wines was estimated by HPLC-UV-fluo analysis, according to Curko et al. (2014). Each wine sample, analyzed in duplicate, was filtered (0.45 µm) and injected directly. The equipment used for HPLC analysis consisted of a Thermo-Finnigan UV-vis detector (Surveyor PDA Plus), a Thermo-Finnigan fluorescence detector (Surveyor FL Plus Detector), a Thermo-Finnigan autosampler (Surveyor autosampler Plus) and a Thermo-Finnigan quaternary pump (Surveyor MS pump Plus) coupled to Xcalibur and ChromQuest softwares for UV-vis and fluorescence data treatment, respectively. Separation was performed on a reversed-phase LiChrospher 100 RP18 column. The mobile phases were 1% (v/v) aqueous formic acid (solvent A) and 1% (v/v) formic acid in acetonitrile (solvent B). The binary elution system was as follow: from 0 to 3% B in 3 min, from 3 to 5% B in 7 min, stay at 5% B for 4 min, from 5 to 7% B in 6 min, from 7 to 10% B in 2 min, stay at 10% B for 5 min, from 10 to 12% B in 5 min, from 12 to 14% B in 2 min, from 14 to 25% B in 11 min, from 25 to 100% B in 1 min, and remain at 100% B for 5 min. Flow rate was set at 1 mL/min, UV-vis detection wavelength at 280 nm and fluorescence detection, at 280 and 320 nm, respectively, for excitation and emission wavelengths. Identification and assignation of each compound were performed by comparing their retention times and UV spectra to authentic standards. Quantification of the flavan-3-ol monomers and dimer is expressed in mg/l.

Total proanthocyanidin content was estimated by spectrophotometer through the Bate–Smith reaction (Ribereau-Gayon et al. 1966), which is based on the transformation of proanthocyanidins in colored anthocyanidins by heating at 100 °C in acid conditions. Absorbance was measured at 550 nm with a Varian Cary 300 Bio UV–vis spectrophotometer. Each determination was performed in triplicate, and the results were expressed in grams of proanthocyanidins per liter of red wine.

Anthocyanin content and composition of wines were estimated by HPLC-UV-fluo analysis, according to Curko et al. (2014). Each wine sample, analyzed in duplicate, was filtered (0.45 µm) and injected directly. Analysis was performed on a Thermo-Finnigan Accela HPLC system consisting of an autosampler (Accela autosampler), a pump (Accela 600 Pump), a diode array detector (Accela PDA Detector) coupled to a Finnigan Xcalibur data system. Separation was performed on a reversed phase Agilent Nucleosil C18 (250 mm x 4 mm, 5 µm) column. A gradient consisting of water/formic acid (99:5, v/v) (solvent A) and acetonitrile/formic acid (99:5, v/v) (solvent B) was applied at a flow rate of 1 ml/min as follows: 10–35% B linear from 0–25 min, 35–100% B linear from 25–26 min, 100% B isocratic from 26–28 min, 100–10% B linear from 28–29 min, with the re-equilibration of the column from 29–35 min under the initial gradient conditions. Detection was conducted at 520 nm and the concentration of each anthocyanin was expressed as malvidin-3-*O*-glucoside equivalent using a calibration curve. The total anthocyanin concentration was calculated as the sum of the concentrations of the free and derivative forms of anthocyanins and also using the SO₂ bleaching method (Ribereau-Gayon and Stonestreet 1965). In the latter case, all analyses were performed in triplicate.

Total soluble polyphenols were measured by spectrophotometer in accordance with the Folin–Ciocalteu method (Singleton and Rossi 1965). The absorbance at 765 nm was measured using a Varian Cary 300 Bio UV–vis spectrophotometer, using distilled water as a blank sample. Gallic acid (0 to 160 mg/L) was used as a standard for calibration, and the phenolic content results were expressed as milligrams of equivalent gallic acid per liter of red wine.

Wine aroma analysis

In 2014 wine aroma analysis was performed after 24 months of aging, while in 2015, wines were analyzed after 12 months of aging. Wine aroma compound concentration was estimated according to methods described in detail by Allamy (2015).

- *Liquid-liquid extraction.* A sample volume of 100 mL was spiked with 100 μ L octan-3-ol (EtOH, 100 mg/L) as internal standard. Wines were extracted three times with 10, 5, 5 mL CH_2Cl_2 (magnetic stirring: 10, 5, 5 min; 750 rpm) in a 250 mL amber flask. The three organic phases obtained were blended, dried over anhydrous sodium sulfate, and concentrated to 0.5 mL under a nitrogen flow (100 mL/L).
- *Gas chromatography coupled to olfactometry and mass spectrometry (GC-O-MS).* The Trace GC Ultra (Thermo Fisher, Waltham, MA, USA) was coupled with a DSQII mass spectrometer (Thermo Fisher, Waltham, MA, USA) functioning in EI mode. 1 μ L of sample extract was injected thanks to an automatic sampler, (AI/AS 3000, Thermo Scientific, Illkirch, France) in a splitless PTV injector (150 $^{\circ}\text{C}$, purge time: 1 min, purge flow: 50 mL/min) onto a BP20 capillary column (SGE, France, 50 m, 0.22 mm i.d., 0.25 μm film thickness). The program temperature was as follows: 45 $^{\circ}\text{C}$ for 1 min, increasing by 3 $^{\circ}\text{C}/\text{min}$ to 230 $^{\circ}\text{C}$, followed to a 20 min isotherm. The carrier gas was helium N 60 (Linde gas, France) with a constant flow rate of 1 mL/min. Source parameters were optimized as follows: source temperature, electron energy and emission current were set at 210 $^{\circ}\text{C}$, 70 eV and 30 μA , respectively. PFTBA (Perfluorotri-*n*-butylamine) was used for mass calibration.

The estimation of the volatile compounds was performed with SIM (Selected Ion Monitoring) modality, selecting the specific ions for each molecule. The dosage was carried out measuring the ratio between the characteristic ion amount for each investigated molecule and the characteristic ion amount of the standard.

Sensory analysis

Sensory analysis of wines were performed in 2014 and 2015, after 12 months of aging, by a panel of 18 and 23 tasters, respectively, from different units of Bordeaux University, selected according to their experience. The evaluation of wines was carried out at room temperature (18 ± 1 $^{\circ}\text{C}$) in individual booths under daylight lighting. Around 20 mL of wine were presented in standard tasting glasses, which was identified by three random codes. Sensory analysis comprised visual (colour intensity), orthonasal (intensity, vegetal, fruitiness) and retronasal feel (body, acidity, astringency). An unmarked line scale was used for the scoring of each parameter on the tasting sheets. Finally, judges classed wine according to quality. A randomized number was attributed to each sample and no specific order was followed when presenting the wines to the tasting panel.

Statistical analysis

Data were analysed using R software (R development Core Team 2015, version 3.2.3). The distribution of berries sorted by Calibaie according to their diameter was estimated by a Kernel Density Estimation (KDE), which is a non-parametric way to measure the probability density function of a random variable. The relationships between investigated variables were determined by linear regression analysis. The coefficient of determination (R^2) for each relationship was reported if statistically significant. Analysis of variance, followed by the Tukey test ($p < 0.05$), was performed to compare the compositional characteristics of berries and wines belonging to different berry size categories.

RESULTS

Preliminary studies

The present work has started in 2012 with a preliminary study carried out on parcels of Merlot and Cabernet franc planted on the gravelly soil (MNG and CFG). The goals were to investigate the efficiency of Calibaie[®] machine to sort mechanically harvested berries and to compare the wine composition made from berries belonging to different berry size categories (small and large). Wines, elaborated in micro-scale, were analysed after malolactic fermentation for major phenolic compounds. These first results showed that wines made by larger and smaller berries were characterized by different phenolic profiles. Both Merlot and Cabernet franc wines made from smaller berries showed higher concentrations of monomer (C: (+)-catechin and EC: (-)-epicatechin) and dimer (B1: [(-)-epicatechin-(4b-8)-(+)-catechin], B2: [(-)-epicatechin-(4b-8)-(-)-epicatechin], B3: [(+)-catechin-(4a-8)-(+)-catechin] and B4: [(+)-catechin-(4a-8)-(-)-epicatechin]) flavan-3-ols compared to wines from larger berries and control. The latter showed intermediate values of each metabolite (table 1).

Total tannin concentration (g/L) showed the same tendency (data not shown). Similarly, differences between wines were observed concerning the anthocyanin profiles. Results reported in table 2 show that the levels of each mono-glucoside anthocyanin and those of anthocyanins derivatives are higher in wines from smaller berries, compared to other wines. Once again, larger berry produced wines with lesser levels of anthocyanin. This was observed on both varieties. Moreover, similar results were obtained when the total anthocyanin concentration (mg/L) of wines was analysed (data not shown).

Table 1 - Effect of berry size on monomeric and oligomeric flavan-3-ol concentrations (mg/L) of wines in 2012

	C	EC	B1	B2	B3	B4	Total
CFG							
Control	18.01 ± 1.42	19.91 ± 2.11	4.59 ± 0.24	0.35 ± 0.54	8.70 ± 0.49	4.40 ± 0.05	55.97 ± 6.88
Large	15.62 ± 1.82	15.16 ± 1.36	3.13 ± 0.13	7.96 ± 0.26	7.02 ± 0.34	2.85 ± 0.04	51.74 ± 4.52
Small	19.30 ± 1.59	22.28 ± 1.19	5.30 ± 0.09	0.52 ± 0.52	9.51 ± 0.30	2.41 ± 0.03	59.33 ± 4.66
MNG							
Control	35.62 ± 1.42	46.83 ± 1.22	7.25 ± 0.26	17.06 ± 0.31	13.75 ± 0.16	7.42 ± 0.03	127.92 ± 4.29
Large	31.48 ± 1.82	42.43 ± 1.36	6.11 ± 0.06	13.51 ± 0.40	12.46 ± 0.33	6.66 ± 0.05	112.65 ± 4.96
Small	37.47 ± 0.58	48.47 ± 0.16	7.66 ± 0.01	19.42 ± 0.06	15.24 ± 0.16	8.02 ± 0.06	136.28 ± 1.33

Wines were analyzed at the end of malolactic fermentation. Results are expressed as mean ± standard deviation of three replicates.

C: (+)-catechin, **EC:** (-)-epicatechin-(4b-8)-(+)-catechin], **B1:** [(-)-epicatechin-(4b-8)-(-)-epicatechin], **B2:** [(+)-epicatechin-(4a-8)-(+)-catechin], **B4:** [(+)-catechin-(4a-8)-(-)-epicatechin]. **Total** was calculated adding up the concentration of individual compounds.

CFG: Cabernet franc on gravelly soil, **CFS:** Cabernet franc on sandy soil, **MNG:** Merlot on gravelly soil, **MNS:** Merlot on sandy soil

Table 2 - Effect of berry size on anthocyanin concentrations (mg/L) of wines in 2012

	Dp	Cy	Pt	Mv	Pn-ac	Mv-ac	Pn-coum	Mv-coum	Total
CFG									
Control	9.43 ± 0.22	0.59 ± 0.02	10.93 ± 0.66	6.81 ± 0.37	85.81 ± 5.84	3.12 ± 0.14	33.44 ± 1.34	0.68 ± 0.03	157.62 ± 7.09
Large	8.21 ± 0.32	0.56 ± 0.02	8.65 ± 0.52	5.00 ± 0.28	71.30 ± 4.85	2.28 ± 0.10	29.67 ± 1.19	0.38 ± 0.02	132.23 ± 5.95
Small	10.55 ± 0.53	0.36 ± 0.01	12.15 ± 0.73	7.24 ± 0.40	96.56 ± 6.57	2.65 ± 0.12	33.52 ± 1.34	1.06 ± 0.04	171.63 ± 7.72
MNG									
Control	15.43 ± 0.52	1.14 ± 0.03	17.01 ± 1.02	8.89 ± 0.49	9593 ± 6.52	2.71 ± 0.12	24.29 ± 0.97	1.39 ± 0.06	174.79 ± 7.87
Large	13.95 ± 0.77	1.03 ± 0.03	16.16 ± 0.97	9.44 ± 0.52	98.49 ± 6.70	3.49 ± 0.16	2.65 ± 0.11	1.53 ± 0.06	156.38 ± 7.04
Small	17.15 ± 0.02	1.40 ± 0.04	19.05 ± 1.14	11.09 ± 0.61	112.33 ± 7.64	3.96 ± 0.18	29.24 ± 1.17	1.04 ± 0.04	205.76 ± 9.26

Wines were analyzed at the end of malolactic fermentation. Results are expressed as mean ± standard deviation.

Dp: Delphinidine-3-O-glucoside, **Cy:** Cyanidine-3-O-glucoside; **Pt:** Pétunidine-3-O-glucoside, **Mv:** Malvidine-3-O-glucoside, **Pn-ac:** Peonidine-3-O-(6-acetyl)-glucoside, **Mv-ac:** Malvidine-3-O-(6-acetyl)-glucoside, **Pn-coum:** Peonidine-3-O-(6-p-coumaroyl)-glucoside, **Mv-coum:** Malvidine-3-O-(6-p-coumaroyl)-glucoside. **Total** was calculated adding up the concentration of each mono-glucoside and the acetyl and *p*-coumaroyl derivatives.

CFG: Cabernet franc on gravelly soil, **CFS:** Cabernet franc on sandy soil, **MNG:** Merlot on gravelly soil, **MNS:** Merlot on sandy soil

Berry fresh mass and tissue relationships

Most of the previous studies addressing the impact of berry size on grape and wine composition express this parameter using mass rather than diameter or volume, probably because it is easier to measure. However, because in this study berries were sorted by machine according to their diameter (hence their volume), we studied first of all the relationship between berry fresh mass and berry volume. This correlation was investigated during the 2014 season only. As expected, the two covariates are highly, positively and significantly correlated (figure 1). Thus, all following observations take into account berry volume rather than berry mass.

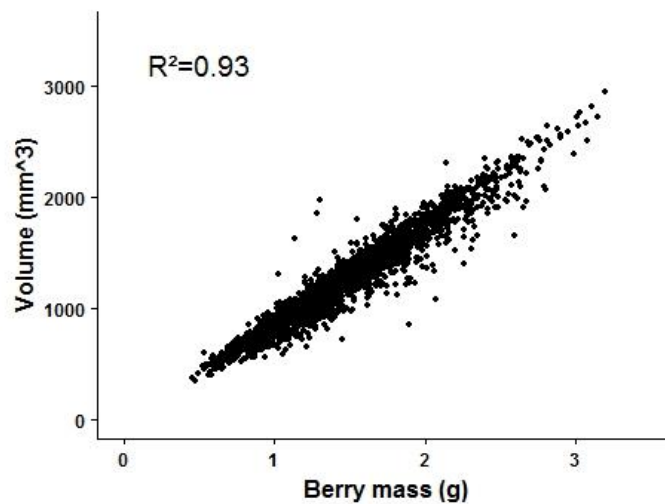


Figure 1 - Berry volume at harvest (calculated from diameter) for Cabernet franc vines as a function of measured berry mass (pooled data across all parcels of the 2014 growing season)

Study of relationships between berry size and berry tissue masses have shown different tendencies according to the measurement unit used to express the tissue mass (absolute mass, i.e. *g per berry*, or relative mass, i.e. *% per berry*).

Despite the fact that the coefficients of determination (R^2) of linear regressions, when significant, slightly varied among experimental years, the relationships between investigated variables showed similar trends. Total seed mass per berry was significantly and positively correlated with berry volume in both years (figure 2A and C).

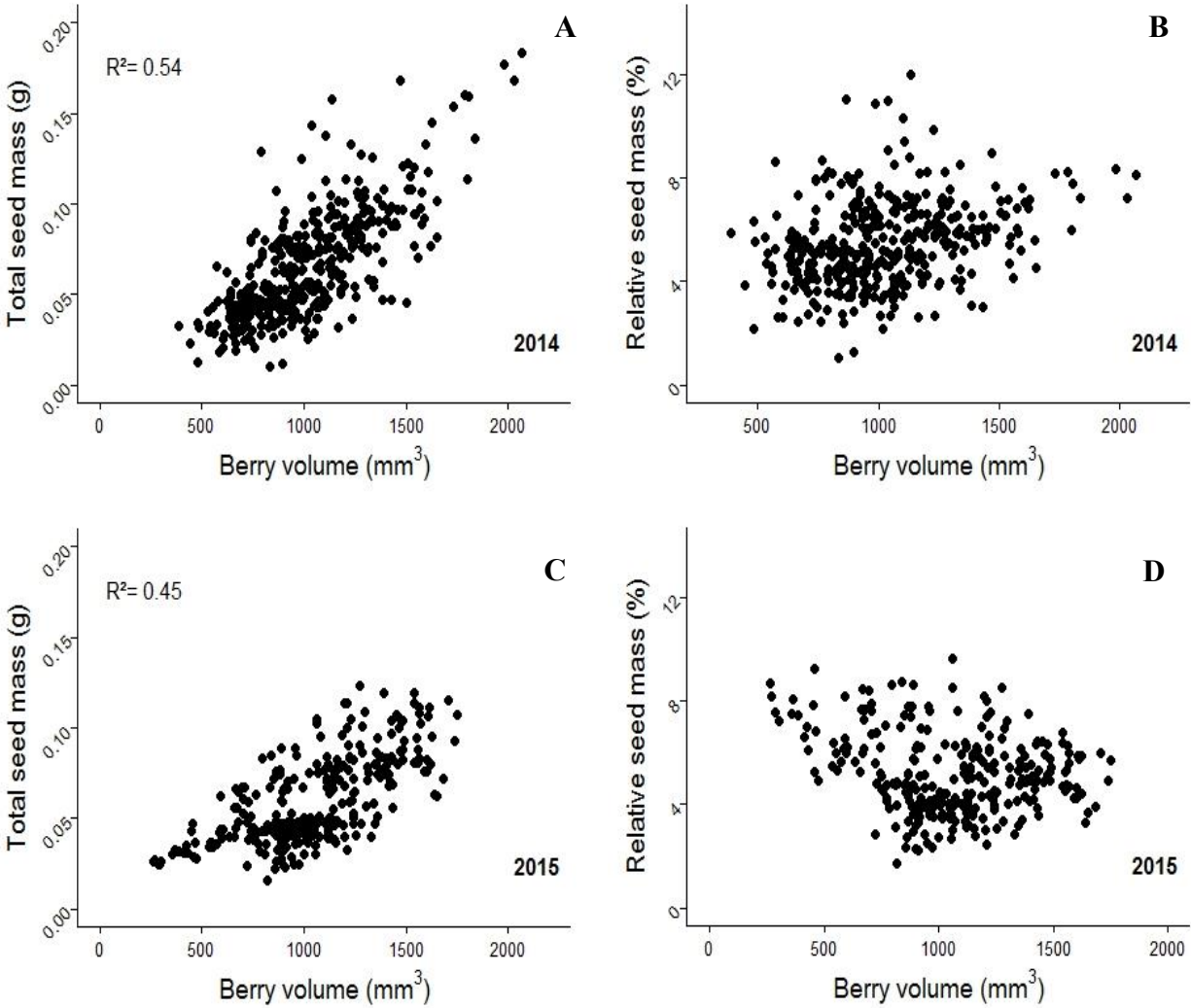


Figure 2 - Seed mass per berry (g) (**A** and **C**) and relative seed mass (%) (**B** and **D**) as a function of berry volume (calculated from diameter) of Cabernet franc vines during the 2014 and 2015 growing seasons.

