MENDOZA, ARGENTINA
APRIL 29, 2014

# NEW OUTLOOK IN VITICULTURE AND THE IMPACT ON WINE QUALITY

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LALLEMAND

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PROCEEDINGS
OF

THE *XXVes ENTRETIENS SCIENTIFIQUES LALLEMAND*

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# FOREWORD

At the ***XXVes Entretiens Scientifiques Lallemand***, viticulture experts updated attendees on current knowledge in viticulture science. The session opened with Alain Deloire from the National Wine and Grape Industry Centre of Charles Sturt University in Wagga Wagga, Australia, who discussed the complexity of fruit composition, including its impact on wine style and consumer preferences. He also discussed vine physiology and the genetic factors influencing grape quality. His introduction to the topic of viticulture gave a great overview of this very complex science. Jorge Perez Peña, from INTA EEA Mendoza, gave an excellent talk on Argentinean viticulture and the many challenges faced by vine growers – most importantly, climate change – and how to adapt to this reality. Heat waves in particular are affecting vineyards, and different strategies are being studied at his research station. Andrew G. Reynolds from the Cool Climate Oenology and Viticulture Institute at Brock University in Canada presented on the different aroma compounds and precursors in grapes and how they are affected by viticultural practices. A summary of the impact of cluster exposure, vineyard irrigation, nitrogen status, fruit exposure, grape maturity, and how these affect the levels of volatile thiols, terpenes, methoxypyrazines, norisoprenoids and volatile phenols was presented.

Hernán Ojeda, also from Argentina but now a researcher at INRA Unité expérimentale de Pech Rouge, in France, discussed the main factors affecting grape and wine characteristics – from biological and natural factors to crop-related issues – and how different solutions need to be researched in order to optimize grape quality in light of these impacts. Gerard Casaubon, the director of Centro de Investigación e Innovación Concha y Toro and Centro de Aromas y Sabores DICTUC in Chile, presented work focused on the other end of the wine spectrum – key aroma compounds and how they affect consumer preferences. In but one example, he discussed how thiols, which are influenced by viticultural practices and other factors, are rated by consumers and orient their choices.

Fernando Zamora from Universitat Rovira I Virgili in Spain began his presentation by describing the changes to anthocyanin and tannin concentrations in red grapes throughout ripening. He then explained the various methods that exist for determining phenolic maturity (e.g., Glories, ITV, AWRI, Cromoenos, etc.), stating the pros and cons of each. He also presented various experimental results from his research group to illustrate the influence of grape phenolic maturity on the composition and quality of red wines.

To close the day, José Ramón Lissarrague of Universidad Politécnica de Madrid presented the results of a study done by his research group on the impact of yeast derivatives on the phenolic maturity and aroma profiles of wine when applied in the vineyard. His studies show that, while the topic is completely new and research is underway, there is a definite trend toward better phenolic maturity, more aromatic complexity and heightened sensory preference upon tasting. A bright future is in store for natural vineyard products.

Learning about viticulture and understanding the concerns and limitations winegrowers face in producing quality grapes was the aim of the ***XXVes Entretiens Scientifiques Lallemand***.

# CONTENTS

# SEQUENTIAL HARVEST AND WINE STYLE: WHAT DOES THE FRUIT PHYSIOLOGICAL INDICATOR HAVE TO OFFER?

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## Abstract

The optimal maturity of grapes depends on multi-faceted criteria. Water status, light (quality and quantity) and temperature (day and night, heat waves) may hasten, delay or enhance ripening. A berry will regulate genes responsible for the biosynthesis of specific compounds (phenolics, aromatic precursors, hormones, organic acids, amino acids, etc.), while other compounds are provided by roots and/or leaves (water, minerals, sugar, nitrogen, etc.). For some compounds, such as tannins, one has to consider the evolution of the structure and the binding of these molecules over the fruit growth and ripening period. The volume of the fruit could determine the concentration or dilution of these compounds. Optimal grape ripeness is defined according to the desired wine style, which in turn is dictated by market demand or by the objective of producing a wine that expresses the typicity of the location. Professionals in the wine sector are therefore obliged to accurately characterize the grapes in order to make an informed decision about the optimum harvest date, and to adapt the winemaking process to obtain the targeted wine style.

The evolution of sugar accumulation per berry in red grape berry cultivars gives an indication of the ripening process from a new perspective. This indicator aims to help winegrowers and winemakers predict the grape harvest dates that will deliver optimum results according to the wine style. Depending on the variety, the harvest can be planned 10 to 40 days before it is due to start, allowing producers to plan ahead for harvest and wine production. This article will present the method developed for the red cultivars, and provide some information on berry development.

## Introduction

The grapevine is an important perennial crop in the world (7.6 million hectares under production), and complex but poorly understood processes occur in the grapevine during berry growth and development. In particular, the determination of final fruit composition from flowering to veraison and from veraison to ripening requires further investigation. It is known that berry development follows a double-sigmoid growth pattern consisting of two distinct growth phases separated by a lag phase (figure 1) (Coombe 1992). Several compounds accumulate during the first growth period, such as tannins, amino acids and some aroma compounds, including methoxypyrazines in the Sauvignon Blanc, Cabernet Sauvignon and Merlot cultivars (figure 2) (Kennedy et al. 2001, Ollat et al. 2002, and Šuklje et al. 2012). Berry growth and organic acid accumulation will cease during the lag phase. According to Terrier et al. (2005) and Pilati et al. (2007), the most significant changes in gene expression occur during the 24-hour transition phase between the lag phase and veraison (i.e., individual berry softening and the onset of ripening). Dal Santo et al. (2013) showed the same effect on Corvina grapes via a study on the plasticity of the berry transcriptome using a network of commercial vineyards to assess the effect of the site (soil x climate) and the vintage. The data of this study suggested that, "veraison is a critical period during which the seasonal climate has its greatest effect whereas the microenvironment and agronomic practices had only marginal impact." The impact of agronomic practices and environmental conditions seemed to have an important effect during ripening. Šuklje et al. (2012 and 2014) demonstrated that canopy manipulation had a considerable effect on fruit and wine composition, which influenced the final wine sensory profile. Leaf removal was practised on Sauvignon Blanc at an early stage of berry growth (berry pea size), and methoxypyrazine and thiol levels were shown to be influenced.

A recent study from Rienth et al. (2014) showed changes in berry gene expression during night development that differed from daytime development. This emphasizes the importance of measuring both day and night temperatures over the entire growth period when the effect of the climate on fruit metabolism and vine physiology is being investigated.

All the above-mentioned studies have demonstrated the importance of the environment for the final grape composition at harvest.

The ideal timing of the harvest is currently being decided by viticulturists and winemakers using the following criteria:

- According to a single criterion, the measurement of total soluble solids expressed as Brix. This requires simple, routine analysis and is the most commonly used indicator in the wine industry today.
- According to berry tasting. This can be relevant but highly subjective as the decision is influenced by the taster's personal experience and training.
- Using a series of indicators and appropriate analysis methods (anthocyanins, titrable acidity, tannins, etc.). This implies that the necessary equipment is available at the estate or at an appropriate laboratory nearby. Knowledge in interpreting analytical results to make the appropriate decision is therefore required. The cost per analysis and per hectare has to be considered.
- To harvest using new decision-making tools and taking into consideration new scientific results. This implies the ability to access the information, understand and assimilate it, then implement it successfully (extension and adoption process). In addition, the ability to afford this new technology, which may be expensive, has to be considered.

This list is not complete. It is also important that skills and information are transferred to the individuals who are using these methods to determine the harvesting date. Such skills include an appropriate sampling procedure, the use of analytical equipment and the ability to interpret the analytical data.

Geographical origin is important for products that lay claim to a *terroir*-linked typicality. Measuring the *terroir* effect on an agri-food product remains difficult for both trained experts and consumers, for whom the appreciation of the product or lack thereof remains the principal criterion in their evaluation. This does not exclude the ability to recognize the product's properties, but it should be remembered that the perceived taste and aromas will be transformed by the individual's experience into a unique overall sensory impression (Deloire et al. 2008).

The quality of the grapes is a determining factor in the quality of the finished wine. But how is grape quality itself determined? What are the relevant parameters of the berry that enable the dynamics of ripening to be monitored?

One of the most important and difficult parts of a viticulturist's and winemaker's job is to predict the wine style from the berries and the oenological process. The classical indicators like Brix, malic and tartaric acids, titratable acidity, tannins, anthocyanins, etc. are relevant indicators of grape ripeness. They are strongly related to the perception of the taste of the wine (mouthfeel), but they give no indication on wine aromatic profile. Therefore, it would be also highly useful to predict or predetermine the future wine style in terms of aromatic profile using fruit-related physiological indicators.

Berry ripening, wine flavours and the elaboration of aromatic low-alcohol wines are, today, among the priorities of the worldwide wine industry, mainly in the context of climate change (i.e., the increase of temperature and unpredictable heat wave events) and scarcity of water.

## Proposed method for red cultivars

This method is based on the indirect relations established between the use of a fruit physiological indicator (i.e., sugar accumulation per berry) and the possible wine styles determined by wine sensory analyses or tasting sessions. The method is based on sequential harvests to understand the relation between the harvest time and the wine composition and profile (Bindon et al. 2013, Deloire, 2011, 2013, and Wang et al. 2003) (figure 3). Sequential harvests using the fruit sugar accumulation profile helped determine four stages during ripening (figure 4).

As shown in figure 4 for Syrah and Cabernet Sauvignon, stage 1 (fresh fruit) always occurs from 12 to 20 days onwards after the sugar per berry has reached a plateau (the termination of berry sugar loading or the slowdown of berry sugar accumulation). Stage 3 (mature fruit) always occurs from 24 to 40 days onwards after the sugar per berry has reached a plateau (figure 4). Between the fresh and mature fruit stages, stage 2 is called neutral (i.e., a deficiency of fruitiness in the related wine, which can enhance the perception of "spicy" and "peppery" characteristics in Shiraz wine), or premature (in some situations, there is an overlap between fresh and mature fruit stages) and may vary according to the site (climate and soil) and cultural practices. In most situations, it is recommended to avoid stage 2 when it is considered neutral as the related wines will show a deficiency of fruitiness and could be judged as one-dimensional. Stage 2 can be determined/predicted using the sugar-loading method. Interestingly, there is a market for "spicy" and "peppery" Shiraz wines produced in Australia, for example in the Grampians region of Victoria (Scarlet et al. 2014). It is suggested that stage 2 of the berry's aromatic evolution could be targeted for this style of wine, meaning that the harvest date chosen according to the relevant physiological indicator could help achieve these production goals.