The increase in total seed mass per berry was probably attributable to seed number per berry, which was bigger in large berries (data not shown). Nevertheless, the relative seed mass, representing around 6 to 10% of total berry mass for both years, doesn't change when berry volume increases (figure 2B and D).

Flesh weight was the main determinant tissue of berry volume and its variations. As a result, the linear regression between these variables was highly significant, showing a proportional increase of flesh mass to the increase of berry volume. This result was observed in both years (Figure 3A and C). Therefore, the relative mass of flesh per berry (%) remained unaffected by berry size (figure 3B and D).

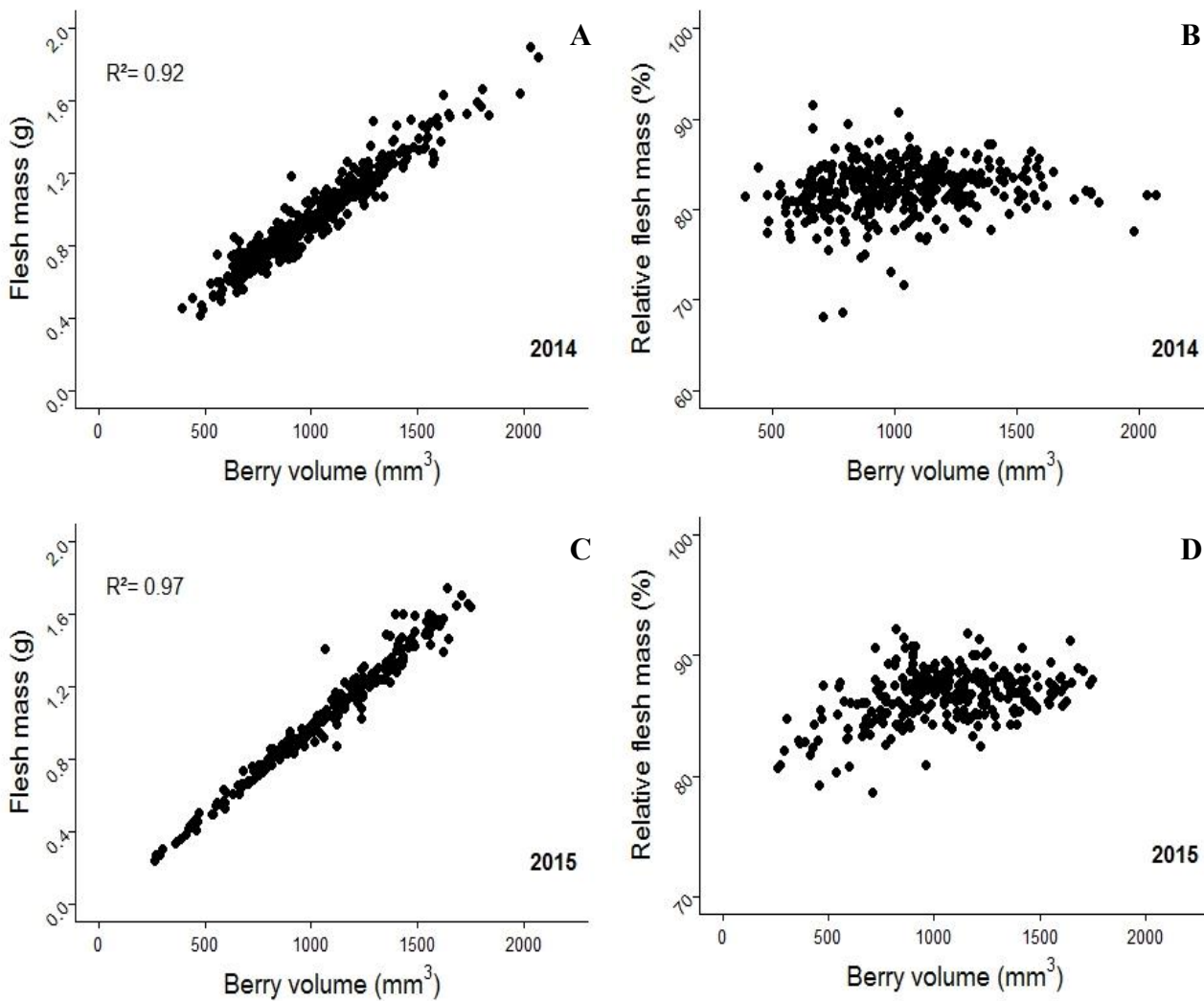


Figure 3 - Flesh mass per berry (g) (A and C) and relative flesh mass (%) (B and D) as a function of berry volume (calculated from diameter) of Cabernet franc vines during the 2014 and 2015 growing seasons

Skin mass per berry was not constant but was linearly related to berry volume in both season, increasing from smaller to the larger berries (figure 4A and C). As a result, the relative skin mass was almost constant among berries of different size (figure 4B and D).

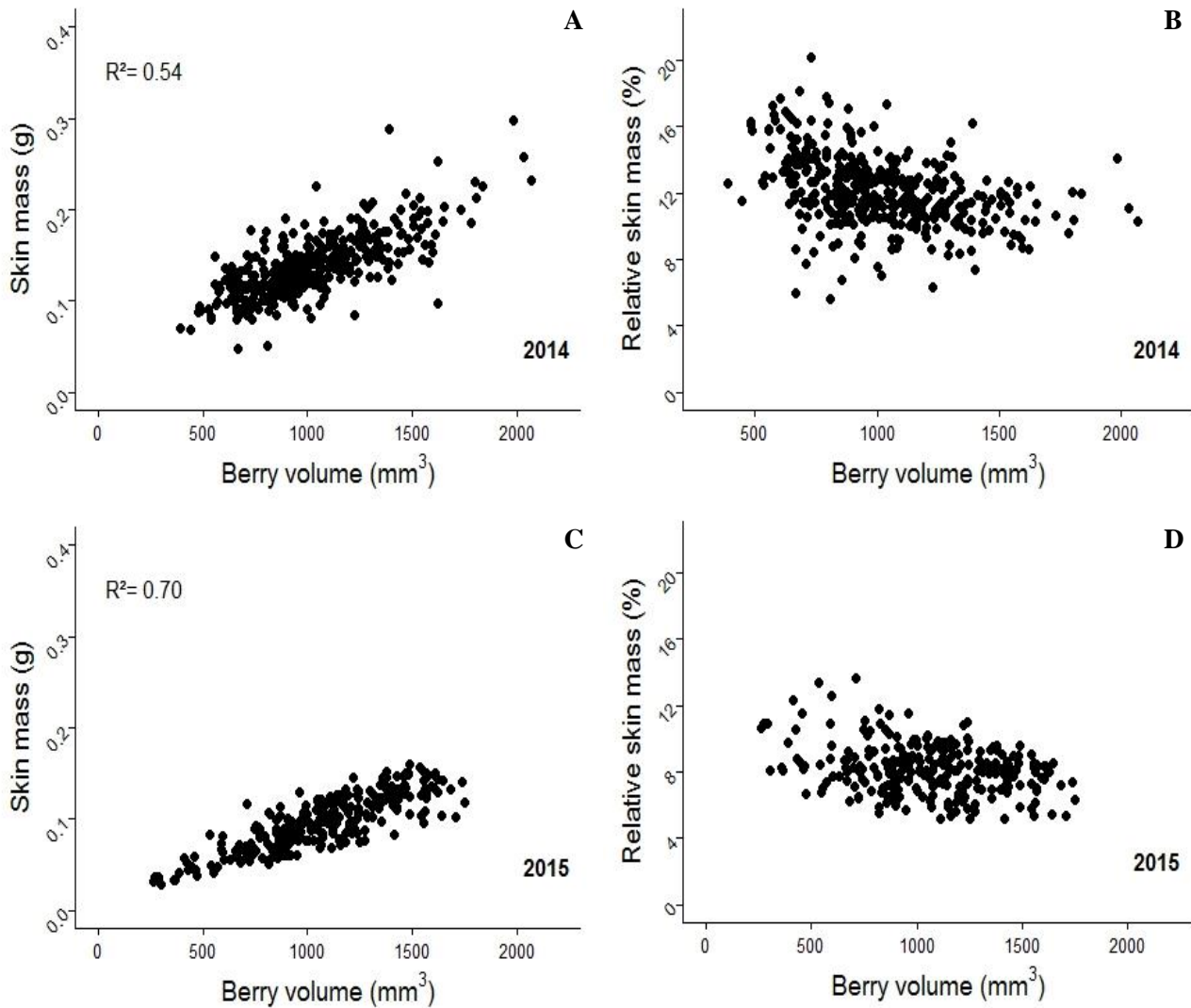


Figure 4 - Skin mass per berry (g) (A and C) and relative skin mass (%) (B and D) as a function of berry volume (calculated from diameter) of Cabernet franc vines during the 2014 and 2015 growing seasons

Although relative mass declined significantly with berry volume, the coefficients of determination were very small (0.15 and 0.07 in 2014 and 2015 respectively).

The skin to flesh mass ratio was not related to berry volume (linear regression not significant, $R^2=12$ in 2014 and $R^2 < 1$ in 2015) and remaining constant as berry size increased. The question if the berry surface (mathematically computed from berry diameter data) per gram of berry changed when the berry volume increases was also investigated. We found a significant and negative relationship between these two variables in both years. However, this relationship was not linear, becoming more and more flat as berry volume increase (figure 5).

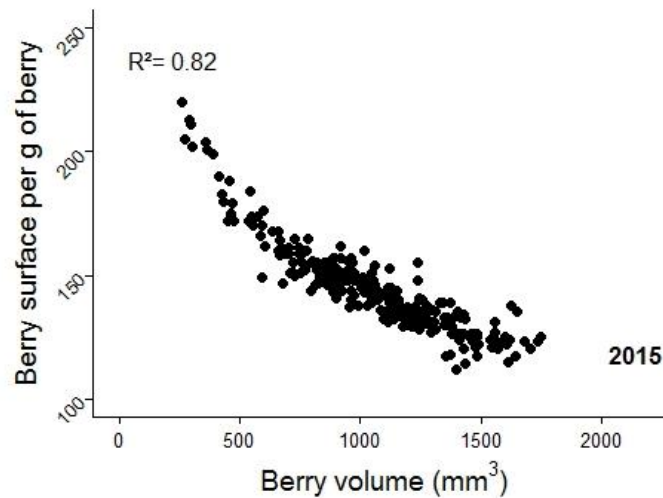


Figure 5 - Berry surface (mm^2) per g of berry plotted against berry volume (calculated from diameter) of Cabernet franc vines during the 2015 growing seasons

Grape sorting and grape juice composition

The berry sorting quality performed by the Calibaie machine was evaluated comparing the distribution of berries from each size category and from control according to their diameter (figure 6). Except for CFS in 2014 (figure 6D), where differences were less obvious, Calibaie was able to perform a satisfactory sorting of berries, according to their size, in two classes: small and large. This was observed in 2014 and in 2015. However, it remains possible to observe that, in most of cases, around one third of small berries is common to one third of large berries (superimposed part of the curves).

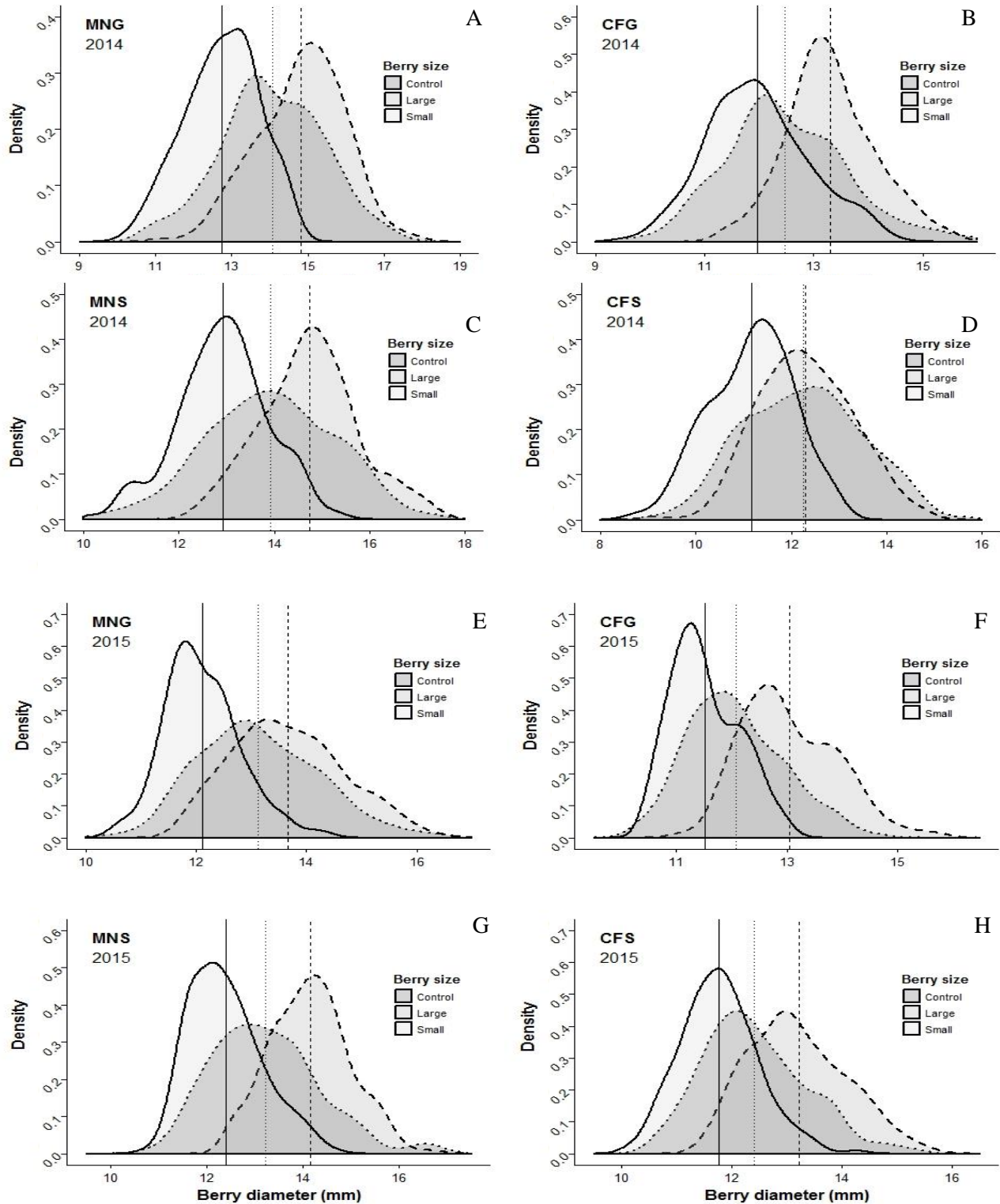


Figure 6 - Density distribution for berry diameter of berries sampled from large and small size categories and from control of each experimental parcel, in 2014 and 2015. **CFG:** Cabernet franc on gravelly soil, **CFS:** Cabernet franc on sandy soil, **MNG:** Merlot on gravelly soil, **MNS:** Merlot on sandy soil

Nevertheless, the study of berry physical parameters, such as berry mass, berry diameter and berry volume (calculated from diameter) confirms that berries belonging to the small category show lower values of each measured parameters, compared to berries from large category (table 3 and 4).

Table 3 - Berry mass, berry diameter and berry volume comparison for each berry size category in 2014

	Berry mass (g)	Berry diameter (mm)	Berry volume (mm ³)
CFG			
Control	1.22 ± 0.29 b	12.48 ± 1.11 b	1040 ± 282 b
Large	1.52 ± 0.26 c	13.31 ± 0.81 c	1247 ± 230 c
Small	1.09 ± 0.26 a	11.97 ± 0.93 a	914 ± 215 a
CFS			
Control	1.11 ± 0.30 b	12.26 ± 1.21 b	992 ± 292 b
Large	1.22 ± 0.29 b	12.30 ± 0.98 b	992 ± 237 b
Small	0.96 ± 0.24 a	11.17 ± 0.88 a	742 ± 170 a
MNG			
Control	1.72 ± 0.45 b	14.06 ± 1.32 b	1492 ± 413 b
Large	2.05 ± 0.44 c	14.83 ± 1.14 c	1735 ± 388 c
Small	1.33 ± 0.29 a	12.75 ± 0.97 a	1103 ± 245 a
MNS			
Control	1.68 ± 0.45 b	13.93 ± 1.32 b	1451 ± 404 b
Large	1.93 ± 0.39 c	14.75 ± 1.01 c	1702 ± 352 c
Small	1.33 ± 0.27 a	12.93 ± 0.94 a	1147 ± 246 a

Results are expressed as mean ± standard deviation. The differences between berry size categories were tested with a one-way ANOVA. Different letters in the same column, for each parcel, indicate statistically significant differences between berry size at P<0.05 by Tukey's HSD test. **CFG**: Cabernet franc on gravelly soil, **CFS**: Cabernet franc on sandy soil, **MNG**: Merlot on gravelly soil, **MNS**: Merlot on sandy soil.

These differences were statistically significant within each parcel and over the two experimental years. Berries from the control category show intermediate values, which were significantly different compared to small and large berries, except for CFS in 2014.

Table 4 - Berry mass, berry diameter and berry volume comparison for each berry size category in 2015

	Berry mass (g)	Berry diameter (mm)	Berry volume (mm ³)
CFG			
Control	0.99±0.31 b	12.07±0.86 b	935±205 b
Large	1.34±0.31 c	13.05±0.86 c	1177±238 c
Small	0.79±0.22 a	11.52±0.61 a	806±131 a
CFS			
Control	1.11±0.33 b	12.42±0.92 b	1018±234 b
Large	1.40±0.33 c	13.21±0.91 c	1224±260 c
Small	0.88±0.25 a	11.77±0.69 a	863±155 a
MNG			
Control	1.37±0.39 b	13.13±1.07 b	1208±302 b
Large	1.56±0.36 c	13.66±1.00 c	1355±301 c
Small	1.00±0.25 a	12.11±0.69 a	938±164 a
MNS			
Control	1.41±0.40 b	13.23±1.10 b	1237±324 b
Large	1.74±0.30 c	14.14±0.81 c	1496±260 c
Small	1.11±0.27 a	12.39±0.75 a	1007±188 a

Results are expressed as mean ± standard deviation. The differences between berry size categories were tested with a one-way ANOVA. Different letters in the same column, for each parcel, indicate statistically significant differences between berry size at P<0.05 by Tukey's HSD test. **CFG**: Cabernet franc on gravelly soil, **CFS**: Cabernet franc on sandy soil, **MNG**: Merlot on gravelly soil, **MNS**: Merlot on sandy soil.

Grape juice composition from each berry size category was estimated by standard analysis. Our results show that in 2014 juice from small berries are characterized by significant higher and lower level of sugar and malic acid concentration, respectively, compared to juice from large berries (table 5). The same results were obtained in 2015, except for CFS where, despite a similar tendency, differences between size categories were not statistically significant (table 6). Grape juice from control berries showed intermediate values, although differences were not always statistically significant. Differences concerning total acidity were less obvious among juices. In 2014, Cabernet franc small berries were characterized by lower levels of acidity compared to large berries, while no significant differences were observed in MNS. The same results were obtained in 2015. In contrast, a different behaviour over two the years was noted in the MNG parcel, where juice from small berries were characterized by significantly lower levels of acidity only in 2015.

Table 5 - Chemical composition of grape juice from different berry size categories in 2014

	Sugar (g/L)	Total acidity (g/L)	Malic acid (g/L)
CFG			
Control	234.13 ± 2.2 b	2.98 ± 0.04 a	1.36 ± 0.06 a
Large	227.13 ± 1.36 a	3.14 ± 0.02 b	1.77 ± 0.03 b
Small	238.80 ± 1.61 c	2.93 ± 0.08 a	1.35 ± 0.05 a
CFS			
Control	239.70 ± 0.75 b	3.02 ± 0.01 b	1.31 ± 0.04 a
Large	230.60 ± 1.21 a	3.14 ± 0.02 c	1.61 ± 0.06 b
Small	245.80 ± 0.95 c	2.95 ± 0.01 a	1.25 ± 0.02 a
MNG			
Control	216.27 ± 0.21 b	3.40 ± 0.00 a	2.23 ± 0.06 b
Large	213.40 ± 0.52 a	3.37 ± 0.06 a	2.40 ± 0.00 c
Small	220.27 ± 1.01 c	3.37 ± 0.06 a	2.10 ± 0.00 a
MNS			
Control	232.23 ± 1.22 b	3.59 ± 0.03 a	2.09 ± 0.04 b
Large	226.97 ± 0.35 a	3.50 ± 0.04 a	2.29 ± 0.04 c
Small	237.07 ± 0.06 c	3.57 ± 0.05 a	1.80 ± 0.01 a

Results are expressed as mean ± standard deviation. The differences between berry size categories were tested with a one-way ANOVA. Different letters in the same column, for each parcel, indicate statistically significant differences between juices at P<0.05 by Tukey's HSD test. **CFG**: Cabernet franc on gravelly soil, **CFS**: Cabernet franc on sandy soil, **MNG**: Merlot on gravelly soil, **MNS**: Merlot on sandy soil

Table 6 - Chemical composition of grape juice from different berry size categories in 2015

	Sugar (g/L)	Total acidity (g/L)	Malic acid (g/L)
CFG			
Control	262.80 ± 0.95 b	2.01 ± 0.01 b	0.73 ± 0.04 b
Large	251.33 ± 1.27 a	2.05 ± 0.02 b	0.80 ± 0.05 b
Small	271.00 ± 1.68 c	1.90 ± 0.06 a	0.56 ± 0.05 a
CFS			
Control	240.37 ± 0.78 a	2.72 ± 0.01 b	1.35 ± 0.02 a
Large	238.80 ± 1.25 a	2.77 ± 0.08 b	1.43 ± 0.04 b
Small	241.60 ± 2.14 a	2.59 ± 0.03 a	1.31 ± 0.01 a
MNG			
Control	241.50 ± 0.98 b	2.86 ± 0.02 a	1.12 ± 0.03 b
Large	235.87 ± 1.19 a	2.98 ± 0.03 b	1.35 ± 0.02 c
Small	246.07 ± 1.04 c	2.85 ± 0.04 a	0.96 ± 0.03 a
MNS			
Control	221.07 ± 1.53 b	1.28 ± 0.03 a	1.60 ± 0.00 a
Large	212.83 ± 1.72 a	1.26 ± 0.07 a	2.03 ± 0.06 b
Small	228.70 ± 2.43 c	1.18 ± 0.03 a	1.60 ± 0.10 a

Results are expressed as mean ± standard deviation. The differences between berry size categories were tested with a one-way ANOVA. Different letters in the same column, for each parcel, indicate statistically significant differences between juices at P<0.05 by Tukey's HSD test. **CFG**: Cabernet franc on gravelly soil, **CFS**: Cabernet franc on sandy soil, **MNG**: Merlot on gravelly soil, **MNS**: Merlot on sandy soil.

Wine phenolic composition

In the 2014 and 2015 vintages, wines were analysed for the standard parameters by WineScan FOSS® at the end of alcoholic and malolactic fermentation (data not shown). As expected, according to juice composition, wines from small berries showed higher alcohol levels compared to those from large berries at the end of each fermentation. Due to the higher levels of malic acid of wines from large berries after alcoholic fermentation, at the end of malolactic fermentation, they showed higher level of lactic acid, compared to wine from small berries. The control wine showed always intermediate values.

In 2014, phenolic profiles of wines were evaluated at the end of malolactic fermentation. The comparison of flavanol concentration of wines from berries belonging to different size categories does not show consistent results (table 7). Few statistically significant differences among wines were observed. These were found only on parcels planted on the sandy soil, where wines produced by smaller berries showed higher levels of total flavanols. These differences concerned procyanidin B2, for CFS and MNS parcels, and procyanidin B4, for MNS and CFG. Nevertheless, when total tannin concentrations were analyzed by Bate Smith method, statistically significant differences, were observed on MNS, MNS and CFG (figure 7A, B and C).

No consistent results were obtained from comparison of molecular anthocyanin profiles of wines produced from small and large berries, in the 2014 vintage. Results of this analysis, reported in table 8, show rare significant differences between wines concerning some monoglucoside anthocyanins, such as Delphinidine-3-O-glucoside (Dp), Cyanidine-3-O-glucoside (Cy), Peonidine-3-O-glucoside (Pn) and Malvidine-3-O-(-6-acetyl)-glucoside (Mv-ac). Despite these erratic differences, the total anthocyanin content, calculated as sum of each individual compounds, was never significantly different. Similar results were obtained by the analysis of the total anthocyanin concentration using the SO₂ bleaching method (figure 7), except for CFG (figure 7C). Wines made from small berries produced on this parcel were characterized by a significantly higher level of total anthocyanins compared to wines from large and control berries.

Despite the low consistence of these results, the analysis of total phenolic compounds of wines showed, for each experimental parcel, statistically significant differences among wines from different berry size categories (figure 7). All wines produced from smaller berries had significantly higher values of total phenolic compounds compared to wines made from large berries. Except for CFG (figure C), control wines showed always intermediate values.

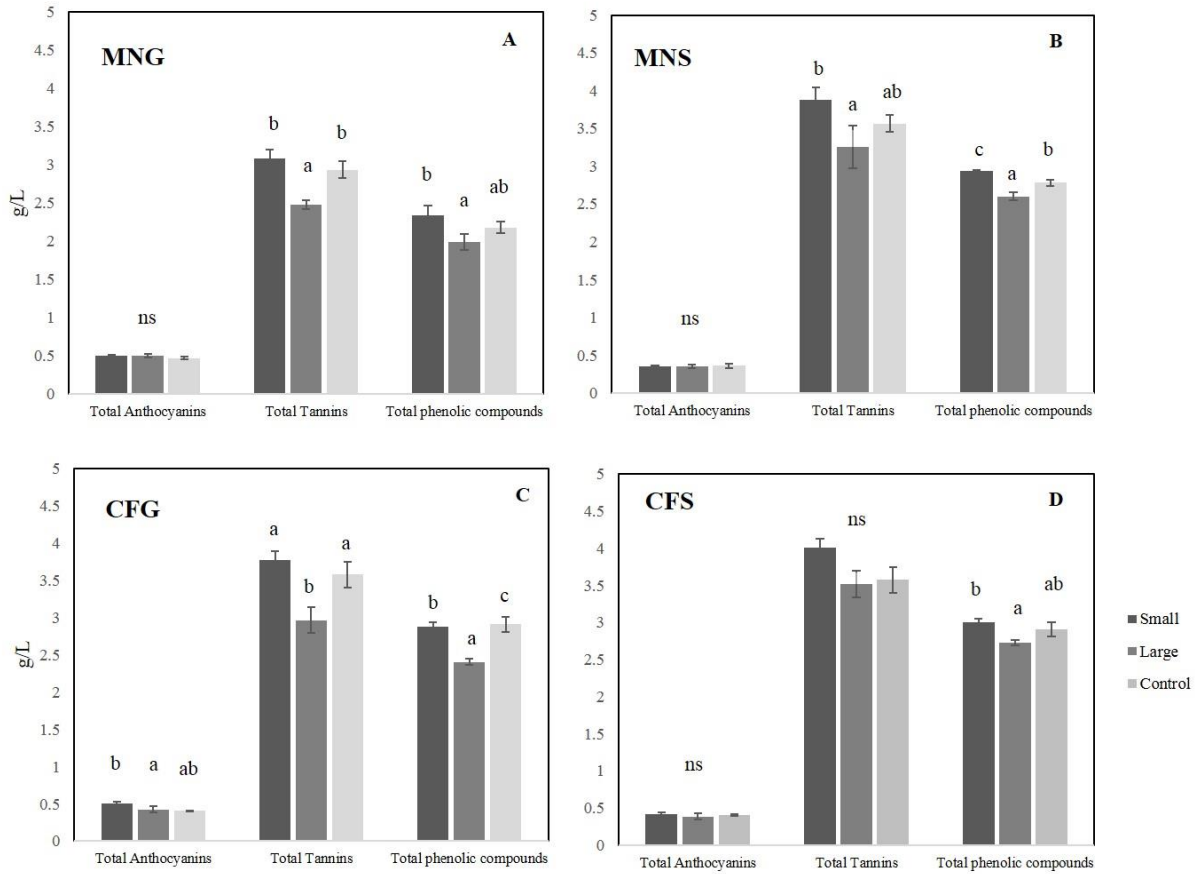


Figure 7 - Total anthocyanins, total tannins and total phenolic compound concentration of wines from small and large berries and from control berries at the end of malolactic fermentation, in the 2014 season. Results are expressed as mean \pm standard deviation of three replicates. The differences between berry size categories were tested with a one-way ANOVA. Different letters in the same column, for each parcel, indicate statistically significant differences between wines at $P < 0.05$ by Tukey's HSD. **CFG:** Cabernet franc on gravelly soil, **CFS:** Cabernet franc on sandy soil, **MNG:** Merlot on gravelly soil, **MNS:** Merlot on sandy soil.

Table 7 - Comparison of wine flavan-3-ols profiles made from berries belonging to different size categories (2014)

	C	EC	B1	B2	B3	B4	Total
CFG							
Control	1.608 ± 0.26	0.990 ± 0.14	1.336 ± 0.21	12.882 ± 2.19	0.247 ± 0.05	0.128 ± 0.03	17.191 ± 2.81
Large	1.648 ± 0.47	1.041 ± 0.29	1.402 ± 0.42	10.619 ± 2.88	0.241 ± 0.06	0.160 ± 0.04	15.112 ± 4.16
Small	1.131 ± 0.09	0.723 ± 0.04	0.935 ± 0.06	10.019 ± 0.51	0.169 ± 0.02	0.185 ± 0.14	13.160 ± 0.46
CFS							
Control	2.008 ± 0.09	0.999 ± 0.03	1.672 ± 0.07	11.761 ± 0.17 b	0.268 ± 0.02	0.129 ± 0.00	16.836 ± 0.22 b
Large	1.885 ± 0.05	0.933 ± 0.02	1.555 ± 0.01	9.665 ± 0.19 a	0.312 ± 0.01	0.150 ± 0.01	14.499 ± 0.26 a
Small	1.939 ± 0.10	0.940 ± 0.04	1.585 ± 0.03	11.269 ± 0.30 b	0.311 ± 0.02	0.138 ± 0.01	16.181 ± 0.46 b
MNG							
Control	1.442 ± 0.72	1.112 ± 0.57	1.122 ± 0.59	6.997 ± 3.80	0.079 ± 0.03	0.074 ± 0.05	10.826 ± 5.77
Large	1.655 ± 0.02	1.211 ± 0.05	1.436 ± 0.01	7.115 ± 0.50	0.114 ± 0.00	0.053 ± 0.00	11.584 ± 0.59
Small	1.998 ± 0.18	1.421 ± 0.17	1.768 ± 0.17	9.461 ± 1.39	0.141 ± 0.01	0.078 ± 0.01	14.867 ± 1.96
MNS							
Control	2.559 ± 0.07	1.591 ± 0.05	2.139 ± 0.14	12.471 ± 0.58 b	0.273 ± 0.03	0.153 ± 0.01 ab	19.187 ± 0.77 b
Large	2.179 ± 0.25	1.300 ± 0.21	1.867 ± 0.26	8.740 ± 1.73 a	0.228 ± 0.05	0.132 ± 0.01 a	14.446 ± 2.53 a
Small	2.593 ± 0.12	1.540 ± 0.05	2.130 ± 0.17	12.753 ± 0.41 b	0.336 ± 0.04	0.183 ± 0.01 b	19.535 ± 0.74 b

Concentration are expressed as mg/L. Wines were analyzed at the end of malolactic fermentation. Results are expressed as mean ± standard deviation of three replicates. The differences between berry size categories were tested with a one-way ANOVA. Different letters in the same column, for each parcel, indicate statistically significant differences between wines at P<0.05 by Tukey's HSD test.

C: (+)-catechin, EC: (-)-epicatechin, B1: [(-)-epicatechin-(4b-8)-(+)-catechin], B2: [(-)-epicatechin-(4b-8)-(-)-epicatechin], B3: [(+)-catechin-(4a-8)-(+)-catechin], B4: [(+)-catechin-(4a-8)-(-)-epicatechin]. Total was calculated adding up the concentration of individual compounds. CFG: Cabernet franc on gravelly soil, CFS: Cabernet franc on sandy soil, MNG: Merlot on gravelly soil, MNS: Merlot on sandy soil.

Table 8 - Comparison of wine anthocyanin profiles made from berry belonging at different size categories (2014)

	Dp	Cy	Pt	Pn	Mv	Pn-ac	Mv-ac	Pn-coum	Mv-coum	Total
CFG										
Control	19.08 ± 1.51 b	2.21 ± 0.21	23.41 ± 1.64	20.16 ± 1.56 b	159.16 ± 10.8	11.11 ± 0.83	43.85 ± 3.47	4.11 ± 0.56	16.74 ± 2.22	599 ± 44
Large	13.34 ± 1.17 a	0.96 ± 0.16	18.41 ± 1.56	12.87 ± 1.06 a	142.43 ± 10.7	9.33 ± 0.88	42.22 ± 3.21	3.41 ± 0.47	15.70 ± 1.77	517 ± 41
Small	19.70 ± 2.29 b	3.48 ± 2.32	25.54 ± 5.51	20.56 ± 1.68 b	159.44 ± 13.5	12.33 ± 3.18	44.00 ± 4.29	4.24 ± 0.79	16.35 ± 1.48	611 ± 69
CFS										
Control	23.22 ± 2.87	6.43 ± 0.68 b	28.90 ± 2.95	34.05 ± 3.62	129.35 ± 12.7	14.44 ± 1.71	34.95 ± 3.45 ab	5.46 ± 0.75	14.47 ± 1.82	582 ± 61
Large	21.68 ± 0.09	3.14 ± 0.05 a	25.94 ± 0.46	30.50 ± 0.34	146.04 ± 1.0	13.55 ± 1.07	39.95 ± 1.01 b	5.05 ± 0.19	16.20 ± 0.68	604 ± 5
Small	23.91 ± 1.47	5.57 ± 1.12 b	27.96 ± 1.69	34.38 ± 2.90	125.92 ± 11.7	13.88 ± 0.83	32.77 ± 2.65 a	5.17 ± 0.13	13.43 ± 0.49	566 ± 40
MNG										
Control	24.28 ± 1.66 ab	4.64 ± 0.37 ab	28.87 ± 2.16	25.26 ± 1.81 b	124.07 ± 8.5	11.77 ± 1.50	29.88 ± 2.14	5.87 ± 0.95	16.72 ± 1.86	542 ± 40
Large	22.42 ± 1.06 a	4.16 ± 0.08 a	27.76 ± 1.61	15.51 ± 0.16 a	128.71 ± 5.15	11.22 ± 0.83	30.14 ± 1.72	5.68 ± 0.42	17.32 ± 0.79	515 ± 9
Small	26.06 ± 1.18 b	5.41 ± 0.44 b	28.82 ± 1.38	26.67 ± 1.30 b	117.10 ± 5.13	11.77 ± 0.77	27.44 ± 1.20	5.73 ± 0.35	15.83 ± 0.80	529 ± 24
MNS										
Control	23.87 ± 2.92	7.63 ± 1.03	25.92 ± 3.13	32.12 ± 4.00	98.52 ± 12.0	10.66 ± 1.45	22.28 ± 2.47	5.10 ± 0.32	11.54 ± 0.87	475 ± 55
Large	22.77 ± 2.56	6.94 ± 0.91	26.31 ± 2.69	29.96 ± 3.04	101.80 ± 8.5	11.33 ± 1.20	23.93 ± 2.12	5.23 ± 0.43	12.56 ± 1.04	481 ± 44
Small	24.73 ± 2.83	8.32 ± 0.87	26.30 ± 2.71	34.30 ± 3.53	95.36 ± 8.9	9.22 ± 1.38	22.61 ± 4.44	5.07 ± 0.93	10.54 ± 1.71	472 ± 48

Concentration are expressed as mg/L. Wines were analyzed at the end of malolactic fermentation. Results are expressed as mean ± standard deviation of three replicates. The differences between berry size categories were tested with a one-way ANOVA. Different letters in the same column, for each parcel, indicate statistically significant differences between wines at $P < 0.05$ by Tukey's HSD test. **Dp**: Delphinidine-3-O-glucoside, **Cy**: Cyanidine-3-O-glucoside, **Pt**: Petunidine-3-O-glucoside, **Pn**: Peonidine-3-O-glucoside, **Mv**: Malvidine-3-O-glucoside, **Pn-ac**: Peonidine-3-O-(6-acetyl)-glucoside, **Mv-ac**: Malvidine-3-O-(6-acetyl)-glucoside, **Pn-coum**: Peonidine-3-O-(6-p-coumaroyl)-glucoside, **Mv-coum**: Malvidine-3-O-(6-p-coumaroyl)-glucoside. **Total** was calculated adding together the concentration of each mono-glucoside and the acetyl and *p*-coumaroyl derivatives.

CFG: Cabernet franc on gravelly soil, **CFS**: Cabernet franc on sandy soil, **MNG**: Merlot on gravelly soil, **MNS**: Merlot on sandy soil.