There is no direct relationship between fruit Brix or titrable acidity levels and the berry aromatic profile evolution, meaning that fresh, neutral and mature stages could be reached at approximately the same sugar concentration. In that regard, the model showed that to harvest using only the Brix value cannot really help predict the harvest date and wine style. (See figure 1 on this page, and figures 2, 3 and 4 on the next pages)

No doubt climate change will influence berry ripening and this may have repercussions for the time of harvest and the style of wine produced. The concept of grape quality at harvest should be considered in terms of the required fruit and wine composition and the winemaking process, resulting in wine with particular sensory properties. Numerous important studies have recently looked at the relationship between grape and wine composition and the wine's aromatic profile (Ristic et al. 2007, Kalua and Boss 2009 and 2010, Sweetman et al. 2012, and Capone et al. 2012), whereas other studies have emphasized some wine markers potentially linked to wine aromatic maturity (Pineau et al. 2009, Lytra et al. 2012, Dubourdieu et al. 2012, and Pons et al. 2013). Despite all these important new insights, the topic is so complex that the relationship between the grape and wine composition, and the wine's sensory profile associated with the different stages of fruit maturity remain poorly understood. The role of the fermentation processes should definitely be considered as strongly impacting the wine composition and sensory profiles.

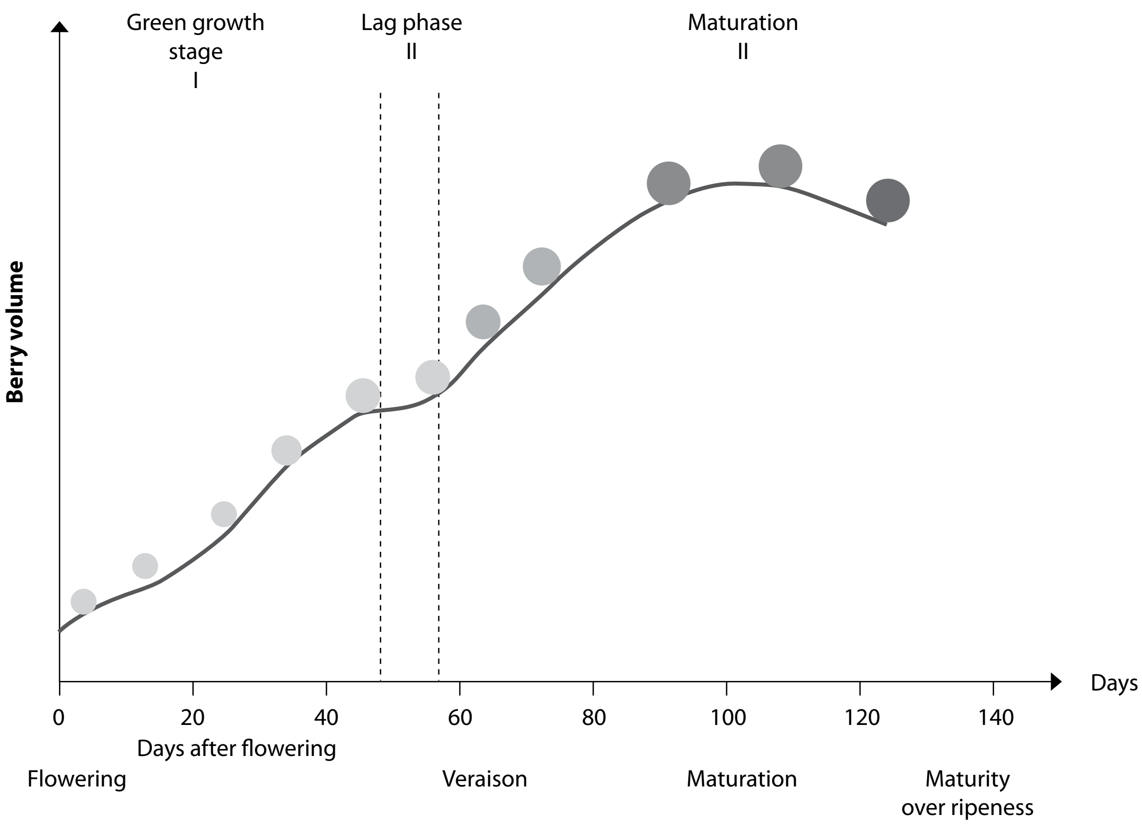


**Figure 1.** Curve of berry growth from flowering to harvest showing the three stages (green growth stage, lag phase and maturation)

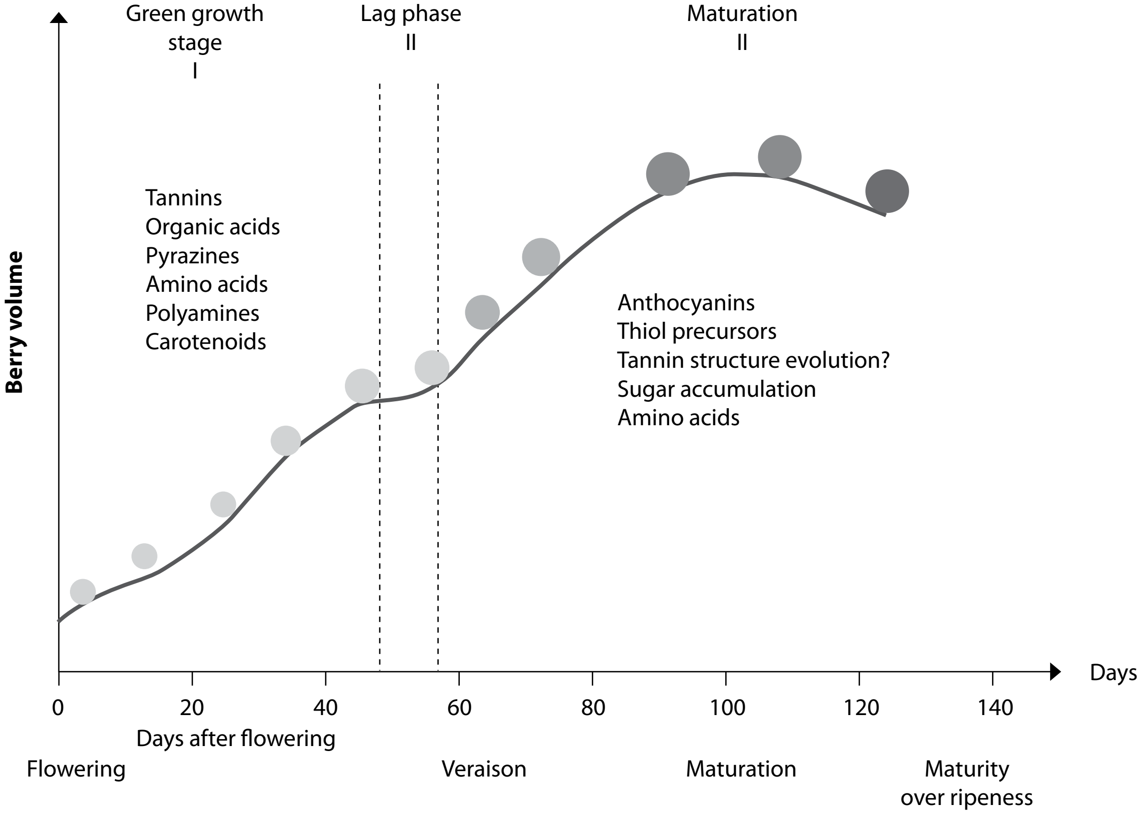


**Figure 2.** Examples of compound biosynthesis during fruit development

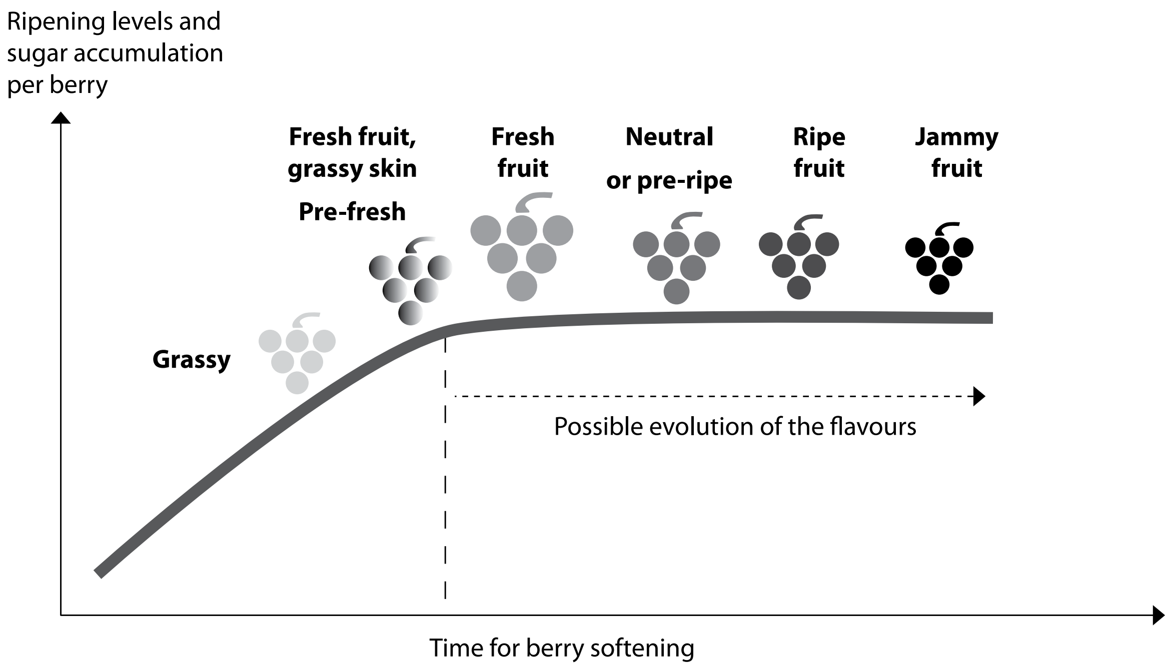


**Figure 3.** A proposed sequence of ripening for red cultivars and possible relations between grape sequential harvest times and wine aromatic profiles