In 2015, which was a supplementary experimental vintage, wines were analyzed after 12 aging months, when biological replicates had been assembled for sensory analysis. Figure 8 reports the flavanol profiles of the wines. Despite the differences between wines seem being more obvious compared to the 2014 vintage, in several cases similar levels of estimated compounds were observed between wines from small and large berries. This was particularly obvious in MNS (figure 8B). Nevertheless, the total flavanol concentrations of wines produced on the remaining parcels, tend to be higher in wines produced from smaller berries (figure 8A, C and D). In contrast, when the tannin concentration was estimated by Bate Smith method, bigger differences were observed in Merlot parcels (figure 9). In all situations, wines produced by smaller berries showed higher level of total tannins. Similarly, these wines contained higher anthocyanin concentrations. This was observed both in molecular anthocyanins (table 9) and in total anthocyanin content (figure 9). The relationship between berry size category and total phenolic compounds was less obvious than in 2014. Nevertheless, except for MNS, higher levels were generally measured in wine from smaller berries.

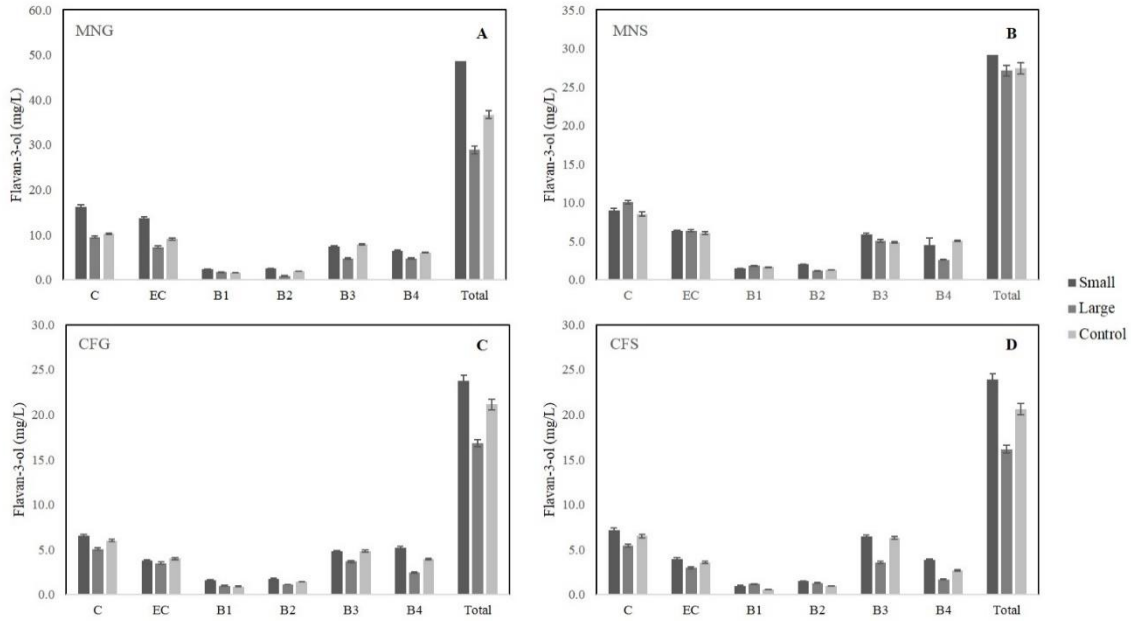


Figure 8 - Comparison of wine flavan-3-ols profiles of wines made from berry belonging to different size categories (2015). **C**: (+)-catechin, **EC**: (-)-epicatechin, **B1**: [(-)-epicatechin-(4b-8)-(+)-catechin], **B2**: [(-)-epicatechin-(4b-8)-(-)-epicatechin], **B3**: [(+)-catechin-(4a-8)-(+)-catechin], **B4**: [(+)-catechin-(4a-8)-(-)-epicatechin]. **Total** was calculated adding up the concentration of individual compounds. Wines were analyzed after 12 months of aging. Results are a mean of two replicate analyses. **CFG**: Cabernet franc on gravelly soil, **CFS**: Cabernet franc on sandy soil, **MNG**: Merlot on gravelly soil, **MNS**: Merlot on sandy soil.

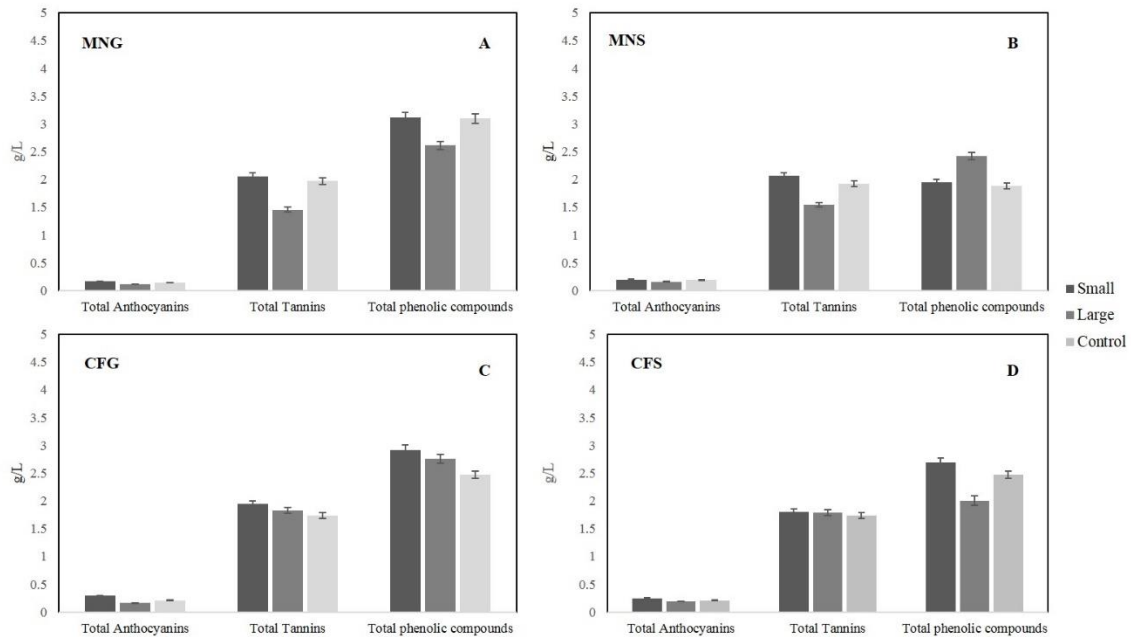


Figure 9 - Total anthocyanins, total tannins and total phenolic compound concentration of wines from small and large berries compared to control berries, in the 2015 season. Wines were analyzed after 12 months of aging. Results are a mean of two replicate analyses. **CFG**: Cabernet franc on gravelly soil, **CFS**: Cabernet franc on sandy soil, **MNG**: Merlot on gravelly soil, **MNS**: Merlot on sandy soil.

Table 9 - Comparison of wine anthocyanin profiles made from berries belonging to different size categories (2015)

	Dp	Cy	Pt	Pn	Mv	Dp-ac	Cy-ac	Pt-ac	Pn-ac	Mv-ac	Dp-coum	Cy-coum	Pt-coum	Pn-coum	Mv-coum	Total
CFG																
Control	8.5±0.22	0.9±0.02	12.1±0.31	6.0±0.19	100.8±2.02	6.9±0.11	1.4±0.03	3.8±0.10	3.0±0.11	31.4±0.91	1.1±0.02	0.3±0.01	0.8±0.02	1.0±0.02	5.9±0.16	184.0±4.52
Large	3.9±0.11	0.5±0.01	5.9±0.14	3.6±0.10	57.2±1.72	4.3±0.11	1.3±0.03	2.8±0.07	1.5±0.03	18.4±0.53	0.6±0.01	0.2±0.01	0.4±0.01	0.9±0.02	3.1±0.09	104.4±2.13
Small	12.3±0.27	0.9±0.03	15.4±0.36	8.1±0.21	119.2±2.58	10.5±0.22	1.1±0.03	5.4±0.15	3.7±0.10	40.0±1.12	1.8±0.05	0.5±0.01	1.4±0.03	1.7±0.05	9.1±0.24	231.2±5.94
CFS																
Control	7.7±0.21	1.1±0.04	10.2±0.36	9.7±0.24	88.0±2.14	9.6±0.19	1.1±0.04	5.1±0.13	2.6±0.07	24.9±0.72	0.8±0.02	0.1±0.01	0.3±0.01	0.7±0.02	3.1±0.10	164.9±3.95
Large	5.8±0.17	1.6±0.05	9.2±0.23	8.9±0.17	68.7±1.06	3.8±0.10	0.8±0.02	2.4±0.06	1.9±0.08	18.1±0.91	0.4±0.01	0.1±0.01	0.3±0.01	0.4±0.01	2.5±0.07	124.8±3.14
Small	11.5±0.25	0.7±0.025	15.0±0.35	9.9±0.2	113.1±2.99	6.7±0.21	1.5±0.03	3.4±0.1	3.9±0.11	33.6±0.91	1.8±0.04	0.4±0.01	1.0±0.02	1.7±0.05	8.6±0.23	213.0±5.39
MNG																
Control	5.8±0.17	0.4±0.01	7.8±0.21	2.4±0.08	60.4±1.21	1.8±0.03	0.4±0.01	3.1±0.08	0.8±0.02	15.2±0.42	0.4±0.01	0.1±0.01	0.5±0.01	0.6±0.02	3.2±0.09	102.8±2.79
Large	5.3±0.14	0.5±0.01	6.5±0.12	3.6±0.11	58.8±1.56	1.3±0.04	0.5±0.01	1.6±0.04	1.3±0.04	11.7±0.36	0.2±0.01	0.1±0.01	0.2±0.01	0.2±0.01	1.7±0.05	93.5±2.10
Small	6.9±0.11	0.5±0.01	9.4±0.18	4.0±0.17	78.2±2.75	2.1±0.06	0.6±0.02	2.7±0.07	1.1±0.02	18.4±0.57	0.4±0.01	0.2±0.01	0.5±0.01	0.5±0.01	4.0±0.11	129.5±2.88
MNS																
Control	13.3±0.34	1.4±0.04	12.1±0.38	11.9±0.40	60.2±1.70	5.2±0.18	0.5±0.01	3.0±0.08	2.0±0.05	13.6±0.31	0.4±0.01	0.1±0.01	0.4±0.01	0.9±0.02	2.7±0.08	127.7±3.33
Large	8.8±0.27	2.3±0.06	9.5±0.23	9.4±0.18	50.0±1.20	6.0±0.16	0.3±0.01	1.7±0.04	1.5±0.04	9.1±0.23	0.2±0.01	0.1±0.01	0.2±0.01	0.4±0.01	1.4±0.04	100.8±3.12
Small	20.3±0.51	1.9±0.05	17.7±0.43	14.6±0.34	72.7±0.8	7.1±0.20	1.3±0.03	4.2±0.12	2.4±0.07	14.9±0.35	0.3±0.01	0.1±0.01	0.5±0.01	1.0±0.03	2.7±0.08	161.6±3.85

Concentration are expressed as mg/L. Wines were analyzed after 12 months of aging. Results are expressed as mean ± standard deviation of two replicate analyses.

Dp: Delphinidine-3-O-glucoside, **Cy:** Cyanidine-3-O-glucoside, **Pt:** Peonidine-3-O-glucoside, **Mv:** Malvidine-3-O-glucoside, **Mv-ac:** Malvidine-3-O-glucoside, **Pn:** Peonidine-3-O-glucoside, **Pn-ac:** Peonidine-3-O-glucoside, **Mv-ac:** Malvidine-3-O-glucoside, **Dp-coum:** *p*-coumaroyl derivatives, **Total** was calculated adding up the concentration of each mono-glucoside and the acetyl and *p*-coumaroyl derivatives. **CFG:** Cabernet franc on gravelly soil, **MNG:** Merlot on sandy soil, **MNS:** Merlot on sandy soil.

Wine aroma compounds

In this study we also investigated whether wines elaborated from berry of different size categories show different aromatic profiles. Five aroma compounds, such as homofuraneol and furaneol, belonging to the class of furanones, and massoia-lactone, γ -nonalactone and δ -decalactone, belonging to the class of the lactones, were estimated on wines produced in 2014 and 2015 after 24 and 12 months of aging, respectively.

Results of this investigation, reported in tables 10 and 11, showed clearer relationships between wines from different berry size categories for lactone compounds, compared to furanone compounds. In most of cases, berry mass did not affect significantly the wine concentration of Homofuraneol and Furaneol compounds. This fact was observed over the two years.

Table 10 - Comparison of aroma compounds in wines made from berries belonging to different size categories (2014)

	Homofuraneol	Furaneol	Massoia_lactone	γ -nonalactone	δ -decalactone
CFG					
Control	9.25±0.21	21.00±4.24	6.38±1.07	22.97±1.17	36.97±1.20
Large	4.00±1.41	23.33±10.12	2.46±1.80	11.96±3.36	20.89±1.00
Small	8.00±0.00	30.67±8.39	5.33±0.86	24.22±1.40	31.32±1.14
CFS					
Control	10.59±3.66	15.50±12.02	8.25±2.64	15.10	1.60
Large	10.95±2.66	18.00±1.00	5.57±2.12	11.24±0.43	1.31±0.34
Small	11.65±1.68	20.67±2.52	5.91±0.09	14.62±1.79	1.52±0.09
MNG					
Control	4.10±0.85	29.67±4.04	2.78±0.03 ab	7.94±0.58 b	10.55±1.65 a
Large	2.93±1.44	16.00±4.36	2.01±0.70 a	3.46±0.75 a	5.34±0.48 b
Small	3.80±1.04	25.00±7.55	3.66±0.49 b	9.77±0.44 c	12.78±0.66 a
MNS					
Control	9.00±5.57	26.00±5.29	5.16±1.25 ab	17.98±1.63 b	21.98±3.43 b
Large	8.67±3.79	21.33±3.79	3.97±1.32 a	9.94±0.64 a	14.75±2.75 a
Small	11.33±2.08	28.00±8.66	7.14±0.47 b	20.79±0.40 c	24.20±1.06 b

Concentration are expressed as $\mu\text{g/L}$. Wines were analyzed after 24 aging months. Results are expressed as mean \pm standard deviation of three replicates analyses. The differences between berry size categories were tested with a one-way ANOVA. Different letters in the same column, for each parcel, indicate statistically significant differences between wines at $P < 0.05$ by Tukey's HSD test. **CFG**: Cabernet franc on gravelly soil, **CFS**: Cabernet franc on sandy soil, **MNG**: Merlot on gravelly soil, **MNS**: Merlot on sandy soil.

Conversely, more differences in lactone compounds concentrations were found among the experimental wines. In 2014, Merlot wines from smaller berries presented significantly higher levels for each measured lactone compounds. In contrast, no significant differences were found in Cabernet franc wines. Similar trends were observed in 2015, indicating a higher concentration of lactone aroma compounds in wines from small berries. However the effect of berry size on lactone concentrations was not always significant. For the δ -decalactone, significant differences were observed only in MNS wines, while the γ -nonalactone showed different levels in Cabernet franc wines. Each experimental parcel showed significant differences in massoia lactone content between wines made from small and large berries.

Table 11 - Comparison of wine aroma profiles made from berry belonging at different size categories (2015)

	Homofuraneol	Furaneol	Massoia_lactone	γ -nonalactone	δ -decalactone
CFG					
Control	20.33±3.79	36.33±2.08 ab	8.10±2.74 a	22.70±4.89 ab	17.60±3.91
Large	12.82±3.05	19.67±12.66 a	2.68±0.27 a	10.38±5.38 a	6.44±1.01
Small	22.03±7.05	46.30±12.52 b	14.69±4.73 b	25.03±6.40 b	15.99±2.53
CFS					
Control	4.95±2.93	15.33±3.06 b	3.22±1.43 ab	6.60±0.60 b	4.96±0.93
Large	6.34±2.95	7.00±2.00 a	1.39±0.70 a	4.08±0.54 a	3.09±3.83
Small	9.06±1.91	17.33±2.89 b	4.60±0.70 b	7.97±1.65 b	7.81±0.02
MNG					
Control	21.17±4.65	58.40±22.48	8.25±0.82 a	10.38±5.74	8.22±0.72
Large	19.51±8.47	49.54±20.21	2.90±0.96 a	5.12±2.50	4.66±1.49
Small	22.67±8.08	49.00±11.53	16.03±4.31b	18.06±7.98	9.92±4.85
MNS					
Control	21.07±7.66	22.16±14.44	9.13±1.92 a	12.94±2.67	2.53±0.38 b
Large	29.48±4.24	19.47±7.75	4.00±1.86 a	7.07±2.62	1.34±0.34 a
Small	23.19±11.82	17.00±7.07	17.90±4.24 b	18.22±8.94	2.41±0.28 b

Concentration are expressed as $\mu\text{g/L}$. Wines were analyzed after 12 aging months. Results are expressed as mean \pm standard deviation of two replicates.

Results are expressed as mean \pm standard deviation of three replicates. The differences between berry size categories were tested with a one-way ANOVA. Different letters in the same column, for each parcel, denote statistically significant differences between wines at $P < 0.05$ by Tukey's HSD test. **CFG**: Cabernet franc on gravelly soil, **CFS**: Cabernet franc on sandy soil, **MNG**: Merlot on gravelly soil, **MNS**: Merlot on sandy soil.

Sensory analysis

In this study, sensory analysis of wines was performed each year after 12 months of aging. Results of panel tasting are resumed in tables 12 and 13. In 2014, only MNG and CFG wines were tasted. Neither in 2014, nor in 2015 statistically significant differences were observed between wines made from berries of different size for all investigated sensory descriptors. Nevertheless, it remained possible to observe some tendencies for many descriptors.

Table 12 - Results of sensory analysis of wines made from berries of different size in 2014

	Orthonasal descriptors			Retronasal descriptors				
	Intensity	Fruity	Vegetal	Acidity	Fruity	Bitterness	Astringency	Body
CFG								
Control	4.9±1.6	3.6±2.1	4.0±2.6	4.1±1.9	4.4±1.7	3.2±2.7	4.0±3.0	4.6±2.1
Large	4.9±1.6	3.8±2.1	3.7±3.0	4.6±2.1	3.8±2.2	3.2±1.9	4.3±2.4	3.9±2.1
Small	5.1±2.2	4.0±1.7	3.9±3.0	4.1±1.9	4.7±2.2	3.8±2	4.6±2.1	4.8±1.8
MNG								
Control	5.6±1.2	4.2±1.8	4.1±1.9	5.4±2.1	4.8±1.7	3.2±1.8	2.6±1.8	4.0±1.4
Large	5.1±1.3	4.6±2.1	3.5±2.3	4.5±2.4	4.7±1.5	3.7±2.6	2.8±1.9	3.4±1.8
Small	5.3±1.6	4.7±1.9	2.7±1.6	4.4±2.0	4.8±1.7	4.1±2.0	3.4±1.9	4.5±1.5

Results are expressed as mean ± standard deviation of 15 replicates, corresponding to tasters of panel. Sensory analysis of wines was performed after 12 months of aging. **CFG**: Cabernet franc on gravelly soil, **CFS**: Cabernet franc on sandy soil, **MNG**: Merlot on gravelly soil, **MNS**: Merlot on sandy soil

Colour intensity was higher in wines made from small berries in 2014. Reverse results for this descriptor were obtained in 2015, except for MNS where similar values between different wines were recorded (data not shown). In 2014, tendencies varied slightly among wines produced on different experimental parcels. In CFG, wines made from small sized berries had highest scores for both orthonasal and retronasal fruity descriptor, vegetal, bitterness, astringency and body. On the other hand, these wines received lower scores for the acidity descriptor. Differences between wines belonging to MNG parcel were less obvious. In this case, wines from small and large berries showed similar scores for fruity orthonasal and retronasal descriptors and for acidity. Other retronasal descriptors showed similar tendencies as reported in the previous case, while a higher score for the vegetal descriptor has been attributed to wines from large berries.