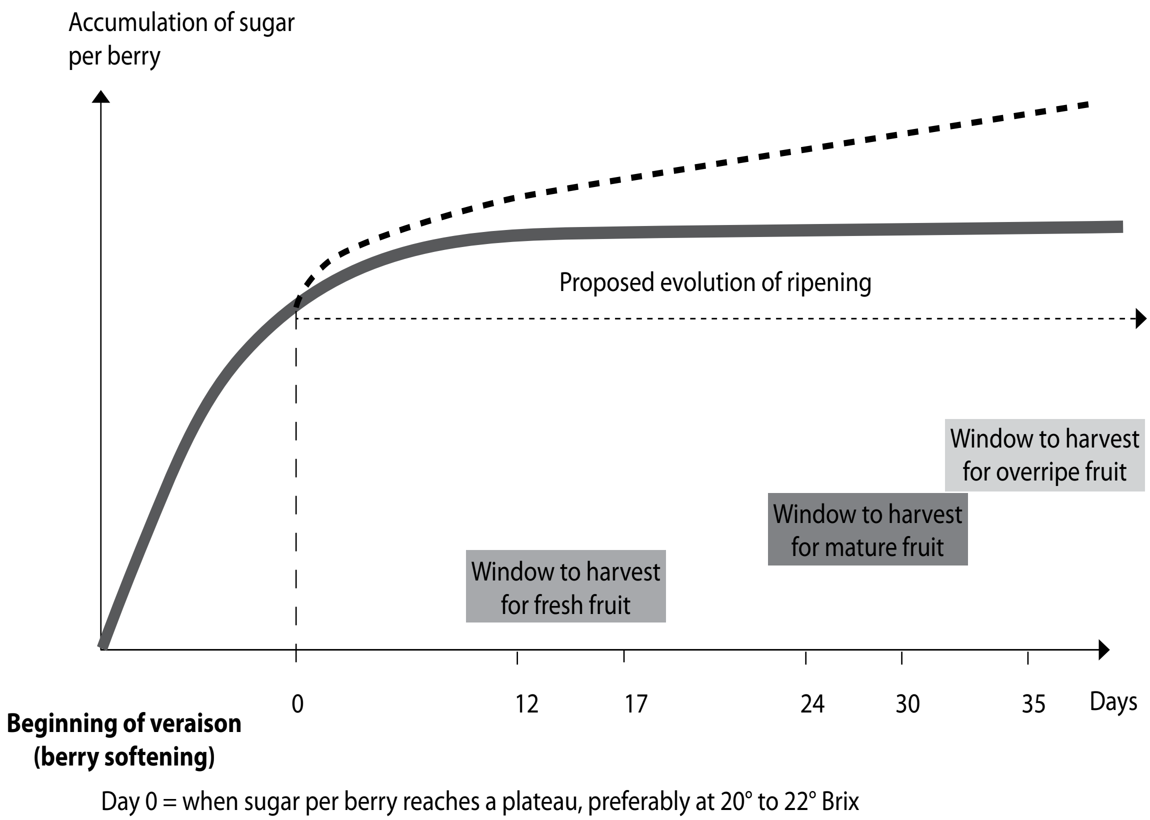


**Figure 4.** Example of Syrah ripening evolution, using sequential harvest. A vine or a vineyard is said to be "in balance" when sugar accumulation stops or slows down around 20° to 22° Brix, 20 to 30 days after berry softening. It is site- and cultural practices-related

The method presented in this article is not exclusive and needs further investigation. It is important to understand the relation between fruit and wine composition and wine style using sequential harvest dates.

## Acknowledgments

We thank Distell, Stellenbosch University (DVO) and Winetech for funding and helping develop the methods for South Africa, and GWRDC (Australia) for funding a project on fruit and wine composition and wine style and predicting the harvest date. We thank Vivelys (France) for providing some of its data and expert knowledge.

## References

Bindon, K., C. Varela, J. Kennedy, H. Holt, and M. Herderich. 2013. Relationships between harvest time and wine composition in *Vitis vinifera* L. Cv. Cabernet Sauvignon 1. Grape and wine chemistry. *Food Chemistry*. 138:1696-1705.

Capone, D. L., D. W. Jeffery, and M. A. Sefton. 2012. Vineyard and fermentation studies to elucidate the origin of 1,8-Cineole in Australian red wine. *Journal of Agricultural and Food Chemistry*. 60:2281-2287.

Coombe, B. G. 1992. Research on development and ripening of the grape berry. *Am. J Enol Vitic.* 43:101-110.

Dal Santo, S., G. V. Tornielli, S. Zenoni, M. Fasoli, L. Faina, A. Anesi, F. Guzzo, M. Delledonne, and M. Pezzotti. 2013. The plasticity of the grapevine berry transcriptome. *Genome Biology*,

Deloire, A. 2011. The concept of berry sugar loading. *Wineland*. 257:93-95.

Deloire, A. 2013. Berry ripening and wine aroma. *Practical Winery & Vineyard*. April, 1-2.

Deloire, A., P. Prevost, and M. T. Kelly. 2008. Unravelling the terroir mystique – An agro-socio-economic perspective. *Cab Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources.* 3(32).

Dubourdieu, D., A. Pons, and V. Lavigne. 2012. Le vieillissement prématuré de l'arôme des vins rouges : identification de nouveaux marqueurs. *Proceedings of Colloque arômes du vin*, Projet VINAROMAS, November 20, 2012, Toulouse, France; November 22, 2012 Zarogoza, Spain (http://www.vignevin-sudouest.com/publications/itv-colloque/documents/actes-complets-francais-espagnol.pdf) 27-30.

Kalua, C. M., and P. K. Boss. 2009. Evolution of volatile compounds during the development of Cabernet Sauvignon grapes (*Vitis vinifera* L.). *Journal of Agricultural and Food Chemistry*. 57:3818-3830.

Kalua, C. M., and P. K. Boss. 2010. Comparison of major volatile compounds from Riesling and Cabernet Sauvignon grapes (*Vitis vinifera* L.) from fruitset to harvest. *Aust. J. Grape Wine Res*. 16:337-348.

Kennedy, J. A., Y. Hayasaka, S. Vidal, E. J. Waters, and G. P. Jones. 2001. Composition of grape skin proanthocyanidins at different stages of berry development. *Aust. J. Grape Wine Res*. 49:5348-5355.

Lytra, G., S. Tempere, G. de Revel, and J. C. Barbe. 2012. Impact of perceptive interactions on red wines fruity aroma. *Journal of Agricultural and Food Chemistry.* 60:12260-12269.

Ollat, N., P. Diakou-Verdin P, J. P. Carde, F. Barrieu, J. P. Gaudillere, and A. Moing. 2002. Grape berry development: a review. *Journal International des Sciences de la Vigne et du Vin*. 36(3):109-131.

Pilati, S., M. Perazzolli, A. Malossini, A. Cestaro, L. Dematté, P. Fontana, A. Dal Ri, R. Viola, R. Velasco, and C. Moser. 2007. Genome-wide transcriptional analysis of grapevine berry ripening reveals a set of genes similarly modulated during three seasons and the occurrence of an oxidative burst at veraison. *BMC Genomics*. 8:428.

Pineau, B., J. C. Barbe, C. Van Leeuwen, and D. Dubourdieu. 2009. Examples of perceptive interactions involved in specific "red-and-black-berry" aromas in red wines. *Journal of Agricultural and Food Chemistry*. 57:3702-3708.

Pons, A., V. Lavigne, P. Darriet, and D. Dubourdieu. 2013. Role of 3-methyl-2,4-nonanedione in the flavor of aged red wines. *Journal of Agricultural and Food Chemistry*. 61:7373-7380.

Rienth, M., L. Torregrosa, M. T. Kelly, N. Luchaire, A. Pellegrino, J. Grimplet, and C. Romieu. 2014. Is transcriptomic regulation of berry development more important at night than during the day? *PLoS ONE* 9(2):1-9.

Ristic, R., M. O. Downey, P. G. Iland, K. Bindon, I. L. Francis, M. Herderich, and S. P. Robinson. 2007. Exclusion of sunlight from Shiraz grapes alters wine colour, tannin and sensory properties. *Aust. J. Grape Wine Res*. 13:53-65.

Scarlett, N. J., R. G. V. Bramley, and T. E. Siebert. 2014. Within-vineyard variation in the 'pepper' compound rotundone is spatially structured and related to variation in the land underlying the vineyard. *Aust. J. Grape Wine Res*. 20:214-222.

Šuklje, K., G. Antalick, Z. Coetzee, L. M. Schmidtke, H. Baša Česnik, J. Brand, W. Du Toit, K. Lisjak, and A. Deloire. 2014. Effects of leaf removal and ultraviolet radiation in the vineyard on the composition and sensory perception of Sauvignon Blanc (*Vitis vinifera* L.) wine. *Aust. J. Grape Wine Res*. 20:223-233.

Šuklje, K., K. Lisjak, H. Baša Česnik, L. Janeš, W. Du Toit, Z. Coetzee, A. Vanzo, and A. Deloire. 2012. Classification of grape berries according to diameter and total soluble solids to study the effect of light and temperature on methoxypyrazine, glutathione, and hydroxycinnamate evolution during ripening of Sauvignon blanc (*Vitis vinifera* L.). *Journal of Agricultural and Food Chemistry*. 60:9454-9461.

Sweetman, C., D. C. J. Wong, C. M. Ford, and D. P. Drew. 2012. Transcriptome analysis at four developmental stages of grape berry (*Vitis vinifera* cv. Shiraz) provides insights into regulated and coordinated gene expression. *BMC Genomics*. 13:691.

Terrier, N., D. Glissant, J. Grimplet, F. Barrieu, P. Abbal, C. Couture, A. Ageorges, R. Atanassova, C. Leon, J. P. Renaudon, F. Dédaldéchamp, C. Romieu, S. Delrot, and S. Hamdi. 2005. Isogene specific oligo arrays reveal multifaceted changes in gene expression during grape berry (*Vitis vinifera* L.) development. *Planta*. 222(5):832-847.

Wang, Z. P., A. Deloire, A. Carbonneau, B. Federspiel, and F. Lopez. 2003. An *in vivo* experimental system to study sugar phloem unloading in ripening grape berries during water deficiency stress. *Annals of Botany*. 92:523-528.

# IMPACT OF VINEYARD MANAGEMENT ON GRAPE MATURITY: FOCUS ON TERPENES, PHENOLICS AND OTHER SECONDARY METABOLITES

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## 1. Introduction

All plants, grapes included, possess a multitude of primary biochemical pathways that are essential for their growth and survival. These include well-known processes such as respiration, photosynthesis, photorespiration, nitrogen metabolism, sulphur metabolism and many others. However, there are numerous other pathways that are utilized by plants to create products that are collectively known as secondary metabolites. These include monoterpenes, phenolics and anthocyanins, norisoprenoids, aliphatic hydrocarbons (esters, aldehydes, alcohols, ketones), alkaloids and perhaps others. None of these compounds are essential for plant survival; however, they are well known to enhance the plant's ability to adapt to its environment—many of these compounds provide the basis for insect or disease resistance, prevention of herbivory, attraction of pollinizing and/or beneficial insects and a plethora of other functions. In wines, many of these secondary metabolites provide the basis for quality—monoterpenes, norisoprenoids and some hydrocarbons are odour-active substances, many phenolics are responsible for bitterness, astringency and wine mouthfeel, and anthocyanins are essential for colour.

## 2. General Impacts of Viticultural Practices on Grapes and Wines

### 2.1 Effects of fruit exposure

The effects of cluster shading on fruit composition can be quite substantial. Enhancement of cluster exposure will increase berry temperature and will lead to upregulation of crucial enzyme systems. Two seminal studies are those of Kliewer and Lider (1968) with Thompson Seedless and Koblet et al. (1977) with Pinot Noir. Both studies found that fruit exposure increased soluble solids (%SS), reduced titratable acidity (TA) and increased pH. Reynolds et al. (1986) found a sizable (1.2ºBrix) difference between shaded and exposed Seyval Blanc clusters on vines of identical training and crop level. They also noted as much as 12ºC difference in berry temperature between western-exposed berries and shaded berries, which diminished the diurnal temperature flux of the exposed berries. Greater malate degradation also occurred in exposed clusters. Similar studies on the effect of cluster shading found higher %SS in clusters of Cabernet Sauvignon from sun-exposed regions of the canopy (Crippen and Morrison 1986a, b). The shaded berries were larger and had higher water content than the exposed berries, effectively lowering %SS. Pre-harvest, anthocyanins were higher on both a berry weight and per berry basis, but by harvest there were no differences (Crippen and Morrison 1986a, b).

Bergqvist et al. (2001) assessed the effects of sunlight exposure on berry growth and composition of two red wine grape cultivars (Cabernet Sauvignon and Grenache) grown in the central San Joaquin Valley of California. Sunlight exposures ranged from mid-day photosynthetically active radiation (PAR) < 10 μmol·m-2·sec-1 (shaded) to > 600 μmol·m-2·sec-1 (fully exposed) from berry set to harvest. Experimental clusters were evenly distributed between the north (afternoon shaded) and south (afternoon exposed) sides of the canopy. Fruit response to sunlight varied based on cluster location within the canopy, and these results were at least partially due to large differences in berry temperature. At the same exposure level or PAR, mid-day berry temperature was generally 3 to 4°C greater for clusters on the south side of the canopy compared to clusters on the north. %SS initially increased with greater sunlight exposure, then declined when mid-day PAR exceeded 31 to 50 and 51 to 100 µmol·m-2·sec-1 respectively for clusters on the north and south sides of the canopy. Juice TA generally declined as sunlight exposure increased, with Cabernet Sauvignon clusters on the north side of the canopy maintaining greater acidity at the same exposure level as clusters on the south. Juice pH declined as exposure increased on the north side of the canopy, while sunlight had little effect on pH for clusters on the south. These results suggested that the effects of light on fruit composition are heavily dependent upon the extent to which berry temperature is elevated because of increased sunlight exposure.

### 2.2 Effects of growing season canopy management

Vertically shoot-positioned canopies are typically hedged at the top and sides of the canopies to eliminate overhanging shoots, reduce canopy width and generally reduce shade in dense canopies. Normally this is done about two weeks after fruit set, but can be repeated one or more times during the growing season. Basal leaf removal is generally done one week or more after hedging by removing the lower two to four leaves at the base of the shoot to expose the clusters. This can be done on both sides of the canopy, particularly in the case of red wine cultivars and during wet growing seasons; typically it is done on the least sunny side (east or north side in the Northern Hemisphere) to avoid the possibility of sunburning the fruit.

Research into the potential effects of hedging and leaf removal on potential wine quality has been quite recent. Many of these studies measured standard fruit composition variables and not aroma compounds. A New Zealand study unequivocally found that basal leaf removal (either 50% or 100% of leaves in fruit zone) increased both total phenol and anthocyanin concentrations in Cabernet Sauvignon, with the greatest increase (≈50% over control) occurring when the treatment was done five weeks after flowering (Smith et al., 1988). Mazza et al. (1999) also found that leaf removal resulted in higher phenolic concentration and colour density over the control. Numerous studies on basal leaf removal have been performed on aromatic white wine cultivars, e.g., basal leaf removal on Chardonnay vines in New York increased %SS and lowered TA (Wolf et al. 1986).

## 3. Monoterpenes

### 3.1 Biogenesis of monoterpenes

Mevalonic acid is the well-known precursor of all terpenoid compounds in the mevalonate pathway. Once formed, mevalonic acid may undergo two successive phosphorylations to form mevalonate-5-pyrophosphate, which is oxidized to form isopentyl pyrophosphate (IPP). IPP may then undergo isomerization to form dimethylallyl pyrophosphate (DMAPP) such that an equilibrium between the two is set up. Additionally, a second pathway known as the methylerythritol phosphate pathway can create DMAPP from glyceraldehyde-3-phosphate and pyruvate. Plants appear to maintain an active pool of DMAPP. Subsequent condensation of the two compounds with prenyltransferase produces the universal terpene precursor, geranyl pyrophosphate (GPP), which can be converted easily to most of the other major monoterpenes. These compounds can occur free, as pyrophosphates, or as glycosides; the latter were first shown in Muscat of Alexandria by Cordonnier and Bayonove (1974), who found that linalool glycosides were cleaved by β-glycosidases into the sugar and the much more organoleptically-active free terpene.

Monoterpene aroma compounds are created in the berries. This is supported by the fact that when clusters of the intensely flavoured Muscat Albardiens were grafted after fruit set to neutral-flavoured Olivette Blanche, the characteristic Muscat flavour still developed in the grafted cluster; the berries, immediately after fruit set, acquire the enzymatic capacity inherent in the genotype for the conversion of translocated precursors to specific aroma compounds (Winkler et al. 1974). Similar results were obtained in other more recent cluster-transfer trials involving Shiraz and Muscat of Alexandria (Gholami et al. 1995).

*V. vinifera* cultivars possess an almost continuous gradation of aroma characters from the very neutral (Grenache, Sylvaner) through pronounced aromatic types (Chardonnay, Riesling) to the very intense Muscat character (Muscat of Alexandria, Gewürztraminer). The latter aroma character, owing to its great desirability, has been investigated very extensively both from the aspect of its flavour chemistry and its inheritance.

Webb and Kepner (1957) used gas chromatography-mass spectrometry (GC-MS) and reported numerous compounds in Muscat of Alexandria but no monoterpenes, which are the compounds that give this cultivar its unique characteristics. Although they failed to detect terpenes, they did identify five alcohols, three aldehydes and eight esters, as well as several unidentified ethyl esters and acetals. Early investigations in Europe (Austerweil, 1946; Cordonnier, 1955, 1956) noted that high concentrations of terpenes, particularly linalool, geraniol, limonene, and α-terpineol, were associated with Muscat-flavoured cultivars. Eventually, other studies found many significant compounds. Bayonove and Cordonnier (1971a) further supported their previous work that terpenes were responsible for the aromas in Muscat and aromatic cultivars by quantifying linalool and other terpenes in several of these cultivars. Volatile compounds could now be targeted specifically and therefore more sense could be made out of all the "noise" of chromatograms.

GC-MS nuanced the study of the volatile components of the Muscat aroma and that of aromatic grape cultivars. Early work utilizing classical methods alluded to the strong possibility that terpenes were major contributors to the aroma. Stevens et al. (1966) identified 19 hydrocarbons, 15 alcohols, 13 esters, 4 aldehydes, 2 ketones and 6 miscellaneous compounds. Linalool, geraniol and hexanol were found to be the major components. Webb et al. (1966), in examining the low-boiling volatile fractions of eight Muscat cultivars, found that linalool concentration varied greatly between the cultivars, from very high to trace amounts. Bayonove and Cordonnier (1970b, 1971a) obtained similar results, but still felt the terpene fraction to be the major contributor to the Muscat aroma, despite its oftentimes low concentration. These same authors (1971b), however, also found large volumes of linalool in non-Muscat-flavoured selections, and so concluded that linalool was "important but not specific" insofar as its role as an aroma constituent in Muscats was concerned. Terrier et al. (1972a, b) substantiated this observation by the identification of several terpenes in aromatic cultivars such as Riesling; no terpenes were detected in neutral-flavoured cultivars such as Grenache. Rodopoulo et al. (1974) conducted an exhaustive study into the composition of the essential oils of Muscat Frontignan, Saperavi and two other Muscat-flavoured cultivars grown in Armenia and the Crimea. Esters and terpenes were the principal components of the essence. Schreier et al. (1976) identified another monoterpene alcohol, hotrienol (3,7-dimethyl-l,5,7-octatrien-3-ol), which they believed to have significance in the Muscat aroma of cultivars such as Müller-Thurgau. In a comprehensive study of the Muscat character, Ribéreau-Gayon et al. (1975) finally collated much of the prior work on the subject into some plausible conclusions. The threshold values of many of the terpenes, especially linalool and geraniol, were found to be extremely low (100 and 132 µg/L, respectively), and so it was felt that the contribution of these terpenes to the aroma was very significant. They also cited the phenomenon of synergism between terpenes in musts and wines, such that a greater intensity of aroma was produced.

Studies on the volatile composition of other aromatic *V. vinifera* cultivars have led to some conclusions. Drawert and Rapp (1966) and van Wyck et al. (1967) both conducted exhaustive studies into the constituents of Riesling aroma. The latter group concluded that the characteristic Riesling aroma could be attributed to specific concentrations of linalool, 2-phenylethyl alcohol, ethyl acetate and 2-methyl-1-butanol. Schreier et al. (1976) identified 81 previously unreported compounds in Riesling, as well as six other popular German cultivars, but they failed to specify from which cultivars the various constituents were isolated. Kormokova and Rodopoulo (1974), in their study of the essential oils of sparkling wine cultivars grown in the USSR, considered geraniol to be the major component of the aroma of Rkatsiteli, a popular Russian cultivar of distinct aromatic character. Sixty-nine other constituents were also identified.

### 3.2. Effects of fruit exposure on monoterpenes

The distillation method of Dimitriadis and Williams (1984) permitted the assessment of viticultural practices on concentrations of monoterpenes in grape berries with rapid throughput. One of the first of these was a study in British Columbia whereby potentially volatile terpene (PVT) concentrations in Gewürztraminer berries were found to be highest in fully exposed clusters throughout the course of fruit maturation, but peaked at about 20 days after veraison (Reynolds and Wardle, 1989b)(Fig. 1).

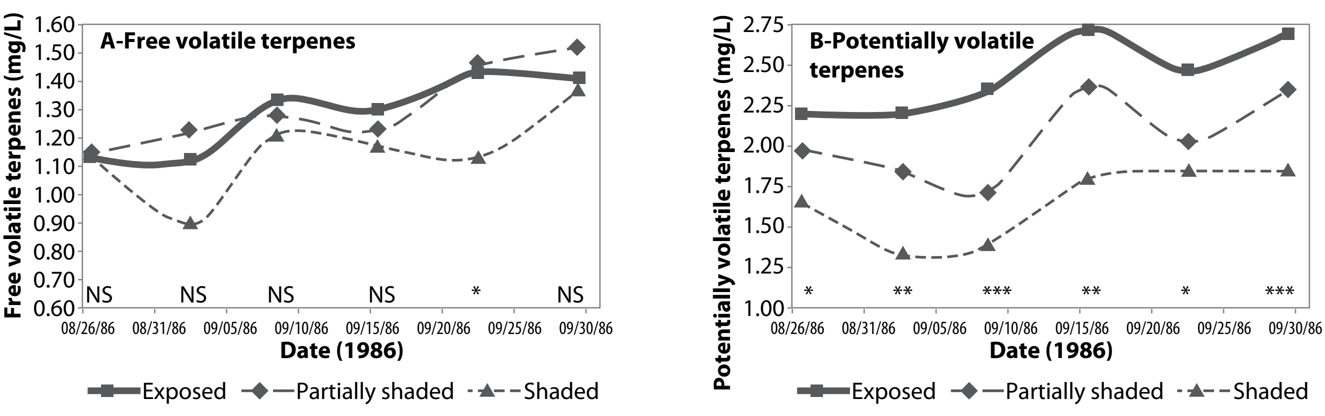


**Figure 1.** Effects of cluster exposure on monoterpene composition of Gewürztraminer, Kaleden, British Columbia, 1986: A: Free volatile terpenes (mg/L); B: Potentially volatile terpenes (mg/L). *,**,***, ns: Significant at $p < 0.05$, 0.01, 0.001, or not significant, respectively. Redrawn from Reynolds and Wardle (1989b).