As observed in 2014, in 2015 tendencies of each descriptor between wines from small and large berries, varied among parcels without clear differences. Only the orthonasal intensity showed similar tendencies in each experimental parcel: all wines from small berries showed higher scores of these descriptors. Wines from small berries produced on MNS, MNG and CFS parcels were

characterized by higher values of red fruits, acidity and body descriptors, while opposite results were found in CFG. In this latter experimental parcel, few descriptors showed differences among wines. For the remaining descriptors, it was not possible to highlight a specific relationship to berry size.

We asked also panel tasters to classify the three wines according to their quality. In 2015 no significant differences were observed between wines from each parcel. Nevertheless, we calculated the percent of judges preferring one wine rather another. Our results, once again for this vintage, showed no consistent relationship among berry size and wine quality. For MNG, most of judges preferred wines from large berries. For MNS, wines from control were mostly appreciated. Concerning CFG, wines from small berries received a positive consensus, while, for CFS a similar number of judges preferred wines from large berries and from control.

Different results were obtained in 2014, where only MNG and CFG were tasted. Friedman's test, performed to investigate a possible significant difference among wines classified according to their global quality, showed that in both parcels, wines produced by large berries were significantly superior compared to wines from small berries. The control was not significantly different.

Table 13 - Results of sensory analysis of wines made from berries of different size in 2015

	Orthonasal descriptors				Retronasal descriptors					
	Intensity	Red fruits	Black fruits	Jammy fruits	Vegetal	Acidity	Fruity	Bitterness	Astringency	Body
CFG										
Control	6.0±2.0	4.1±2.6	4.4±2.3	4.0±2.8	2.2±2.4	3.6±2.0	2.0±2.1	4.6±2.5	2.1±2.3	3.2±2.1
Large	5.0±2.1	4.2±2.4	4.2±2.4	3.9±2.7	2.3±2.2	4.0±2.0	2.0±1.8	3.7±2.5	1.8±2.3	3.4±2.0
Small	5.6±1.6	4.0±2.0	4.4±2.0	3.9±2.6	2.3±2.6	3.8±2.3	2.3±1.7	4.1±2.5	1.7±2.5	3.4±1.9
CFS										
Control	5.1±1.5	3.8±2.2	3.4±1.9	2.8±2.2	3.6±2.7	4.2±2.1	2.1±2.2	3.7±2.1	2.2±1.9	2.9±2.2
Large	5.2±1.7	4.3±1.8	4.8±2.3	3.0±2.3	3.2±2.3	4.1±2.1	2.1±2.2	4.5±2.0	2.2±2.2	2.8±2.4
Small	5.5±1.5	5.2±1.7	4.8±2.0	3.3±2.4	2.8±2.3	4.4±1.9	1.9±1.8	4.7±2.2	1.8±1.9	3.1±1.8
MNG										
Control	5.6±1.7	4.8±2.1	4.9±1.9	3.4±2.0	2.4±2.5	3.9±1.9	1.9±1.8	5.0±1.7	1.8±1.8	3.0±1.9
Large	5.5±1.8	3.7±2.1	4.4±2.3	3.3±2.9	2.6±1.8	3.9±2.0	2.0±1.9	3.9±1.7	1.9±1.7	3.1±1.7
Small	6.5±1.6	4.3±2.4	5.4±2.3	3.9±2.6	2.8±2.1	4.3±1.8	1.8±2.1	5.2±2.3	2.1±1.8	3.7±1.8
MNS										
Control	5.1±1.6	3.6±2	4±2.5.0	3.1±2.3	2.8±2.0	4.3±1.8	1.8±2.2	4.4±2.4	2.2±1.9	3.7±2.1
Large	5.2±1.9	4.6±2.3	5.1±2.7	4.3±2.8	2.1±1.7	4.3±2.1	2.1±1.9	5.2±2.3	1.9±1.6	3.6±2.0
Small	5.4±2.0	4.8±2.2	4.8±2.1	3.5±2.5	1.8±1.6	4.7±2.0	2.0±2.1	5.0±2.3	2.1±2.4	3.8±2.2

Results are expressed as mean ± standard deviation of 23 replicates, corresponding to tasters of panel. Sensory analysis of wines was performed after 12 months of aging.
CFG: Cabernet franc on gravelly soil, **CFS**: Cabernet franc on sandy soil, **MNG**: Merlot on gravelly soil, **MNS**: Merlot on sandy soil

DISCUSSION

Relationships among berry volume and berry tissues were studied during two subsequent years (2014 and 2015). Data was obtained on Cabernet franc berries produced on two different soil types.

During both years, berry seed mass was significantly correlated with berry volume, increasing proportionally with it, which is consistent with Ferrer et al. (2014). This correlation was due to the increase in seed number from smaller to larger berries, according to Scienza et al. (1978) and Carwthon and Morris (1982). A part of this relationship is linked to the fact that because seeds are a part of berries, a part of the correlation between whole berry size and berry seed content can be considered as an auto-correlation. Nevertheless, several studies demonstrated that the influence of berry seed content on berry size is explained by growth regulators supplied by the seeds, which promote cell multiplication and enlargement (Ristic and Iland 2005, Walker et al. 2005, Friend et al. 2009, Gray and Coombe 2009, Attia et al. 2010). Conversely to previous studies (Roby and Matthews 2004, Walker et al. 2005, Barbagallo et al. 2011), our results show that the relative seed mass (% per berry) remains constant as berry volume increases.

Dilution of skin solutes during processing can be approximated by relative skin and flesh mass, assuming no differences in skin solute concentrations among berries of different size (Roby and Matthews 2004). However, Ojeda et al. (2002) showed that anthocyanins expressed as mg/g of skin increases significantly when berries are produced by water stressed vines.

In this study, the amount of skin and flesh per berry increases approximately in proportion to the berry size (figures 3A, 3C, 4A and 4C). Despite we observed that surface per g of berry sharply decreases with berry volume (figure 5), the relative flesh mass does not change with berry size, while relative skin mass only very slightly decreases with berry volume (figures 3B, 3D, 4B and 4D). This would mean that, when berry size increases, skin becomes thicker: growth of tissues of berries, produced under similar environmental conditions, is coordinated. Thus, skin to flesh ratio remains approximately constant as berry volume increases. Our observations are consistent with Roby and Matthews (2004), Walker et al. (2005), Barbagallo et al. (2011), Ferrer et al. (2014). Hence, our results are in contrast with one of the *BBB myth* principles, which affirms that, assuming the grape berry as a sphere, as its diameter increases, the skin to flesh ratio decreases: implicit in this principle is that the amount of skin tissue remains fixed while berries are growing (Matthews 2015).

Calibaie performances, evaluated by berry physical measurement, showed satisfactory results. However, the berry sorting was not always perfect, especially when large berries were compared to berries from control. This fact is probably linked to setting the parameters of the machine. In the present work, we adjusted Calibaie to obtain the same amount of berries per size category per time of unit. However, it is well known that the final berry size is the result of the integrated effect of biotic and abiotic factors (Fernandez et al. 2006). As a result, the machine parameters, such as distance between rollers and/or feed rate, set by grapevine growers, have to take into account the degree of berry size variability specific for each parcel and vintage. Thus, in order to improve the machine efficiency, grapevine growers could perform a simple preliminary study of berry size distribution (vintage by vintage, parcel by parcel) and set the machine parameters according to their production goals.

The results of the present work, during 2014 and 2015 season, show that sugar concentration is higher in juice from smaller berries, which is consistent with Scienza et al. (1978), Cartwthon and Morris (1982), Roby et al. (2004). Similar observations have been made in 2012, when we carried out a preliminary study on the same experimental parcel. These results are instead in contrast with Glynn (2003), who measured the sugar concentration berry by berry. Because we also did not observe a clear relationship between berry size and °Brix when measured with a similar protocol (cf. article III, in result section of the present thesis), we suppose that the observation scale and the analysis approach (berry by berry vs batch of berries) could explain the absence of consensus between these results.

Moreover berries which were separated into size categories follow a negative significant relationship of the concentration of malic acid to size, in contrast to results found by Walker et al. (2005). Although the relationships with total acidity were not so clear, in most of cases juice from berries belonging to small size class showed lower TA.

The fact that previous studies showed no consensus on the relationship between berry size and wine composition could be due to the fact that berry composition is dependent on physiological processes other than growth (Matthews 2015). Also our results concerning the effect of berry size on phenolic composition of wines are not easy to interpret. Nevertheless, in most of cases smaller berries produced wines characterized by higher levels of phenols, such as flavanols and anthocyanins, which is consistent with other studies (Gil et al. 2015, Melo et al. 2015). These

results were particularly obvious in 2015, which was a warmer and drier vintage compared to 2014. This fact seems to confirm the conclusions of several authors studying the effect of berry size on berry composition. They reported that the way in which berry mass is reduced is more important than the berry mass itself (Matthews and Anderson 1988, Roby et al. 2004, Walker et al. 2005, Holt et al. 2008). Thus, in the present study, the vintage effect on the concentration of skin tannin and anthocyanin could be interpreted as a direct effect of berry size on those variables.

Most of studies consider that superior wines are made from grapes with high solute concentrations. This is particularly true with regard to phenols for red wines production. The objective of this study was to investigate possible compositional differences between wines made from berries of different sizes. Our results confirm this hypothesis. However, we do not necessarily consider that “small berries make superior red wine”, because grape growers have to judge the potential quality of berries according to their production goals.

Most of the published works on berry diameter of red grape cultivars have focused on phenolic compounds. Few data are available concerning the effect of berry size on wine aroma compounds (Friedel et al. 2016). However, these works were carried out on white varieties, such as Sauvignon blanc and Riesling, and considered other metabolites compared to aroma compounds investigated in the present study. The effect of berry mass on wine flavor concentrations vary with the class of aroma compounds (furanone or lactone), with the vintage and with the variety. However, neither vintage, nor variety showed a consistent effect on the relationship between berry size and wine aroma compounds.

The relationships between berry size and lactone compounds in wines were clearer compared to those concerning the furanone compounds. For the latter, berry mass did not seem to affect their concentration in wine.

In 2014, Merlot wines from smaller berries presented significantly higher levels for all measured lactone compounds. Similar trends were observed in 2015, although the effect of berry size was not always significant. Among aroma compounds investigated in this study, massoia lactone, responsible for coconut flavor, seems to be the compound most impacted by berry size. All wines produced by small berries showed higher levels of massoia lactone concentration: in almost all cases the berry size effect was significant. Because of the interest of these preliminary results, the

relationship between berry size and wine aroma compounds in red wines should be further investigated.

In this study, sensory analysis of wines were performed each year after 12 months of aging. Differences between wines made from berry different in size were not statistically significant. However, it remained possible to observe some tendencies for some descriptors. These tendencies were not always consistent with regard to berry size, because they varied from one year to another and from one parcel to another. Colour intensity, for example, was higher in wines made from small berries in 2014. Opposed results were obtained in 2015. Moreover, in some parcels, the number of descriptors showing differences between wines were higher than in other parcels. This was the case, for example, of MNG in 2014 and CFG in 2015.

In 2015, only the orthonasal intensity descriptor showed consistent results with regard to berry size effect: in all experimental parcel wines from small berries showed higher scores. The other descriptor values varied between wines with a less consistency. Only on three parcels, we observed that wines from small berries were characterized by higher values of red fruits, acidity and body descriptors. For the remaining descriptors, it was not possible to highlight a specific relationship with berry size.

The global wine quality, judged by the panel of tasters through the classification of wines according to their appreciation, was not significantly different in 2015. Moreover when results were expressed in percent of judges classifying a given wine as the first among the three samples, we did not found any consistent relationship between berry size and wine quality. These results are consistent with Walker et al. (2005). However, in 2014, we found that wines produced from large berries were judged significantly superior to wines from small berries. Hence, as reported in literature, there is no consensus on whether smaller berries make superior wines, because the relationship between grape composition and wine quality is neither linear, nor simple (Hunter et al. 1991). Despite the fact that some differences between wines have been highlighted in the present work, the direct effect of berry size on wine quality needs further investigation.

CONCLUSION

The goal of this study was to investigate the existence of a compositional difference between wines made from berries differing in size. Our results seem to confirm this hypothesis.

Calibaie machine allows to sort berries according to their size, creating two significant different classes. However, because of the high variability of final berry size, specific to each parcel and vintage, specific machine parameters have to be set by grape growers for each batch of grape according to their production objectives.

Study of the relationships between berry size and berry tissues shows that seed, skin and flesh masses increase proportionally with berry growth. As a result, the relative mass of each tissue, expressed as percent per berry, only very slightly decreases as berry volume increases. The proportional developing of skin and flesh in a berry seem to explain the little variation in skin to flesh ratio when plotted against to berry size, invalidating to the theoretical relationship between the surface and the volume of a sphere. Nevertheless, grape juice extracted from small berry show higher sugar and lower malic acid concentration. Despite the effect of berry size on wine phenolic composition not being simple, in this study most of wines produced from small berries show higher flavanol and anthocyanin levels. This was particularly obvious in the warm and dry 2015 vintage, confirming that the way in which berry mass is reduced has a bigger impact on berry composition than the direct impact of berry size itself.

The effect of berry mass on wine flavors composition was also investigated. Lactone compounds, and particularly the massoia lactone, show higher concentrations in wines made from small berries. Despite the fact that the physical analysis of small and large berries does not show significant differences with regard to tissue mass, grape juices and wines composition seem to be impacted by berry size. However, the origin of this effect is still to be understood. It is certainly influenced by the grapevine variety and terroir factors. The integrated effect of all these factors could be the reason of the no linear relationship between berry size and wine taste quality, which needs further investigation. The absence of a simple linear relationship between berry size, wine quality and sensory attributes can be the result of the fact that these factors both influence berry size and berry composition and their effect on berry composition can be direct, or indirect, mediated through berry size.

Chapter V

Effect of water deficit on berry mass and skin to flesh ratio

ABSTRACT

Water status of grapevines is widely recognized as a primary determinant of vigour, source to sink ratio, berry size and berry chemical composition. It is well known that water deficit during berry growth reduces final berry size, because it limits cell division and expansion. The relationship between final berry size and berry tissue masses provides information on the oenological potential of the wine grape. Different berry tissues may respond differently to stress conditions. Under field conditions, the interaction between the factors impacting berry growth and development, make the study of the variability of berry mass difficult. Sicily is a region in the south of Italy where growing seasons are generally dry and warm and characterized by high solar radiation. As a result, the evaporative demand is high. Under these semiarid conditions, water availability play a major role in the regulation of berry growth. In this conditions, it was possible to isolate the effect of vine water status on the final berry mass and to perform a special focus on berry tissue masses. This study was carried out in a commercial vineyard located in the Alcamo DOC. Area (Sicily), during the 2014 and 2015 growing seasons. The experiment was a randomized block design, where two irrigation treatments were established in three replicates: Non-irrigated (NI), in which irrigation was withheld and Irrigated (I), in which vines were irrigated in order to maintain midday stem water potential higher than -1.0 MPa until harvest. Vine water status was estimated by two physiological indicators: stem water potential and carbon isotope discrimination. Dynamic evolution of berry mass was investigated and, at harvest, average final berry mass per block was quantified by recording the mass of individual berries. Berry physical characteristics were analyzed only in 2015. The three berry tissues were separated and their mass was recorded. Seed number per berry was also recorded.

Our results showed that berries produced under moderate to severe water deficit were smaller than berries produced under weak water deficit stress. However, independently from vine water status, growth of flesh and skin appears coordinated. Hence, small and large berries, produced under similar water conditions, are characterized by a similar skin to flesh ratio. This observation implies that the propensity of flesh solutes to dilute skin solutes during winemaking is generally similar for various berry sizes. However, berries produced under moderate to severe water deficit showed higher skin to flesh values, independently from berry mass. Hence, regulated water deficit could represent a useful instrument to increase the skin to flesh ratio, in order to limit the dilution of anthocyanins and skin flavanols during winemaking, which are important compounds for the production of high quality red wines.

INTRODUCTION

The grape berry is a non-climacteric fruit characterized by a double-sigmoidal growth curve. The latter can be divided into two major phases of growth, separated by a lag phase, where pericarp growth is arrested and the embryo completes its development (Coombe 1992). The first stage of development is characterized by a rapid increase in berry size due to high rates of cell division and expansion in the berry pericarp, while, during the last stage, berries experience a period of rapid cell expansion as the pericarp grows to its final size (Ojeda et al. 1999, 2001, 2002).

Berry size has always been considered as an important quality factor in wine production. In this study, for practical reasons, berry size was expressed as mass. Final berry mass depend on several factors. Some of them are directly related to the individual berry itself. However, several external factors, such as environment and cultural practices, can also play a key role (Wong et al. 2016).

Water status of wine grapevines (*Vitis vinifera* L.) is widely recognized as a primary determinant of vigour, source to sink ratio, berry size and berry chemical composition (Chaves et al. 2007, Shellie 2014). Hence, the impact of a water deficit, measured on fruit at harvest, is the result of the changes in sensitivity of vegetative and reproductive tissues to water deficit during different developmental stages (Ojeda et al. 2002, Shellie 2006, Keller et al. 2008).

It is well known that water deficit during berry growth reduces final berry size, mostly when low water supply occurs during the first stages of development, as a result of an increase in abscisic acid (ABA), which limits cell division and expansion (Ojeda et al. 2001, 2002, Ferrer et al. 2014). Different berry tissues (skin, flesh and seed) respond to stress conditions according to the variety, the flesh having been represented being the most decisive one in reducing berry size (McCarthy 1997, Ojeda et al. 2001, Roby and Matthews 2004, Dai et al. 2011).

In viticulture, it is often assumed that wines produced from smaller berries are higher in quality because of a higher skin to flesh ratio (Singleton 1972). However, this concept is primarily based on assumptions and traditional beliefs, rather than on scientific evidences (Roby and Matthews 2004, Roby et al. 2004, Walker et al. 2005, Matthews and Nuzzo, 2007). Most of grapevine growers believes, in fact, that “if berry shape can be considered a sphere, then the surface:volume ratio could be related to the formula $(3/r)$, inversely correlated with the berry radius”. In other words, the berry skin to flesh ratio would decrease when berry size increases (Gil et al. 2015).