Partially exposed clusters (those shaded by one layer of leaves) contained a lower concentration of PVT than exposed clusters, but much more than shaded clusters. Free volatile terpenes (FVT) were not as responsive to fruit exposure as PVT, but followed the same trends. By the final sampling date, %SS, TA and pH were similar among the three exposure treatments, but exposure-related differences in PVT remained until after commercial maturity. Conclusions were that fruit exposure enhances monoterpene concentrations in grape berries and that FVT and PVT concentrations may not necessarily be correlated to %SS, TA or pH. These results were confirmed in Arkansas by Macaulay and Morris (1993) using *V. labruscana* Golden Muscat.

Belancic et al. (1997) monitored the effect of sun exposure on the aromatic composition of two Muscat grape cultivars (Muscat of Alexandria and Pink Muscat) over two seasons in Chile. Fully exposed, semi-shaded (20% shaded) and fully shaded (80% shaded) clusters were sampled. Both cultivars contained similar levels of total free terpenols but Muscat of Alexandria was richer in total bound terpenols. The highest concentration of free terpenols was obtained from the semi-shaded treatment, although the differences between exposed and semi-shaded were negligible for Muscat of Alexandria. Shaded grapes had the lowest concentration of terpenols, with poor Muscat typicity. Linalool was the most sensitive to sun exposure. Berry temperature was considered critical for maximizing monoterpene concentrations and Muscat flavour in the fruit.

### 3.3 Effects of growing season canopy management on monoterpenes

A few studies have examined leaf removal effects on monoterpene compounds. Smith et al. (1988) were among the first to demonstrate the effectiveness of basal leaf removal on increasing both monoterpene concentration and wine sensory scores of Sauvignon Blanc in New Zealand.

Work on Gewürztraminer in British Columbia indicated that FVT and PVT were responsive to leaf removal, which could also be increased by cluster thinning and hedging (Reynolds and Wardle, 1989a). Neither FVT nor PVT were dependent on %SS, TA or pH. In a multisite trial, leaf removal consistently increased berry PVT, and in one year, FVT as well, regardless of site (Reynolds et al. 1996a). Must FVT and PVT were also increased by leaf removal treatment. Increased monoterpene concentration was, in some cases, associated with lower TA, pH and potassium, but slightly lower %SS as well. Tasters found more Muscat aroma and flavour in both the hedged and leaf removal wines than in the control.

FVT and PVT in the berries of Pearl of Csaba, Bacchus, Schönburger and Siegerrebe were responsive to basal leaf removal during the veraison-to-harvest period at several sites in British Columbia (Reynolds et al. 1995a). Musts displayed greater treatment differences, and basal leaf removal musts usually contained higher FVT and PVT. %SS and TA were largely unresponsive to basal leaf removal in the berries and must, but must pH was in many cases lower in leaf-pulled treatments. At warmer Oliver, BC sites, aroma differences occurred between control and leaf-pulled wines for two of four cultivars, and flavour differences were apparent for three of four. Tasters almost overwhelmingly indicated that the leaf-pulled treatments contained the most Muscat and/or floral flavour. These distinctions could be made based on differences in PVT of 1.45, 0.10 and 0.87 mg/L for Pearl of Csaba, Schönburger and Siegerrebe respectively. Also, in a multiyear experiment testing training system, vine spacing and leaf removal, basal leaf removal consistently increased both FVT and PVT (Reynolds et al. 1996b). These results suggested that berry or must monoterpene concentrations may be used as indicators of potential wine varietal character.

A multiyear project in Ontario found positive effects of basal leaf removal and cluster thinning on berry and must concentrations of FVT and PVT in Chardonnay Musqué (Reynolds et al. 2007a). Elevated monoterpene concentrations were measured in berries and musts from basal leaf removal and cluster-thinned plots, but basal leaf removal had by far the greatest magnitude of effect. No differences were found in berry FVT across treatments, but basal leaf removal berry PVT were higher than control and thinned samples. Must FVT and PVT concentrations showed differences among treatments with basal leaf removal > cluster thinned > control in both cases. There were substantial differences in sensory profiles of the wines resulting from the viticultural practices. Cluster thinning enhanced dry fruit aroma and colour and reduced the citrus component to the aroma. Basal leaf removal enhanced citrus aroma and lychee and dry fruit flavour. The data also showed that regardless of winery treatment (various yeasts and enzymes had also been tested), veraison thinning had the highest dry fruit aroma.

The use of the glycosyl-glucose (G-G) assay permitted an assessment of viticultural practices whereby all glucoconjugates could be accurately measured by an enzymatic method (Abbott et al. 1993). Target compounds included monoterpenes, norisoprenoids and some phenolic compounds. Zoecklein et al. (1998a) found higher glycosides (measured by G-G assay) as well as higher monoterpenes and aromatic alcohols in leaf-pulled treatments of Riesling in Virginia. In a related trial, they found that both the total G-G concentration and the phenol-free fraction were highest in leaf-pulled treatments of Chardonnay and Riesling (Zoecklein et al. 1998b). The concentrations of total and phenol-free glycosides were higher in Riesling and Chardonnay fruit from leaf-pulled vs. control vines on three of four harvest dates. Phenol-free glycosides averaged 80% of the total in Riesling juice and 66% of the total in Chardonnay.

### 3.4 Effects of shoot density and crop level on monoterpenes

McCarthy and Coombe (1985), McCarthy (1986), McCarthy et al. (1987) and Coombe and Iland (1987) showed that PVT in Riesling berries were responsive to cluster thinning and reduced irrigation in Australia. It was apparent that these treatment differences were closely related to yield. Eschenbruch et al. (1987) demonstrated increases in PVT of Müller-Thurgau berries resulting from cluster thinning and shoot thinning. They concluded that PVT development in that cultivar closely paralleled %SS accumulation and hence afforded no better indication of ultimate grape and wine quality. They were also unable to demonstrate a clear relationship between PVT concentration and wine quality.

In a Riesling shoot density x crop level trial, Reynolds et al. (1994a, b) showed that despite large yield and shade increases, increases in shoot density actually increased Riesling berry and must PVT. Reducing crop level had a minor effect on PVT concentration. Tasters found 26 shoots/m row wines equal to wines of lesser shoot densities in terms of sensory quality, despite higher yields. This may have been due to higher PVT concentration in the original berries and musts. Some monoterpenes, including linalool, linalool oxides, α-terpineol and citronellol, were associated with lower crop levels and low to moderate (16 or 26 shoots/m row) shoot densities and increased in concentration during aging.

In a similar trial testing Riesling shoot density x cordon age, Reynolds et al. (1994c) found that increasing volume of "old" wood increased berry and must FVT and PVT. Wines produced from vines containing more "old" wood were higher in floral aroma and flavour, with less vegetal character. Shoot density had less of a magnitude of effect than volume of old wood.

A multiyear project in Ontario found positive effects of crop reduction on berry and must concentrations of FVT and PVT (Reynolds et al. 2007c) (Fig. 2). The thinning treatments were carried out at five different times during fruit development (bloom, post-set, mid-Stage I, lag phase, veraison). Berry FVT increased 10 to 15% over non-thinned vines in vines thinned at bloom, set and Stage I. Berry PVT increased in all thinning treatments except bloom. Both FVT and PVT concentrations were lower in must samples than in berry samples. Must samples had lowest FVT con-

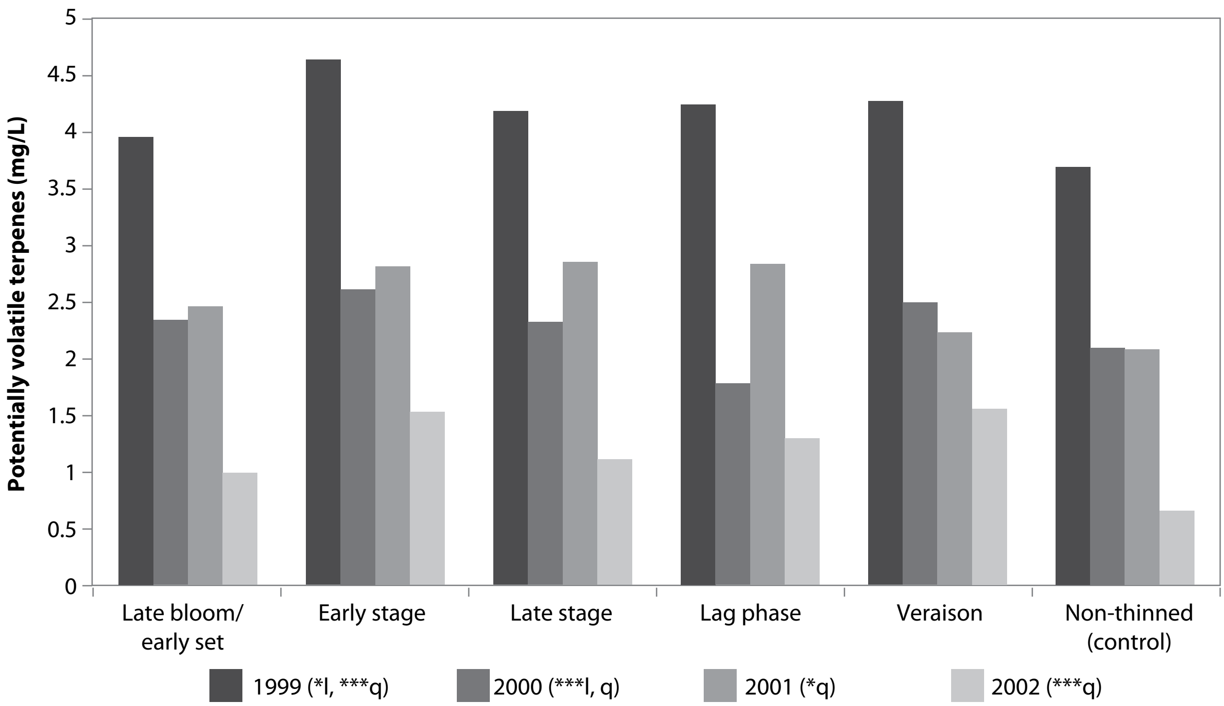


**Figure 2.** Impact of six thinning times on Chardonnay musqué berry potentially volatile terpene concentration 1999 to 2001. Asterisks indicate significant difference from the control, $p < 0.05$, Dunnett's t-test. From Reynolds et al. (2007c).

centrations from Stage I and lag phase-thinned vines, and the lowest PVT in bloom, post-set, Stage I and veraison-thinned vines. Overall, it was possible to increase the concentration of both FVT and PVT through thinning. Their concentrations were increased by up to 15% and 24% respectively. Non-thinning maximized dry fruit (raisin, fig) aroma and colour and did not differ from thinning treatments with respect to most other sensory descriptors. Bloom thinning maximized citrus (as did veraison thinning) and grassy aroma.

### 3.5 Influence of training systems on monoterpenes

Numerous training systems are used around the world for wine grapes. One of the many objectives of a successful training system affords optimal fruit exposure that is appropriate for the region in which the grapes are grown. Enhanced fruit exposure might lead to increased concentrations of aroma compounds. A great many studies have compared the effects of training systems on fruit composition and wine quality.

In British Columbia, a divided canopy system (alternate double crossarm or ADC) produced yields as high as 33 t/ha, along with lower TA and higher FVT and PVT than standard *pendelbogen* and bilateral cordon systems (Reynolds et al. 1996b) (Fig. 3). Fruit exposure and berry temperatures were considerably higher in ADC vines than in bilateral cordon vines. However, despite significant increases in cluster exposure resulting from canopy division, basal leaf removal still reduced TA and increased PVT, even in the ADC system. This suggests that natural fruit exposure can be augmented by cultural practices to increase potential wine quality.

Zoecklein et al. (2008) found that divided canopies generally produced wines higher in some terpenes and in overall glucoconjugates. Fruit showed consistent differences in linalool, α-terpineol, β-damascenone and *n*-hexanol concentrations among training systems. The Smart-Dyson vertically divided system had the highest concentration of most free volatiles in both juice and wines, while Geneva double curtain wines frequently had the highest concentration of phenol-free glycosides. GDC wines generally had higher fruity and floral aromas compared with the other systems.

### 3.6 Influence of irrigation on monoterpenes, esters and higher alcohols

Few irrigation studies have extended their focus to include aroma compounds. Reynolds et al. (2006) showed that irrigation deficits applied at veraison resulted in higher concentrations of FVT and PVT in Gewürztraminer than in early and mid-season deficit treatments (Fig. 4). Related floor management treatments (clean-cultivated, total herbicide, permanent sod) also produced differences, whereby FVT were highest in clean-cultivated treatments but PVT were highest in sod plots.

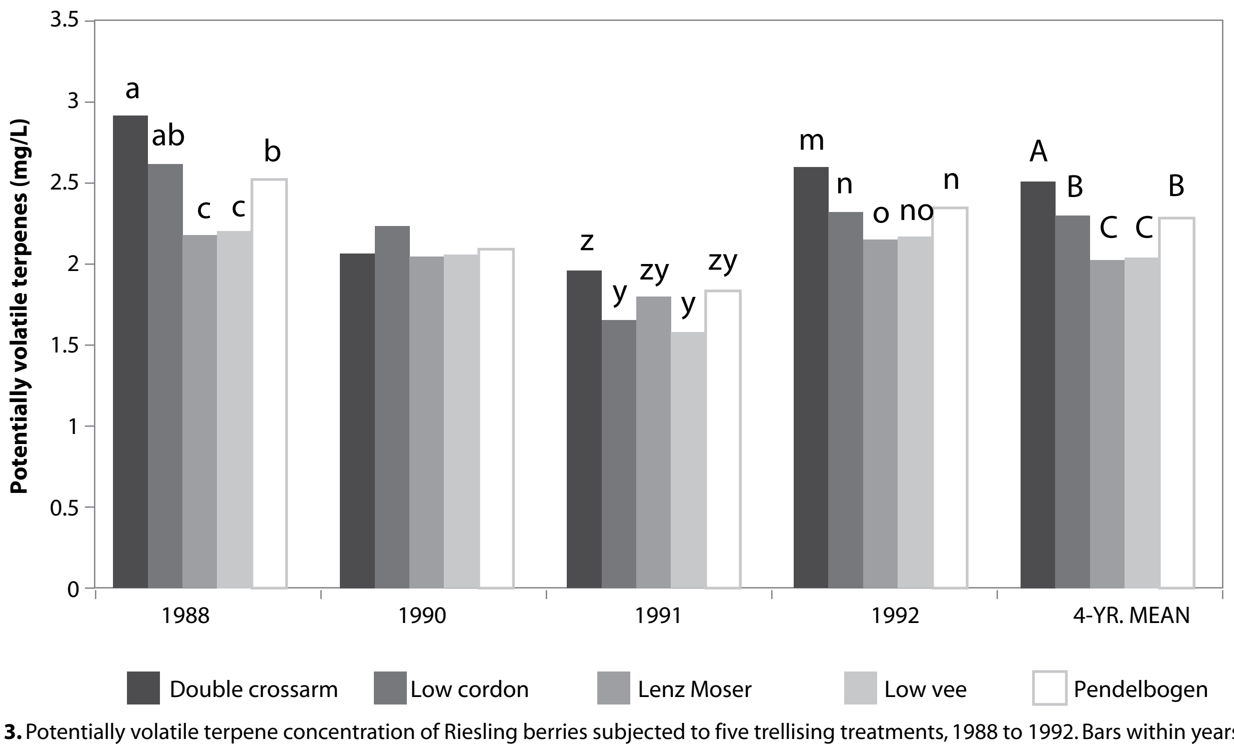


**Figure 3.** Potentially volatile terpene concentration of Riesling berries subjected to five trellising treatments, 1988 to 1992. Bars within years containing different letters are significantly different at $p < 0.05$, Duncan's multiple range test. Figure was redrawn from Reynolds et al. (1996b).

The influence of irrigation on grape and wine composition was investigated for Agiorgitiko in the Nemea appellation area in southern Greece by Koundouras et al. (2006). Three non-irrigated plots were studied during vintages that were very hot and devoid of summer rainfall. Limited water availability increased glycoconjugates of the main aromatic components of grapes. Wines produced from grapes of stressed vineyards were also preferred in tasting trials.

Dos Santos et al. (2007) investigated the impacts of partial root-zone drying (PRD) irrigation on Moscatel vine water relations, vegetative growth, plant microclimate, berry composition (including aroma compounds) and yield components compared to conventional deficit irrigation (50% ETc), full irrigation (100% of ETc) and non-irrigated vines. The PRD vines had a better microclimate in the cluster zone with higher incident photosynthetic photon flux density and higher berry temperatures than deficit irrigation and full irrigation. PRD improved berry composition with higher flavour precursor concentrations, without any yield reduction compared to deficit irrigation and full irrigation.

Vineyard fertilization may have indirect effects on aroma composition. Ough and Bell (1980) in California showed that increased fertilization rates between 0 and 440 kg/ha increased concentration of higher alcohols in Thompson Seedless wines. Ough and Lee (1981) showed that increased vineyard fertilization rates could likewise increase most fermentation esters such as isoamyl acetate in Thompson Seedless. Riesling vines in Washington fertilized between 0 and 224 kg/ha affected concentrations of free and bound monoterpenes in the aged wines (Webster et al. 1993). Other compounds, including esters and alcohols, were also impacted. Generally, most monoterpenes decreased with increasing fertilization rates, perhaps as a shade response, whereas esters and alcohols increased, as a likely effect of increased must amino nitrogen and resultant transamination.

### 3.7 Impact of vineyard site on monoterpenes

The influence of site on fruit composition is difficult to define objectively when site-based differences in canopy density, phenology, soil type and cultural practices are involved. Di Stefano and Corino (1984, 1986) found only minor differences in terpene concentrations between Moscato Bianco and Moscato Giallo grapes grown on several sites in the Piedmont and Val d'Aosta regions in Northern Italy. Subsequent work (Corino and Di Stefano, 1988) showed that higher terpene concentrations were associated with warm sites. Likewise, Noble (1979) found few differences between Chardonnay wines whose origins included Monterey (Region I), Oakville (Napa County, Region III) and Livermore (Alameda County, Region III). Larrechi and Ruiz (1987) and Larrechi et al. (1988) used multivariate analysis to distinguish between winegrowing regions in Catalonia, Spain. Ewart (1987) found that a cool, high elevation site (High Eden, South Australia)

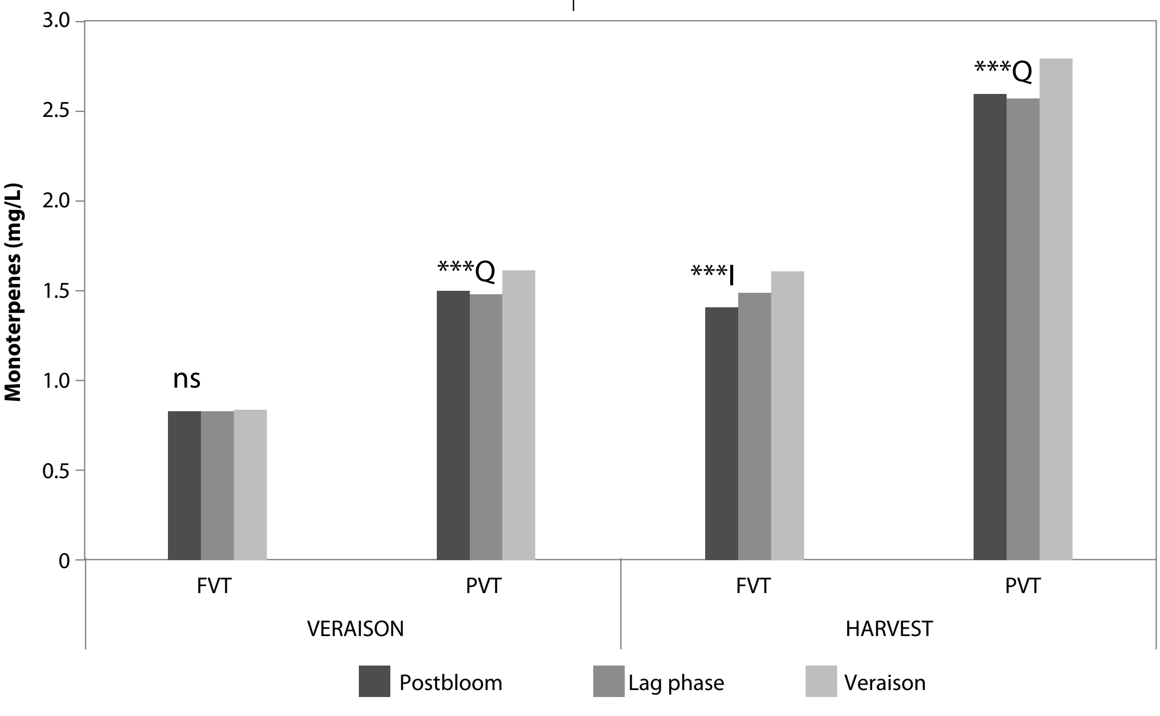


**Figure 3.** Potentially volatile terpene concentration of Riesling berries subjected to five trellising treatments, 1988 to 1992. Bars within years containing different letters are significantly different at $p < 0.05$, Duncan's multiple range test. Figure was redrawn from Reynolds et al. (1996b).

produced Riesling fruit with the highest terpene concentration, but terpene concentrations could not be linked to wine scores. Thus, although great volumes of anecdotal evidence exist for differentiating sites, very few objective studies have been carried out to quantitate these differences.