The relationship between final berry size and berry tissue masses, providing information on the oenological potential of the wine grape (Chaves et al. 2007), has been described by many

researchers (Walker et al. 2005, Matthews and Nuzzo, 2007, Ferrer et al. 2008, Friend et al. 2009, Gray and Coombe 2009, Barbagallo et al. 2011). Among the tissues of the berry, flesh represents the largest proportion of mass (Matthews and Nuzzo 2007, Attia et al. 2010, Barbagallo et al. 2011, Pisciotta et al. 2013). Seed number and their mass have a direct impact on total berry size due to the hormonal regulation exerted by the seeds on cell proliferation and expansion (Ristic and Iland 2005). Finally, the skin, where anthocyanins are synthesized (Coombe and Iland 2004) has a mass that grows proportionally to berry size increase (Attia et al. 2010, Pisciotta et al. 2013) and represents the smaller portion of the grape fruit. However, water deficit during berry development appears to alter these relationships (Ojeda et al. 2001, 2002, Roby and Matthews 2004, Shellie 2010, Girona et al. 2009, Attia et al. 2010, Ferrer et al. 2014, Zsofi et al. 2014). Roby and Matthews (2004), comparing different irrigation treatments, showed that in berries produced under non limited conditions, the skin to flesh ratio did not change with berry size. Conversely, in berries produced by vines subjected to water stress after veraison, the skin to flesh ratio varied according to the relationship between surface and volume of a sphere. In many wine producing regions in the world that experience seasonal drought, irrigation is commonly used to stabilize yield and maintain or improve grape quality (Chaves et al. 2010). The present work is supplementary to a study simultaneously carried out in the Bordeaux region (South-West of France). In the latter, in most of AOC (*Appellation d'Origine Contrôlée*) regulation the irrigation of the vines is forbidden, because the negative relationship between this practice and wine quality (Koundouras et al. 2006, van Leeuwen et al. 2009). In these conditions, it is difficult to compare two different water status conditions, especially during a rainy vintage (i.e. 2013 in Bordeaux). For this reason, in 2014, we decided to carry out simultaneously a study in a Mediterranean region, where irrigation is allowed. Sicily is a region in the south of Italy characterized by a semi-arid climate. Growing seasons are generally dry and warm and characterized by high solar irradiation. As a result, the evaporative demand is high. Under semiarid conditions, water availability plays a major role in regulation of berry growth (Santesteban and Royo 2006). Hence, in order to increase grape yield and quality, Sicilian grapevine growers apply irrigation, an essential practice to reach their production goals. Because of the difficulty, under field conditions, to isolate the singular effect of each factor potentially impacting berry size, the aim of this study was to accomplish a focus on vine water status effects on final berry mass and to investigate on the interrelationships between berry size at harvest and fresh mass distribution between seed, skin and flesh tissues.

MATERIAL AND METHODS

Location and plant material

This study was carried out in a commercial vineyard located in the Alcamo D.O.C. area, in the hinterland of western Sicily at 300 m a.s.l. (37°55'10'' N – 13°04'08'' E) The experimental parcel, which covers an area of about one hectare, is characterized by an average slope of 4-6 %. Measurements were performed during the 2014 and 2015 growing seasons on *Vitis vinifera* L. cv Cabernet franc, grafted onto 1103P rootstock. Practices were uniform in terms of vineyard floor management and fertilization. Vines were trained to a vertical shoot positioning with spur pruning system. The plant density is 4389 plants/ha and rows are oriented NE to SW. During the summer season, canopy management practices, such as topping, vertical shoot positioning, and desuckering, were performed.

The pedological study was carried out through opening and observation of two soil profiles. According to the French “*Référentiel Pédologique*” (Baize and Girard, 1995), soil was classified as *Vertisol*. The soil was very deep (> 170 cm) and the profile was Ap-B-C. The structure is polyedric in almost all layers. The porosity is high in the top soil and it decreases with depth. The amount of skeleton is generally low, while the amount of total limestone is from medium to little active lime. Vine roots are present over the total depth of the profile.

The experiment was a randomized block design with two irrigation treatments in three replicates. Each experimental block had 4 rows with 16 vines per row. Inside each block, 9 plants with a similar architecture were chosen. All measurements were carried out on the basal bunch of the central shoot. According to Carbonneau et al. (1991) this was the most appropriate bunch to represent the plant.

In order to determine the sanitary status of the plants, leave samples were collected during two seasons from selected vines of each block. Leaves were analysed by ELISA test, according to Beuve et al. 2013, to check possible presence of *Grapevine FanLeaf Virus* (GFLV, genus *Nepovirus*) and *Arabic Mosaic Virus* (ArMV, genus *Nepovirus*). Results showed that all plants were healthy.

Irrigation treatment and vine water status assessment

Two water regimes were established: (i) Non-irrigated (NI), in which irrigation was withheld and (ii) Irrigated (I), in which vines were irrigated in order to maintain midday stem water potential higher than – 1.0 MPa until harvest. Irrigation water was applied with drip emitters (4.0 L/h). The

number of treatment hours (generally 7h= 28 litres/plant) and their frequency, varied according to the measured stem water potential values. The total amount of water supplied to irrigated plants was around 250 L (110 mm) per vine and around 200 L (90 mm) per vine for 2014 and 2015, respectively. Measurements were performed exclusively on vines located on the two central rows of each block. Irrigation did not start until veraison (defined as the stage of development where berries begin to soften and colour).

Vine water status was estimated by two physiological indicators: stem water potential (Ψ_{stem}) and carbon isotope discrimination ($\delta^{13}\text{C}$).

Ψ_{stem} was measured with a pressure chamber (Scholander et al. 1965). Measurements were carried out with almost regular weekly intervals from half June until the end of August during both vintages. Measurements were taken on three fully expanded leaves from primary stems of different individual vines per block, just prior to irrigation in I blocks. Leaves were enclosed in a reflective plastic envelope for at least one hour before measurement. Stem water potential values reflect soil water availability, but they also depend on climatic parameters. Because stem water potential represents whole vine water status during the day, it is a particularly useful tool for irrigation management (van Leeuwen et al. 2009).

Ambient CO_2 contains 98.9% of ^{12}C isotope and 1.1% of ^{13}C isotope. ^{12}C isotope is preferentially used by the enzymes involved in photosynthesis (Farquhar et al 1989). Therefore, the sugar produced by photosynthesis contains a higher rate of the ^{12}C isotope than ambient CO_2 . This process is called “carbon isotope discrimination or $\delta^{13}\text{C}$ ”. Under water stress conditions, this discrimination is reduced due to stomatal closure (Farquhar et al. 1989). Hence, in these conditions, sugars produced contain more ^{13}C compared to those produced when plant water status is not limiting. Therefore, the $^{12}\text{C} / ^{13}\text{C}$ ratio in products of photosynthesis can be used as an integrative indicator of water deficit experienced by vine during grape ripening (Gaudillère et al. 2002). $^{13}\text{C} / ^{12}\text{C}$ ratio is expressed as $\delta^{13}\text{C} = [(\text{Rs} - \text{Rb})/\text{Rb}] * 1000$, where Rs is the ratio $^{13}\text{C} / ^{12}\text{C}$ of the sample and Rb is the $^{13}\text{C} / ^{12}\text{C}$ of the PDB (Pee Dee Belemnite) standard (Farquhar et al 1989). $\delta^{13}\text{C}$ ranges from -27 ‰ (no water deficit) to -20 ‰ (severe water deficit stress, van Leeuwen et al. 2009). This indicator is more and more used in the study of grapevine water relations, as it has been proven to be highly related to plant water status (Gaudillère et al. 2002, Koundouras et al. 2006, van Leeuwen et al. 2009, Santesteban et al. 2011a, 2014).

In this study, $\delta^{13}\text{C}$ measurements were carried out on grape juice extracted from 6 individual berries of different parts of three individual bunches per block (18 berries per block). 5 μL of berry juice were put in a tin capsule and dried. Carbon isotope content was measured by stable isotope mass spectrometry (Europa Scientific Ltd, Crewe, UK).

Grape sample and berry mass at harvest

In order to follow the seasonal dynamics of fresh berry mass, samples of 200 berries were collected from vines of each experimental block and their total mass were weighed. Average berry mass was determined dividing the total mass by the number of berries. Measurements were carried out from veraison to one week before harvest: four and three measurements were carried out in 2014 and 2015, respectively. At harvest, average final berry mass per block was quantified by recording the mass of each individual berry of nine experimental bunches.

Berry physical analysis

Berry physical characteristics were analyzed only in 2015 on 18 berries collected on tree plants per block on the basal bunch of the central shoot (54 berries per block). Berries were collected in different parts of bunches, taking into account the possible effect of berry position on berry characteristics (Pisciotta et al. 2013). Mass of each individual berry was recorded. Then the three berry tissues were separated and their mass was recorded. Seed number per berry was also recorded.

Statistical data analysis

Data were analysed using R software (R development Core Team 2015, version 3.2.3). Correlations between investigated variables were performed by a partial least squares regression analysis. The distribution of points was observed and, when possible, a linear regression was performed. The effect of vine water status on studied dependent variables was tested with a one-way ANOVA followed by Tukey's HSD test.

RESULTS

Weather conditions during the 2014 and 2015 seasons

Climatic data were recorded by an automatic weather station based on one of vineyard parcels. Temperature and rainfall values, during the summer of each experimental year are plotted in figure 1 and compared to average values recorded during the last 20 years (1996-2015).

Climatic conditions varied during the two vintages studied (Figure 1), especially with regard to the seasonal trends of monthly rainfall.

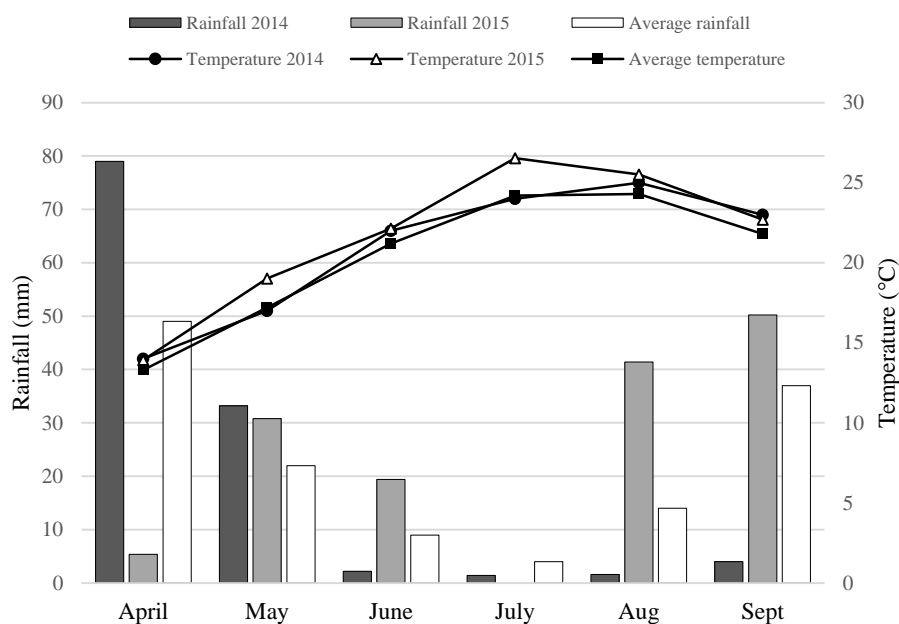


Figure 1 - Seasonal trends (1 April-30 September) of monthly temperature and rainfall recorded in 2014 and 2015; comparison with average values (1996-2015)

Temperature recorded from April through September in 2014, were close to long-term mean. Conversely, this vintage was exceptionally dry from June to September, while the month of April was rainy. Rainfall levels of May were similar in comparison to average and 2015 season. 2015, in contrast, was characterized by higher levels of rainfall compared to long-term mean during the months of May, June, August and September, while very low data were recorded in April and July. The latter month was also warmer compared to 2014 and average temperatures, which was also the case in May. Hence, these seasons reflected the typical Mediterranean climate of the south of Italy and they can be considered as warm and dry vintages.

Vine water status

According to the seasonal dynamics of Ψ_{stem} , reported in the figures 2A and 2B, vine water status varied during each season from one block to another depending on irrigation treatment.

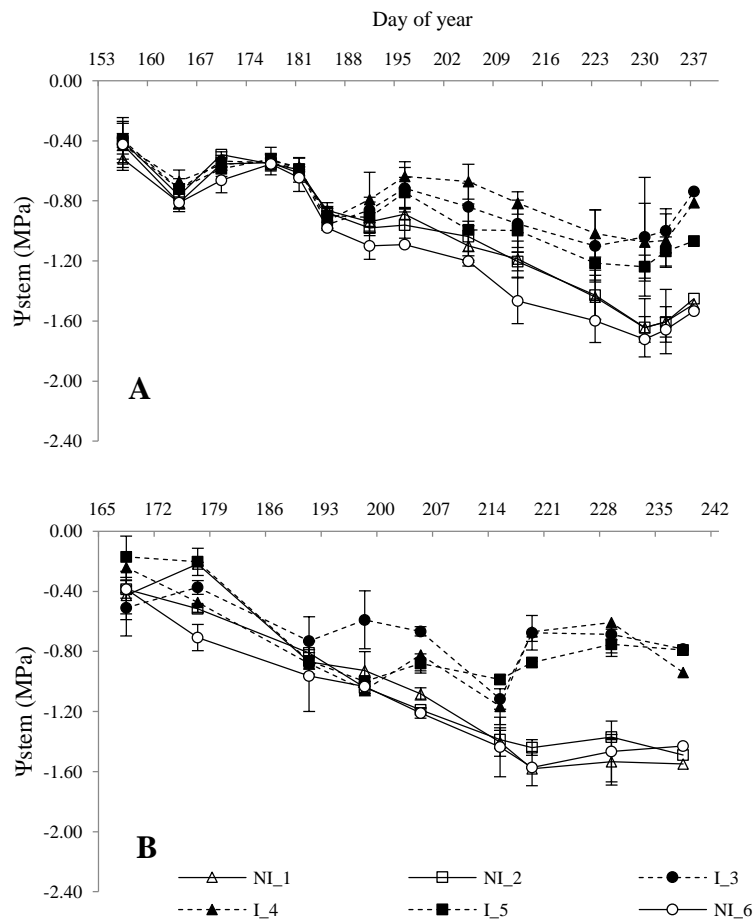


Figure 2 - Seasonal dynamics of stem water potential (Ψ_{stem}) measured in the experimental blocks in the summer of 2014 (A) and 2015 (B). Each point represents the mean of tree replicates.

Vines were irrigated according to the stem water potential values recorded. However, the trends of seasonal dynamics of vine water status varied also depending on seasonal weather conditions. Stem water potential values recorded in 2014 were lower compared to 2015, because of the variation of amount of rainfall observed during this season. As a result, nine irrigation treatments were performed in 2014, two more than in 2015. Moreover, in 2014, vines belonging to “non-irrigated” treatment were irrigated also because the exceptional low values of stem water potential recorded at the end of season (< -1.5) MPa, which could potentially damage the vines. Conversely, rainfall during the second part of the 2015 growing season, explained the stability of water status

of vines belonging to two treatments (I and NI) during the last weeks prior the harvest, around -0.8MPa and -1.5 MPa respectively.

Despite these small differences between seasons, vines submitted to the two irrigation treatments, showed significantly different water status. Non-irrigated vines faced severe water deficit during the second part of each season. In contrast, at the end of each season, irrigated vines faced weak water deficit in 2014 and weak to no water deficit in 2015. These results were confirmed by $\delta^{13}\text{C}$. Values reported in table 1 correspond to weak water deficit and moderate to severe water deficit for irrigated and non-irrigated vines, respectively (van Leeuwen et al. 2009). This was observed both in 2014 and 2015.

Table 1 - Vine water status in the experimental blocks, measured by Carbon isotope discrimination

Block	$\delta^{13}\text{C}$ (‰)	
	2014	2015
NI_1	-22.96 ± 0.25 d	-22.99 ± 0.34 d
NI_2	-23.28 ± 0.14 cd	-23.36 ± 0.18 c
I_3	-25.45 ± 0.96 ab	-25.72 ± 0.43 a
I_4	-25.89 ± 0.22 a	-24.56 ± 0.23 b
I_5	-25.04 ± 0.46 b	-25.62 ± 0.37 a
NI_6	-23.46 ± 0.55 c	-23.21 ± 0.52 cd

Results are expressed as mean ± standard deviation of 18 replicates. The differences between blocks were tested with a one-way ANOVA. Different letters in the same column denote statistically significant differences at $P < 0.05$ by Tukey's HSD test.
NI: Non-Irrigated; I: Irrigated.

Berry growth and final berry mass

In order to track the seasonal dynamics of berry growth during each growing season, several measurements of average berry mass were carried out inside each block. The first berry sampling occurred at veraison, while the last one was accomplished one week before harvest. Results of this investigation are reported in figures 3A and B.

In both seasons, in almost all sampling dates, irrigated vines showed bigger berries compared to vines facing to water deficit. These difference were particularly striking in 2015. At harvest, a more meticulous study of berry mass variability was performed. Mass of each individual berry, belonging to nine different bunches per block (each of them on a different vine), was recorded. Berry mass data, reported in table 2, confirmed the tendencies observed during the second phase of berry growth. Final berry mass was slightly lower in 2015 than in 2014, despite the latter being

a warmer and dryer vintage. Statistically significant differences, with regard to berry mass, were observed between berries produced under different water status conditions.

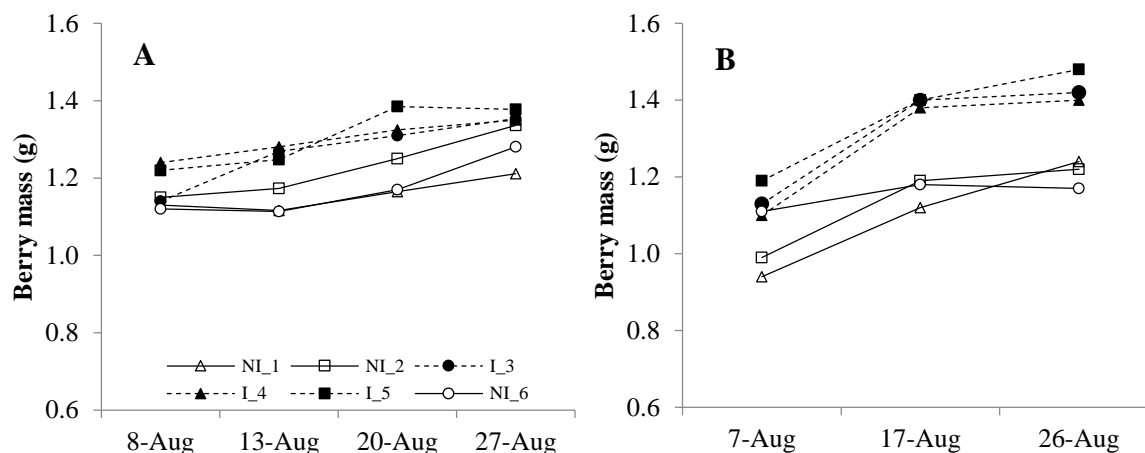


Figure 3 - Seasonal dynamic of berry mass (g) measured in the experimental blocks in the summer of 2014 (A) and 2015 (B). Each point was determined dividing the total mass of 200 berries by the number of berries itself.
NI: Non-Irrigated; **I:** Irrigated

In 2014, higher values of berry mass were recorded in irrigated vines. In almost all cases, these differences were significant. However, two particular cases were observed. Berries produced on I_3 showed significantly higher mass compared to berries produced on vines submitted to the same irrigation treatment. Similarly, berries of NI_2 block were significantly heavier than NI_1 and NI_6 berries. As a result, differences between NI_2 and I_4 and I_5 berries were not significant. This was likely related to the position of blocks within the parcel.

Table 2 - Effect of water treatment on berry mass at harvest

Block	2014	2015
	Berry mass (g)	Berry mass (g)
NI_1	1.23 ± 0.34 a	1.22 ± 0.41 bc
NI_2	1.30 ± 0.34 b	1.12 ± 0.37 a
I_3	1.35 ± 0.36 c	1.32 ± 0.42 d
I_4	1.31 ± 0.34 b	1.24 ± 0.37 c
I_5	1.32 ± 0.39 b	1.24 ± 0.39 c
NI_6	1.22 ± 0.33 a	1.15 ± 0.35 a

Berry mass is expressed as mean ± standard deviation of all berries belonging to nine bunches harvested within each block. The differences between blocks were tested with a one-way ANOVA. Different letters in the same column denote statistically significant differences at P<0.05 by Tukey's HSD test. **NI:** Non-Irrigated; **I:** Irrigated.

Similar results were obtained in 2015. Berries produced under weak water deficit were heavier compared to berries produced on vines facing moderate to severe water deficit, except for NI_1. Once again, berries from I_3 showed higher mass values compared to berries growing on all remaining blocks.

In order to confirm the significant effect of vine water status on berry size, average berry mass per bunch was plotted against average $\delta^{13}\text{C}$ values, measured berry by berry in the same samples. Linear regressions reported in figure 4, show the significant and negative relationship between $\delta^{13}\text{C}$ and berry mass at harvest, confirming that berries produced under limited water conditions are smaller compared to berries produced on irrigated plants. These results were obtained over the two experimental vintages. Despite the coefficient of determination of linear regressions being lower in 2015, relationships were highly significant in both years.

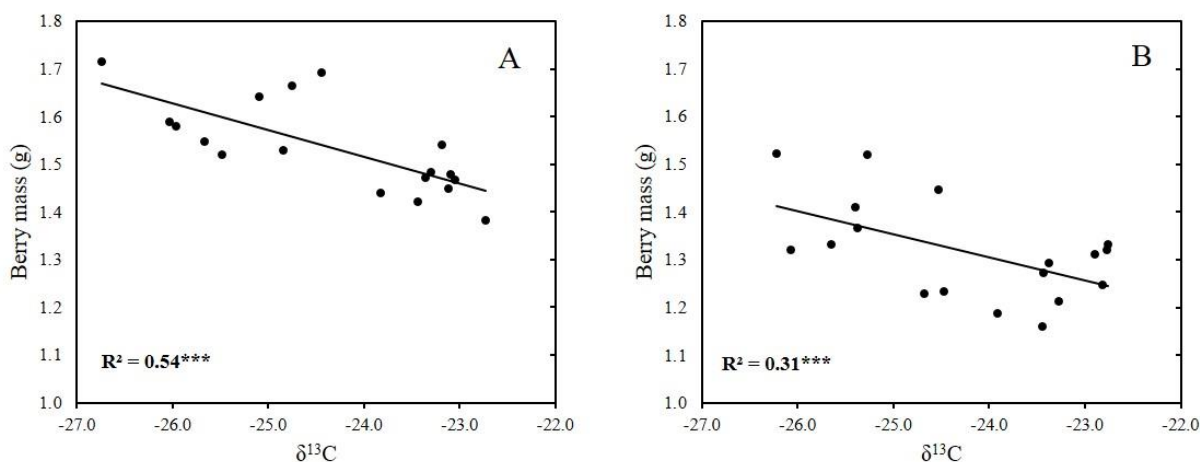


Figure 4 - Relationship between berry mass and $\delta^{13}\text{C}$, used as indicator of vine water status, in 2014 (A) and 2015 (B). Each point is the mean of six replicates.
* indicates the significance of the linear regression.

Vine water status and berry tissue masses

The effect of vine water status on berry tissue mass was investigated in 2015 only. In table 3, each berry tissue mass, analysed berry by berry, was expressed as absolute mass (g/berry) and relative proportion (g/g of berry * 100).

Table 3 - Berry tissue mass and their relative proportion measured at harvest, in 2015

Block	Seed mass (g)	Skin mass (g)	Flesh mass (g)	Seed proportion (% per berry)	Skin proportion (% per berry)	Flesh proportion (% per berry)	Skin:Flesh ratio
NI_1	0.084±0.05 a	0.108±0.03 b	1.182±0.35 ab	5.54	7.92	86.53	0.092±0.02 c
NI_2	0.079±0.04 a	0.090±0.02 a	1.052±0.26 a	6.36	7.61	86.03	0.088±0.01 c
I_3	0.084±0.04 a	0.095±0.03 ab	1.281±0.36 b	5.40	6.45	88.16	0.073±0.01 a
I_4	0.081±0.03 a	0.090±0.03 a	1.136±0.30 ab	5.97	6.95	87.08	0.080±0.01 ab
I_5	0.081±0.04 a	0.087±0.02 a	1.195±0.33 ab	5.75	6.50	87.75	0.074±0.01 a
NI_6	0.083±0.01 a	0.090±0.02 a	1.075±0.28 a	6.46	7.32	86.22	0.085±0.01 bc

Mass of each berry tissue is expressed as mean ± standard deviation of 54 berries belonging to three bunches per block. The differences between blocks were tested with a one-way ANOVA. Different letters in the same column denote statistically significant differences at $P < 0.05$ by Tukey's HSD test. The proportional mass of each tissue was determined as the ratio between their mass and berry mass, and expressed in percent.

NI: Non-Irrigated; I: Irrigated.

Seed mass per berry (g) did not vary among blocks. Similar results were observed with regard to skin mass (g), except for berries belonging to NI_1 block, which showed a significantly higher value. Not all differences in flesh mass between berries produced under different water conditions were significant. Only berry from I_3 showed significantly different higher values. Nevertheless, it was possible to observe some tendencies, indicating that flesh mass of berries produced under water deficit conditions is lighter. This result means also that flesh represents the only berry tissue being affected by limited water conditions.

Some differences appeared clearer when tissue mass was expressed as relative proportions of berry (g of tissue/g of berry, in %), comparing data from each treatment. Skin proportion, representing around 6.5-8% of total berry mass, significantly increased under water limited conditions. Seed represented 5.5-6.5% of total berry mass and was not significantly different among treatment. Confirming our first hypothesis, flesh represents a berry tissue clearly affected by vine water status. It was the main determinant of berry weight, representing 86-88% of total berry mass, with significantly lower values recorded on berries produced on non-irrigated vines. As a result, skin to flesh ratio was significantly different between berries belonging to non-irrigated and irrigated blocks. The latter showed a skin to flesh ratio significantly lower compared to ratio recorded on berries produced under stressed conditions.

Relationships between berry mass and mass of each tissue

The relationship between berry mass and skin, flesh and seed masses, was investigated only in 2015, by carrying out a study berry by berry. Results of this analysis are reported in figure 5, where each tissue mass (expressed as g/berry and as g/g of berry) is plotted against berry mass.

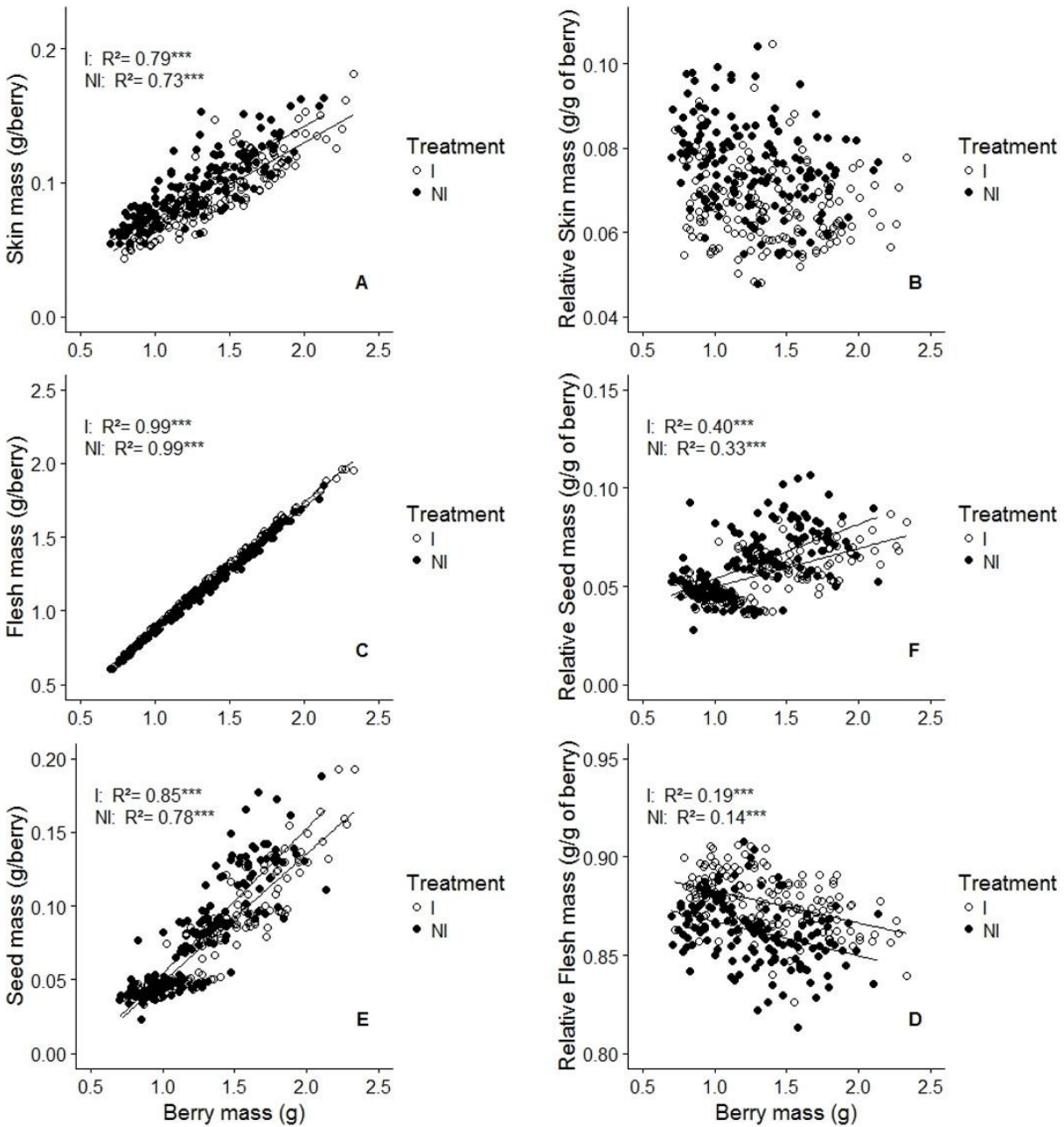


Figure 5 - Total skin, flesh and seed mass (g) per berry (A, C and E, respectively) and their relative mass (g/g of berry) (B, D and F, respectively), plotted as function of berry mass (g). Each point represent an individual berry; berries were sampled in three bunches per block. **I**: Irrigated, **NI**: Non-Irrigated. N= 54. *** indicates the significance of linear regression

Our results show that mass of skin, flesh and seed, expressed in g/berry, increases as berry mass increases (figure 5A, C and E). All tree relationships are highly significant, but the highest R^2 were obtained for the relationships between berry mass and flesh mass, confirming that among the berry tissues, flesh represents the largest proportion of mass. These relationships were similar for irrigated and non-irrigated vines. Nevertheless, the intercept varied between I and NI treatment for the relationship between skin mass and berry mass and the slope varied between I and NI treatment for the relationship between seed mass and berry mass.

Relationships between berry mass and relative proportion of each tissue showed different trends. Relative skin mass (g/ g of berry) did not vary with berry mass (figure 5B). Hence, its growth is coordinated to growth of other berry tissues. However, for a given berry mass, relative skin mass is significantly higher for NI treatment compared to I treatment. A slight slope was observed when relative flesh mass per berry was plotted against berry mass (figure 5D). Relative flesh mass significantly decreases as berry mass increases. For a given berry mass, relative flesh mass is significantly lower for NI treatment compared for I treatment. Relative seed mass best fitted with berry mass and increased proportionally to berry mass (figure 5F). On average, relative seed mass is significantly different between NI and I treatment. However, relative seed mass increase more rapidly with berry mass for NI treatment.

Because we found that skin to flesh ratio was higher on berries produced on water stressed vines, we decided to investigate the relationships between skin to flesh ratio and berry mass, calculated berry by berry (figure 6).

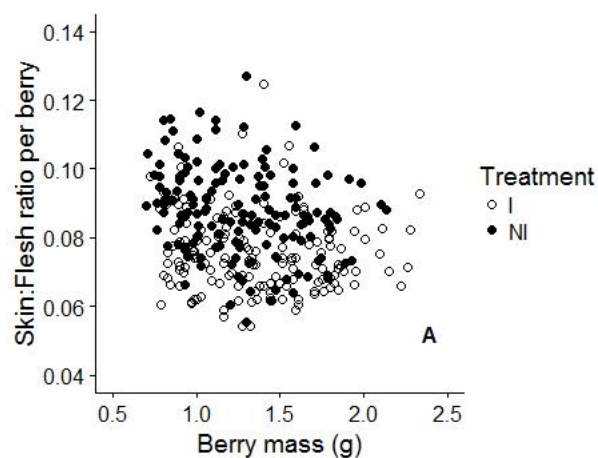


Figure 6 - Skin to flesh ratio per berry as a function of berry mass. Each point represent an individual berry; berries were sampled in three bunches per block.
I: Irrigated, **NI**: Non-Irrigated.

Results of this analysis showed that skin to flesh ratio did not change with berry mass, it remains constant as berry size increases. This was observed in both treatments. However, skin to flesh ratios were higher on berries facing severe water deficit.

Following, the average of skin to flesh ratio and $\delta^{13}\text{C}$ values for each experimental bunch (n=18) was calculated and one against the other plotted these variables together (figure 7).

Skin to flesh ratio increases proportionally to $\delta^{13}\text{C}$. In other words, berries produced under limited water status conditions, showing higher (less negative) values of $\delta^{13}\text{C}$, are characterized by higher skin to flesh ratio

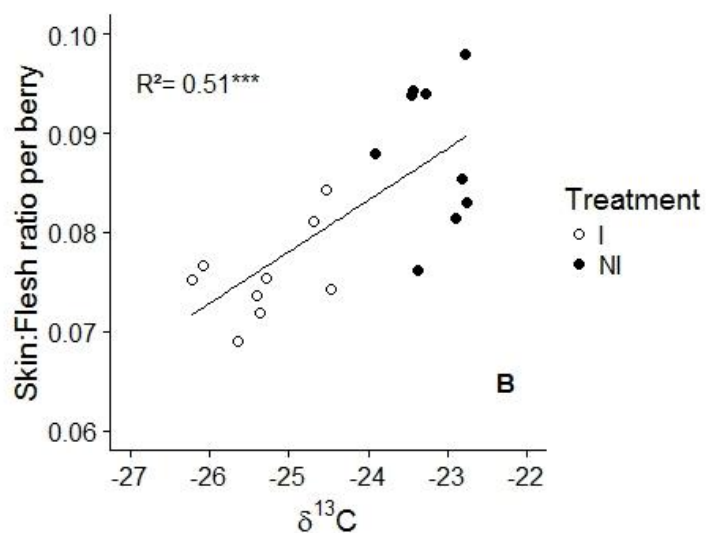


Figure 7 - Relationship between Skin to flesh ratio and $\delta^{13}\text{C}$

Each point represents a bunch; values are the mean of data collected berry by berry inside of each bunch.

I: Irrigated, NI: Non-Irrigated. n= 18. *** indicates the significance of linear regression

DISCUSSION

The present work was complementary to a study simultaneously carried out in the Bordeaux region (South-West of France), on the same grapevine variety (*Vitis vinifera* L. cv Cabernet franc). Its objective was to isolate the effect of vine water status on the final berry mass, with a special focus on berry tissue masses. Berry size was measured on irrigated vines and compared to a non-irrigated control.

Sicily is a region in the south of Italy characterized by a semi-arid climate. Seasonal weather conditions of the two vintages reflected the typical Mediterranean climate of these zones, although the 2014 summer had been particularly dry. Nevertheless, berry mass values, recorded at harvest, were lower in 2015. This was observed for berries produced on irrigated and non-irrigated vines. To explain this difference, the average seed number of berries produced during the two experimental vintages was investigated. Average seed number per berry was significantly higher in 2014 compared to 2015 (respectively 2.13 and 1.87). This result explains the fact that berries in the dryer 2014 vintage were heavier than berries produced in 2015. This result highlights the fact that final berry mass is the result of the combined effect of several factors (Fernandez et al. 2006; Coombe 1962).

Vines submitted to different irrigation treatments (Irrigated and Non-Irrigated) showed, at the end of each season, significantly different water status. Irrigated vines faced weak water deficit, while non-irrigated vines showed moderate to severe water deficit. These results were obtained both with stem water potential and $\delta^{13}\text{C}$. These two physiological indicators of vine water status were highly correlated (data not shown), confirming results reported in earlier studies (Gaudillère et al. 2002, van Leeuwen et al. 2009, Santesteban et al. 2012).

Berry mass at harvest was significantly different between blocks, depending on irrigation treatment. Severe water deficit in grape vines inhibited berry growth. This result, consistent with several previous studies (Ojeda et al. 2001, Roby and Matthews 2004, Roby et al. 2004, Chaves et al. 2007, Matthews and Nuzzo 2007, Ferrer et al. 2008, Girona et al. 2009, Attia et al. 2010, Ferrer et al. 2014), was confirmed by the significant relationships obtained between $\delta^{13}\text{C}$ and fresh berry mass.

Slight differences among blocks submitted to the same irrigation treatment were also observed. This fact was likely related to block position. The experimental parcel is characterized by a slope

of 4-6%, which varies within the parcel itself. Hence, I_3, located after I_5 and I_4, perhaps received a part of water volume from other blocks due to its position. This fact could explain why berries produced in I_3 were heavier. Similar hypothesis can be formulated with regard to NI_2, when, in 2014, mass of berries produced in this block, was heavier compared to berry mass of the other non-irrigated blocks.

In this study, neither skin mass, nor flesh mass, expressed as g/berry, were significantly affected by irrigation treatment, except in one or two rare cases. With regard to skin mass, similar results were obtained by Ojeda et al. (2002), who found that skin mass was affected only when the water deficit was applied between flowering and veraison, while even strong dehydration applied between veraison and harvest did not modify skin mass.