Work in British Columbia attempted to distinguish between sites based on monoterpene concentrations by locating vineyards of similar soil type and vine vigour and by maintaining the vines using identical cultural practices. Fruit maturation proceeded faster at Oliver, BC sites on a daily basis, and FVT and PVT were therefore usually higher in Oliver berries on any given sampling day (Reynolds et al. 1995a). Cooler Kelowna, BC sites matured their fruit more quickly when expressed on a per growing degree–day basis. Oliver musts tended to be higher in FVT and PVT, although harvested at similar TA and pH. Tasters distinguished between wines from the Oliver and Kelowna sites on the basis of aroma for only one of the four cultivars studied (Reynolds et al. 1995a), but the sites could be distinguished on the basis of flavour for three of the four cultivars. For Bacchus and Schönburger, the Oliver sites were clearly identified as having the more intense Muscat flavour.

In a three-site experiment with Gewürztraminer, no clear pattern emerged regarding the relationship between site and FVT, but berries from both the Oliver and Kelowna sites were highest in PVT in two of five years (Reynolds et al. 1996a). Must FVT and PVT were highest from the Kelowna site. The young wines from the Oliver and Kelowna sites were identified as most spicy. Aged wines from Oliver and Kelowna sites were high in citrus aroma; those from the Kaleden site were primarily vegetative, acidic and astringent; and wines from the Oliver site were characterized by butter, cedar and Muscat flavours as well as high astringency, aftertaste and body. These results corresponded with the PVT concentrations measured in the berry samples taken at harvest.

### 3.8 Spatial variation of aroma compounds

Recently there has been great interest in using geomatic tools for the assessment of spatial variation in vineyards, including variation in aroma compounds. Elucidation of unique spatial patterns for aroma compounds in specific vineyard blocks could lead to the identification of sub-blocks of potentially higher value for economic exploitation. There might also be implications from this type of study for precision viticulture, if spatial variability in vine vigour and yield were found to be highly correlated, and if spatial variation in yield were found to be temporally consistent within individual vineyard blocks.

A study in a 4 ha Riesling vineyard (Reynolds et al., 2007c) was an attempt to resolve the continuing question of direct and independent soil and vine vigour effects on yield components, berry, must and wine composition and wine sensory attributes. Monoterpenes were specifically chosen as indicators of fruit maturity and as variables to associate with wine sensory attributes. Geographic information systems (GIS) delineated spatial variation in soil texture, soil and vine tissue composition, yield components, weight of cane prunings (vine size, an estimate of vine vigour) and berry composition including monoterpenes. Correlations were observed between soil texture and composition vs. berry weight and PVT. However, there were no consistent soil texture or vine size effects on berry, must or wine composition. High vine size increased berry TA, berry PVT and wine FVT and decreased must pH. Sandy soil (vs. clay soil) reduced wine TA and must PVT and increased berry TA and must %SS. Percent sand was positively correlated in one or more years with several variables of potential significance to wine quality: berry weight, %SS, TA, pH and PVT (Reynolds et al. 2007c). Percent clay was also correlated in one or more seasons with %SS and PVT and inversely correlated with berry weight, TA and PVT. These results suggest that soil texture might play a role in fruit composition and varietal typicity, although non-consistent temporally. No fruit composition variables consistently correlated with vine size, and very few correlative relationships of consequence were displayed except those between vine size and certain yield components. This suggests that vine size, at least in the range used in this study, does not play a major role in determining fruit composition.

Other soil or nutritional factors may have an impact on variables associated with varietal typicity; however, little to no work has focused on the impact of vine nutrition on concentrations of aroma compounds in grapes. Since low berry weight is frequently considered desirable from a winemaking standpoint, soil and vine nutritional factors associated with berry weight may also be determinants of the terroir effect. Soil variables found inversely correlated with berry weight (other than soil texture) included pH, organic matter, P, K, Mg, Ca, CEC and % base saturation as Ca; petiole variables included K, Mg and Mn (Reynolds et al. 2007c). Positive soil vs. monoterpene correlations included several with PVT (soil pH, P, K, Ca, Cu, B, CEC and % base saturation as Ca) as well as petiole B. Few studies have found correlations between mineral nutrition and fruit composition, and direct connections between soil nutrients and aroma compounds have been difficult to determine. Webster et al. (1993) found an increase in PVT in Riesling with increased nitrogen fertilization, but there was little impact on FVT except where increased vegetative growth lead to greater shading (and thus lower FVT).

Vine size and soil texture did not consistently affect wine sensory attributes across vintages (Reynolds et al. 2007c). However, several sensory attributes were affected by vine size in at least one season. For example, high vine size decreased mineral aroma and citrus flavour and increased apple attributes. Clay soil increased mineral aroma and citrus attributes, but decreased apple aroma. Vine vigour and soil texture sometimes affected composition of berries, must and wines and impacted sensory perception of aroma, flavour and mouthfeel in wine, but neither variable did so consistently (Reynolds et al. 2007c). It must therefore be concluded that within the scope of this trial's conditions, wine sensory attributes cannot be ascribed to either vine size or soil texture exclusively. Factors other than those tested apparently impacted wine sensory attributes and hence form much of the basis for so-called terroir effects.

### 3.9 The influence of vine water status on spatial variation in monoterpenes

More recently, we have focused on water status as a potential determinant of terroir. Mild "water stress" may be beneficial to wine "quality" but sustained water stress can have many negative consequences, including diminished winter hardiness, delayed maturity and reduced yields, to name a few. In a trial initiated in 2005, we chose 10 vineyard blocks for each Riesling (and Cabernet Franc in a related study). The main objectives were (1) to ascertain the impact of vine and soil water status on aroma compounds and wine sensory attributes, (2) to enumerate the comparative magnitude of effects of soil texture, water status and vine vigour and (3) to elucidate relationships between these variables and wine sensory quality. By meeting these objectives, we intended to elucidate the basis for terroir by integration of several years of soil, plant nutrition, water relations, yield, fruit composition and sensory data.

Using GPS and GIS applied to several vineyards with heterogeneous soil types in Niagara Peninsula, Ontario, we tested the hypothesis that vine water status plays a major role in aroma compound concentration and wine sensory attributes. Riesling data were analyzed by analysis of variance, and GIS-generated maps were analyzed by spatial correlation analysis. In some instances, FVT and PVT were correlated with leaf water potential ($\gamma$) and/or soil moisture, suggesting that mild water stress may be beneficial for wine flavour (Fig. 5 on next page). In most cases, the sand and clay content of the soils were inversely correlated. Soil moisture content was typically higher in clay-dominated areas of the vineyards, while vine water status (leaf $\gamma$) also tended to be higher in clay soils. Leaf $\gamma$ was often inversely correlated with vine size, i.e., vine water status was improved in low vine size areas. Berry weight and %SS were both correlated with vine water status, while TA was inversely correlated. Spatial relationships in vine water status appeared to be temporally stable, and patterns observed in one vintage appeared for the most part to be similar in the next despite different weather conditions. In addition to these reasonably good spatial correlations between soil moisture and leaf $\gamma$, there were some excellent spatial relationships between leaf $\gamma$ and both vine size and soil texture. This validates our original idea that vine size and soil texture are major contributors to terroir.

Wines were subjected to sensory analysis. The results suggest that vine water status may in fact have an influence on wine sensory attributes. A sorting task was initially performed using expert judges. The judges were asked to group wines in terms of similar sensory characteristics. Statistical analysis using multidimensional scaling demonstrated that for the most part wines of similar water status were grouped together. Subsequent descriptive analysis showed consistent differences in wines produced between regions and within vineyards with lower and higher water status.

### 3.10 Impact of pre-fermentation decisions on monoterpenes

#### 3.10.1 Harvest date

Many odour-active compounds clearly continue to accumulate in grape berries long after commercial maturity has been reached. Work by Hardy (1970), Bayonove and Cordonnier (1970a) and Gunata et al. (1985) showed that terpenes could increase in fruit long after the point of commercial maturity, while Marais and van Wyk (1986) and Marais (1987) indicated that delayed harvest of Bukettraube and Riesling, and Gewürztraminer, respectively, led to higher terpene concentrations in musts and wines. In many cases, these differences could be distinguished by sensory evaluation. Ewart (1987), on the other hand, found that wine quality was reduced in late-harvested Riesling, even though the late-harvested fruit attained the highest total terpene concentration.

In a trial in British Columbia, FVT and PVT increased in three of six *V. vinifera* cultivars with delays in harvest dates between 10 and 20 days (Reynolds et al. 1993). Tasters could distinguish between wines from "early" and "late"-harvested fruit in five of six cultivars based on aroma, and three of the six based on flavour. In many cases, these tast-