Despite the fact that in this study water deficit did not strongly affect skin and flesh mass per berry, vine water status conditions seemed to change their relative distribution within the berry (% of tissue per berry). Berries produced under severe water deficit showed higher and lower proportion of skin mass and flesh mass, respectively, compared to berries produced from irrigated vines. Although it is possible that water deficit stimulated post-veraison skin growth, it is more likely that expansive growth of the inner mesocarp was more inhibited by water deficits than was the skin tissue itself (Roby and Matthews 2004). As a result, vine water status affected the skin to flesh ratio of berries, which was higher under severely limited conditions. This result is consistent with Ojeda et al. (2002) and Roby et al. (2004). The latter reported that flesh growth was inhibited more than skin growth. Hence, under stress conditions, berry mass loss could be due almost exclusively to flesh mass loss, which is also reported by Ferrer et al. (2014).

Seed mass, expressed as % of tissue of the whole berry is not affected by vine water status conditions. This result is in contradiction with previous studies. Roby and Matthews (2004) and Attia et al. (2010) reported that water stressed grapevines showed significantly higher total seed mass compared to well-watered grapevines. Ferrer et al. (2014) found that seed mass decreased under drought stress, although this relationship varied according to the variety. Hence, further study is needed to understand the relationship between water deficit and relative seed mass per berry.

Total seed mass, skin mass and, in particular, flesh mass were strongly linearly and positively correlated to final fresh berry mass, which is consistent with earlier studies (Roby and Matthews

2004, Walker et al. 2005, Attia et al. 2010, Barbagallo et al. 2011, Ferrer et al. 2014). These relationships were not affected by vine water status. The increase in total seed mass per berry, observed also when their mass was expressed as relative proportion of total berry mass, was attributable to both seed size and seed number per berry (data not shown). The correlation between berry mass and berry seed content is explained by growth regulators supplied by the latter, which promote cell division and expansion (Ristic and Iland 2005, Walker et al. 2005, Friend et al. 2009, Gray and Coombe 2009).

In contrast, skin mass as a proportion of total berry mass was constant among berries of different size. Hence, relative skin mass was not affected by berry mass, which is consistent with Roby and Matthews (2004). However, for a given berry mass, relative skin mass was significantly higher in water deficit vines.

Relative flesh mass was affected by vine water status conditions and was lower in water deficit vines. Water deficit did not change the relationship between relative flesh mass and berry mass, which showed a slight decreasing trend with a similar slope but a different intercept for NI and I treatment. As a result of the coordinate growth of berry tissues and the consequent minor variations of their proportion among berry of different size, skin to flesh ratio was not affected by berry mass, remaining almost constant among berries. Our results show that skin to flesh ratio does not change when berries are produced by vines under similar water uptake regimes, be it well watered or water stressed. Similar results were reported by Roby and Matthews (2004) for well-watered vines. In those conditions (even water status), smaller berries are not likely to produce more concentrated wines.

When vines are subject to water deficit, berry flesh expansion is more restricted than berry skin expansion. Hence, skin to flesh ratio increase with water deficit. We obtained higher skin to flesh ratio in NI treatment compared to I treatment. This observation was confirmed by the highly significant correlation between $\delta^{13}\text{C}$ and skin to flesh ratio. This observation, obtained in field conditions, is consistent with results obtained by Ojeda et al. (2002) on potted vines. As a result, when berry size is restricted by water deficit, small berries are likely to produce more concentrated wines.

CONCLUSION

The experimental design of this study allows carrying out a focus on the impact of vine water status on final berry mass, recognized as one of the most important factors on berry growth and development in field conditions. The relationships between berry size and relative proportion of seed, skin and flesh tissues were also investigated. Our results showed that, independently from vine water status, growth of flesh and skin appears coordinated, leaving the relative skin mass per berry basically unchanged. Hence, small and large berries, produced under similar water conditions, are characterized by a similar skin to flesh ratio. Hence, the theoretical model, based on the assumption that skin mass per unit area remains constant independently on berry mass, is not validated. This observation implies that the propensity of flesh solutes to dilute skin solutes during winemaking is generally similar for various berry sizes.

However, berries produced under moderate to severe water deficit were smaller than berries produced under weak water deficit stress and showed higher skin to flesh ratio values, independently from berry mass. Hence, regulated water deficit could represent a useful instrument to increase the skin to flesh ratio, in order to limit the dilution of skin solutes during crushing and fermentation of wines and increase the concentration of most anthocyanins and skin flavanols, which are important compounds for the production of high quality red wines.

Chapter VI
General discussion and Conclusion

General discussion and conclusion

The aim of the present work was to study the origin of berry size variability at different scales and to understand the direct and indirect relationships between berry size and grape and wine composition, under different environmental conditions.

The study was carried out simultaneously on two different commercial vineyards located in the Saint-Emilion area (Aquitaine, South West of France) and Alcamo area (Sicily, South of Italy), during two consecutive growing seasons (2014 and 2015). The results obtained at both locations were complementary and allowed to reach the goals that were set.

Variability of berry size can be observed at different scales, because final berry mass is the result of the combined effect of several impacting factors. Depending on the observation scale, the hierarchy of these factors may vary. Most of previous works examined the effect of a single factor on berry size and/or berry composition. Moreover, in most cases, these studies were conducted under controlled experimental conditions. This approach does not allow to obtain a full understanding of berry mass and composition variability under field conditions, where environmental and plant related factors interact with each other. In this study, for the first time, factors impacting grape berry mass have been clearly hierarchized in field conditions.

The impact of vine water uptake conditions, vine nitrogen status and berry seed content on berry mass and its composition have been investigated at different scales: intra-bunch (berry by berry), intra-plant (bunch by bunch), intra-parcel (block by block) and inter-parcel. For each scale, with the aid of appropriated statistical models, we graduated the contribution of each factor and created a hierarchy of them according to their degree of impact.

This part of the study was carried out in experimental parcels located in Saint Emilion AOC, where vines were neither irrigated nor fertilized. Hence, their water and nitrogen status were exclusively dependent on variability in soil characteristics, which can be considered as a key environmental factor. Its impact on vine water and nitrogen uptake was clearly shown in this study. Moreover, a vintage effect on vine water status was also observed, confirming that the intensity and the timing of vine water deficit do not depend only on SWHC, but also on weather conditions of the year. Conversely, YAN level and berry seed content did not change in magnitude over the two experimental years.

In order to fit the best model, necessary to hierarchize the different factors according to their level of impact on berry mass, the variability of each considered variable was observed for each observation scale: block, plant and bunch.

Vine water status exhibited a significant variability at inter- and intra-parcel level, because it is highly dependent on soil water availability, which in turn reflects the soil characteristics. Thus, on the gravelly soil, characterized by a higher spatial variability in soil composition compared to a sandy soil, vine water status was variable at the intra-parcel scale. On the sandy soil, which was highly homogenous, block showed similar vine water conditions, but significantly different compared to blocks on the gravelly soil. In our experiment, vine water status was higher on all blocks of the sandy soil compared to the gravelly soil, except for G1 block (located on the gravelly soil). The latter, because of its soil characteristics showed similar vine water conditions to sandy soil in 2014, while in 2015, which was a warmer and drier vintage, G1 block showed an “intermediate” water condition compared to the remaining blocks. In this study, vine water status was also estimated berry by berry by $\delta^{13}\text{C}$. This innovative approach was used to study the berry water status. In this study, possible variability of this indicator within a bunch (berry by berry) and/or within a plant (bunch by bunch) was investigated. Our results show that the variability within a bunch is lower compared to variability within a vine, which, in turn, is generally lower than variability among vines inside a block and among blocks. Hence, we conclude that all berries belonging to the same bunch are subject to the same water uptake conditions, in relation to the SWHC of the block and the rooting depth of the studied vine.

Similar results were observed when YAN variability was investigated at different scales. YAN levels were highly variable among experimental parcels. Once again, the intra-parcel variability of this factor was low or not existing in the sandy soil. In our experiment vine nitrogen status was higher on the gravelly soil compared to the sandy soil, indicating that it was related to the soil type and to the soil depth. Without addition of nitrogen fertilizer vine nitrogen status depends on soil organic matter content and its mineralization rate. The latter increases with soil temperature and soil aeration increase. In this study, the gravelly soil is a warm and well aerated soil, favouring the turn-over of organic matter. The sandy soil is cooler and less well aerated, due to water logging during spring. These differences were confirmed by a significant relationship between vine water and nitrogen status, which was shown in this study.

YAN variability within a bunch was small in most cases. No consistent results were obtained when the possible relationship between YAN and berry mass was investigated at this scale. Thus, YAN values measured berry by berry reflected the real nitrogen availability of the block, depending on soil characteristics. Hence, these observations support our hypothesis that, as observed for $\delta^{13}\text{C}$, all berries of an individual bunch are submitted at the same nitrogen uptake conditions.

Among considered factors, intra-bunch level berry seed content showed high coefficient of variation (cv) values. However, when berry seed content was investigated at a larger scale, we observed that most berries contained one or two seeds. This was observed on all blocks and for both years. Hence, at the intra-parcel scale, this factor shows a low variability.

Variability berry mass and potentially impacting factors were finally investigated within a plant, by the comparison of average values computed bunch by bunch. Berry seed content shows low variability among bunches from one single plant and among different plants. In contrast, YAN and $\delta^{13}\text{C}$ level varied between bunches of the same vine and between bunches of different plants. Thus, at plant level, compared to bunch level, $\delta^{13}\text{C}$ and YAN variability increases, while berry seed content variability decreases. We investigated a possible effect of bunch position on the observed intra-plant variability of $\delta^{13}\text{C}$ and YAN among bunches. No consistent effect was evidenced. Hence, the origin of the variability of YAN and $\delta^{13}\text{C}$ variability among bunches of a single plant needs further investigation. The inter-block variability of $\delta^{13}\text{C}$ and YAN is higher compared to that observed at the intra-block and the intra-plant scale.

Differences in berry size within a parcel, within a block, within a plant and within a bunch, are the result of the combined impact of several factors. For a given scale of observation, berry mass variability reflects the variability of the most impacting factor. In this study, berry mass variability within a plant was lower than the one observed within a bunch. Bigger differences of berry mass were observed among blocks, in relation to variability in vine water and nitrogen status.

The major objective of this work was to create a hierarchy among the impacting factors on final berry mass, by quantifying the contribution of each covariate to the variance of berry mass. At intra-parcel scale, results of a multiple regression showed that vine water availability, vine nitrogen status and berry seed content impacted significantly the final berry size. Berry seed content was the less impacting factor among those considered. In contrast, vine water status was the most important factor. Its impact on fresh berry mass was much greater compared to the effect of

nitrogen availability. Moreover, a significant interaction between vine water and vine nitrogen status was also observed. The combined effect of these two impacting factors on fresh berry mass was particularly obvious on the G1 block. This block showed an “intermediate” profile compared to remaining blocks. It was characterized by an unlimited nitrogen supply during both experimental years and by a high water availability, which was limited however at the end of the 2015 growing season. Berries produced under these conditions were bigger compared to those produced on the other blocks. In contrast, berries produced under limited water and/or nitrogen conditions, were smaller. Berries produced on the sandy soil (limitation in nitrogen, not in water), were smaller than G1 berries, but bigger than G7 and G8 berries, where vines were subjected to severe water deficit. The influence of vine water and nitrogen status was highlighted by a vintage effect. In 2015, which was a warm and dry vintage, the inter-block differences were bigger and occurred early in the season.

Within a bunch berry mass was closely correlated with berry seed content. Conversely, at this level, no significant differences of $\delta^{13}\text{C}$ and YAN among berries of different sizes were observed. Results of the multiple regression model, performed in order to hierarchize the degree of impact of the three factors on berry mass, confirmed that, at bunch level, berry seed content is the most important determinant of berry mass, while neither $\delta^{13}\text{C}$ nor YAN have a significant effect on berry mass variability. Hence, although water and nitrogen conditions are known to affect berry growth, berry mass in a cluster seems to be homogeneously influenced by these factors. These observations support our hypothesis that all berries of an individual bunch are submitted to the same water and nitrogen uptake conditions.

Within a plant, no consistent results were observed when berry mass was plotted against $\delta^{13}\text{C}$ and YAN level bunch by bunch. In contrast, significant relationships were observed between berry seed number and berry mass, although, due to the low variability of this factor at this level, R^2 values were lower than those observed inside a bunch. Nevertheless, results obtained by multiple regression models, confirm that berry seed content is the driving factor of berry mass variability within a plant. The absence of a significant effect of nitrogen and water status within a plant supports the hypothesis that all bunches of a vine are submitted to the same water and nitrogen uptake conditions.

Although the relationship between berry size and grape composition is still a subject of debate, a large number of studies showed a significant impact of berry mass on berry composition. Another important goal of this study was to investigate the effect of berry mass on major berry compounds, in relation to the factors responsible of the berry mass variability.

Observing the accumulation and degradation curves of sugar and malic acid concentration during the ripening period, it was possible to conclude that berries produced under water deficit conditions, which were generally smaller, were characterized by higher sugar levels and lower malate levels. This result was confirmed when the relationships between berry composition and impacting factors were investigated by applying an appropriated statistical model. This approach allowed to hierarchize the factors impacting berry sugar and malate concentration, according to their degree of influence. Our results showed that, despite the fact that not all the covariates did significantly impact sugar and malic acid concentration simultaneously, vine water status was the most important factor.

The relationships between berry mass and grape composition at harvest, investigated within a bunch and within a plant were unclear. Conversely, when data were pooled together (independently from the bunch or plant origin), the relationships between berry mass and major berry compounds were consistent. Results obtained berry by berry showed that smaller berries are characterized by higher sugar and anthocyanins levels. This fact was particularly obvious in 2015 on bunches belonging to vines subjected to severe early water deficit. The effect of berry size on berry composition was also confirmed by results obtained by the analysis of grape juices extracted at harvest from berries belonging to a single parcel, sorted in two different size categories by Calibaie® machine. Smaller berries, independently from variety, soil and vintage, were characterized by higher sugar concentration levels and lower levels of malic acid.

The fact that, within a bunch and/or within a plant, the relationships between berry mass and grape composition was not consistent confirms that the way in which berry size is reduced is more important than the berry size itself. Thus, a part of the berry size effect on grape composition is an indirect effect, related to the factors responsible of berry size variability. In order to confirm this hypothesis, with the aid of appropriated statistical models, we separate the direct effect of factors impacting berry sugar and malate concentration from those mediated through their impact on berry size (i.e. indirect effect). Our statistical model series, implemented on data collected under field

conditions, allowed to prove that all factors had a direct impact on berry composition, independently from the one mediated through their impact on fresh berry mass.

Berry size also affected the concentration of some secondary metabolite of wines, such as polyphenols and flavor aroma compounds. This part of the study was carried out on experimental parcels located in the Saint Emilion area. Our results showed that most of wines made from small berries, sorted by Calibaie[®] machine, had higher flavanol and anthocyanin levels. This was particularly obvious in the warm and dry 2015 vintage, highlighting the hypothesis that the way in which berry mass is reduced has a bigger impact on berry composition than the direct impact of berry size itself. Moreover, wine made from small berries were characterized by higher levels of lactone compounds and, particularly, of massioa lactone compound. Most of the published works on berry diameter of red grape cultivars have focused on phenolic compounds. Few data are available concerning the effect of berry size on wine aroma compounds. Moreover, most of the published works were carried out on white varieties and considered other metabolites compared to aroma compounds investigated in the present study. Thus, for the first time, the relationship between berry mass and wine lactone concentration was shown.

Despite the fact that grape juice and wine composition seems to be impacted by berry size, the origin of these relationships is still to be understood.

In viticulture, it is often assumed that wines produced from smaller berries are higher in quality because of a higher skin to flesh ratio. In this study, the physical analysis of small and large berries does not show significant differences with regard to tissue mass. This was observed both in berry produced in Saint-Emilion AOC and in Alcamo DOC areas. On both sites, the study of the relationships between berry size and berry tissues shows that the growth of tissues of berries, produced under similar water uptake regimes (well-watered or water stressed), is coordinated. In fact, neither skin mass, nor flesh mass, expressed as g/berry, were significantly affected by irrigation treatment. As a result of the coordinated growth of berry tissues and the consequent minor variations of their proportion among berries of different sizes, skin to flesh ratio remains approximately constant as berry volume increases, invalidating to the theoretical relationship between the surface and the volume of a sphere. This observation implies that the propensity of flesh solutes to dilute skin solutes during winemaking is generally similar for various berry sizes.

Our hypothesis that the way in which berry mass is reduced has a bigger impact on berry composition than the direct impact of berry size itself, was confirmed by results obtained by the experiment carried out on parcels located in the Alcamo DOC area. This study, in fact, was performed in order to carry out a focus on effect of wine water deficit on berry growth and, in particular, to obtain clear results on its impact on berry tissue development and their relationship. Berry mass at harvest was significantly affected by irrigation treatment. Severe water deficit in grape vines inhibited berry growth.

Vine water status changes the relative distribution of the berry tissues (% of tissue per berry). For a given berry mass, berries produced under severe water deficit showed higher and lower proportion of skin mass and flesh mass, respectively, compared to berries produced from irrigated vines. Although it is possible that water deficit stimulated post-veraison skin growth, it is more likely that expansive growth of the inner mesocarp was more inhibited by water deficits than was the skin tissue itself. As a result, vine water status affected the skin to flesh ratio of berries, which was higher under severely limited conditions. Hence, regulated water deficit (either happening naturally or induced by deficit irrigation) represents a useful instrument to increase the skin to flesh ratio, in order to limit the dilution of skin solutes during winemaking and increase the concentration of most anthocyanins and skin flavanols, which are important compounds for the production of high quality red wines.

This would mean that, for a given vintage, small and large berries, produced from a single parcel with homogenous water conditions, tend to have similar enological profiles. In contrast, in the parcels where the spatial variability of water availability is high, small berries are likely to have significant compositional differences compared to large berries. This is more likely to happen in a dry vintage. This conclusion is confirmed by results obtained berry by berry in the Saint-Emilion experimental site. They showed that the negative relationship between berry size and anthocyanin concentration per berry, was particularly obvious on bunches belonging to vine subjected to severe early water deficit stress and especially in 2015, which was a dryer vintage. Thus, the hypothesis that the way in which berry size is reduced is more important than the berry size itself with regard to grape composition is confirmed.

In this study we demonstrated that the final berry size is the result of the integrated effect of biotic and abiotic factors. The results of this work indicate that when the variability of berry mass is

driven by the variability of berry seed content, which was the case at intra-bunch or intra-plant level, the berry mass effect on grape composition seems to be only marginal. Conversely, when the variability of berry mass is driven by external factors, varying at large scales and from one vintage to another, the berry mass effect on grape composition is more obvious. The performances of the Calibaie[®] machine, evaluated by the measurement of physical berry parameters, showed satisfactory results. However, the berry sorting was not always perfect. This fact is probably linked to the setting of the parameters of the machine. Thus, in order to improve the efficiency of the machine, grapevine growers could perform a simple preliminary study of berry size distribution (vintage by vintage, parcel by parcel) and set the machine parameters according to their production goals.

The objective of this study was to investigate possible compositional differences between wines made from berries of different sizes. Our results show that this is the case on a heterogeneous parcels, but much less so when vine water uptake and vine nitrogen status is homogenous inside a parcel. However, we do not necessarily consider that “small berries make superior red wine”, because grape growers have to judge the potential quality of berries according to their production goals.

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