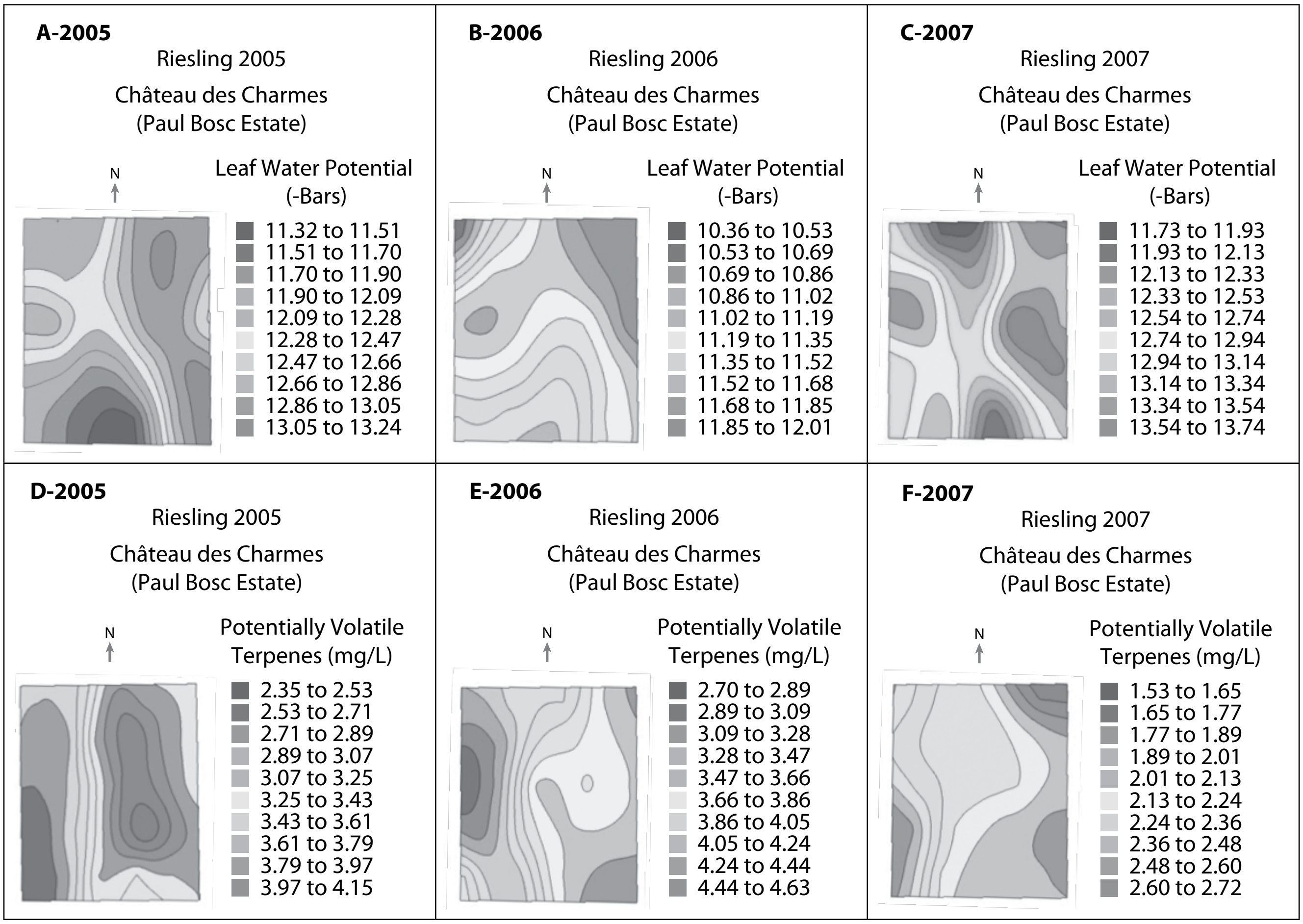


**Figure 5.** GIS-derived maps of the Riesling block on the Paul Bosc Estate, St. Davids, ON, 2005 to 2007. A to C: Leaf water potential, 2005, 2006 and 2007 respectively; D to F: Potentially volatile terpenes (PVT), 2005, 2006 and 2007 respectively. Note how (a) spatial variation in both leaf water potential and PVT are temporally stable across the three seasons; (b) the patterns of leaf water potential and PVT are very similar spatially. These observations suggest that high flavour zones in vineyards are temporally stable and are related to vine water status. Maps are courtesy of Dr. Jim Willwerth.

ers indicated that the late-harvested treatments had either the strongest Muscat and/or strongest floral character.

### 3.10.2. Pressing

Effective extraction of free terpenes, as well as liberation of free terpenes from their glycosidic precursors, may be achieved through pre-fermentation practices such as skin contact, pressing and the use of enzymes (Cordonnier and Bayonove 1981, Strauss et al. 1986, and Reynolds et al. 2007a, b). Pressing treatment was shown by Cordonnier and Bayonove (1979) as well as Kinzer and Schreier (1980) to have an impact on terpene concentration in musts. In British Columbia, pressing had no effect on terpenes of Müller-Thurgau, but PVT in press juice of Muscat Ottonel and Gewürztraminer were higher than in their free run fractions (Reynolds et al. 1993). FVT were not affected. Tasters could distinguish between the aromas of Müller-Thurgau and Muscat Ottonel wines made from free run and press juice. Despite the lack of difference between treatments in FVT and PVT concentration in the Müller-Thurgau, tasters indicated that the press wines had stronger floral character than the free run wines. Concentrations of FVT and PVT decreased substantially from the berry to the juice stage; losses in FVT were 52%, 41% and 22% for Muscat Ottonel, Gewürztraminer and Kerner respectively, and losses in PVT were 16%, 52%, 13% and 28% for Müller-Thurgau, Muscat Ottonel, Gewürztraminer and Kerner respectively.

### 3.10.3. Skin contact

Use of skin contact is controversial among some winemakers. Bayonove et al. (1976), Marais and van Wyk (1986), Marais (1987) and Marais and Rapp (1988) have indicated that use of and duration of skin contact can appreciably increase the concentration of specific terpenes in must and wine. Skin contact increased FVT and PVT in three of four *V. vinifera* cultivars (Reynolds et al. 1993). Only Siegerrebe produced large enough aroma and flavour differences to allow tasters to distinguish between the two treatments. There was no clear indication of whether skin contact resulted in more Muscat or floral character in the aroma or flavour.

## 4. Methoxypyrazines

Methoxypyrazines are a group of heterocyclic aromatic organic compounds that are naturally present in green plant tissue, including grape berries that are associated with green, vegetal or herbaceous characteristics. Important methoxypyrazines found in grapes include 3-isobutyl-2-methoxypyrazine (IBMP), 3-sec-butyl-2-methoxypyrazine (SBMP) and 3-isopropyl-2-methoxypyrazine (IPMP). Bordeaux cultivars such as Sauvignon Blanc and Cabernet Sauvignon contain methoxypyrazines at significant concentrations and owe much of their distinct aroma to these potent aroma compounds (Bayonove et al. 1975, Kotseridis et al. 1998, and Lacey et al. 1991). IBMP is the most abundant methoxypyrazine found in grapes (Sala et al. 2000). Bayonove et al. (1975) first reported IBMP in Cabernet Sauvignon, and subsequent studies (Augustyn and Rapp 1982; Allen et al. 1991, 1995; Lacey et al. 1991; and Kotseridis et al. 1998) identified IBMP, SBMP and IPMP in grape berries and wines. Concentrations in the fruit range from 0 to as much as 42 ng/L (Roujou de Boubée et al. 2000).

One of the most significant aspects of methoxypyrazines is that their sensory thresholds are extraordinarily low. For example, the human sensory threshold for methoxypyrazines is 1 to 2 ng/L (Buttery et al. 1969a, b). In red wines, IBMP and IPMP are detected at 10 ng/L (Tominaga et al. 1998a, b) and 2 ng/L (Kotseridis et al. 1998) respectively.

Relatively little is known about the biochemistry of methoxypyrazines. Consequently there has not been much literature published about their synthesis or degradation. Methoxypyrazine concentrations diminish greatly during the berry expansion phase and are relatively high at veraison, but can decrease dramatically during maturation from as high as 78 ng/L at mid-veraison to < 2 ng/L at harvest (Lacey et al. 1991). The current belief is that sunlight and/or heat can lead to degradation of pyrazines. Allen et al. (1991) showed a decrease in methoxypyrazine concentrations in grapes via photodecomposition due to sunlight. Hashizume and Sumuta (1999) showed that light exposure has two opposite effects on the concentration of methoxypyrazines in grapes: (a) promoting the formation of methoxypyrazines in immature grapes and (b) non-enzymatically photo-decomposing the methoxypyrazines in ripening grapes. A balance between biological formation and photo-degradation may determine the methoxypyrazine concentration in grapes throughout the ripening process (Roujou de Boubée et al. 2000). Methoxypyrazines might form largely in the earlier stages of grape development, and photo-degradation might be more important in the ripening fruits (Sala et al. 2005).

Sauvignon Blanc wines from cooler climates such as New Zealand have much higher concentrations than those from Australia, South Africa or Bordeaux (Allen et al. 1991, 1995; Lacey et al. 1991). In Europe, Kotseridis et al. (1999) analyzed Merlot and Cabernet Sauvignon wines from various Bordeaux regions and wine samples from Greece (Xynomavro) for 2-methoxy-3-isobutylpyrazine concentration. Cultivar, level of maturation and duration of maceration affected the concentration of IBMP. Higher humidity and cooler years yield higher IBMP concentration in grapes than sunnier and less humid years (Roujou de Boubée et al. 2000).

Bayonove et al. (1975) reported that a considerable proportion of IBMP resided in grape skins. More recently Roujou de Boubée et al. (2002) observed that inside the berry, IBMP is found mainly in the skin (72%) and seeds (23.8%), and the pulp contains very little (4.2%).

### 4.1 Effects of fruit exposure on methoxypyrazines

Cultural decisions and practices that decrease shading and increase fruit exposure should be beneficial in reducing methoxypyrazine concentration in fruit. These include the use of divided canopies, basal leaf removal, cluster thinning, deficit irrigation, vine spacing and trellising, hedging and shoot thinning. These practices can produce a more open, manageable canopy that improves fruit exposure. A few studies indicate that methoxypyrazines can be manipulated in the vineyard and within the winery. In fact, all of the studies targeting manipulation of methoxypyrazine concentration in the vineyard have been indirectly due to better fruit exposure. Since vigorous canopies that limit sunlight exposure are associated with higher concentrations of methoxypyrazines, good canopy management can lower these methoxypyrazine concentrations. Since methoxypyrazines have been shown to have negative impacts on wine quality at high concentrations, studies have focused on minimizing methoxypyrazines in grapes and wine. Research seems to indicate that conditions during grape maturation are primarily responsible for the methoxypyrazine concentration in wines (Allen et al. 1991, 1995; Lacey et al. 1991; Roujou de Boubée et al. 2000). Shade seems to have a major effect on methoxypyrazine concentrations in grapes. Exposed clusters can have three times lower concentrations of methoxypyrazines than shaded fruits (Roujou de Boubée et al. 2000). Sala et al. (2004), however, found that during ripening, IBMP concentrations in grapes exposed to sunshine were not different from those covered with pieces of sackcloth. Nonetheless, clusters protected from sunlight from the beginning of veraison resulted in wines with a substantially lower concentration of this compound than the control samples.

#### 4.2. Effects of growing season canopy management on methoxypyrazines

It has become clear that methoxypyrazine concentrations can be reduced by canopy management. Arnold and Bledsoe (1990) in California showed that severe fruit zone leaf pulling reduced vegetal sensory descriptors in Sauvignon Blanc, suggesting that the leaf pulling treatments had reduced methoxypyrazines. The canopies in question were manipulated using several leaf removal treatments. Leaves were removed on three different occasions at three severity levels, whereas control grapevines received no leaf removal. Descriptive sensory analysis showed large differences among the wines for two vegetal aromas (celery/fresh vegetable and canned green bean) as well as the vegetal-flavour-by-mouth. The greatest reduction in the vegetal components was found with the middle timing/ most severe leaf removal treatment. Early, severe leaf removal was nearly as effective as the middle timing treatment in reducing vegetal flavours, but late, severe leaf removal was not. Fruit composition was also altered, %SS were increased and TA was reduced in the leaf pulled treatments (Bledsoe et al. 1988).

#### 4.3 Effects of shoot density and crop level on methoxypyrazines

The effect of crop level on methoxypyrazines has heretofore not been a well-researched topic. Crop thinning (Marais 1994, and Chapman et al. 2004a, b) can also help decrease methoxypyrazine concentration in grapes because excessive crop-to-leaf area can delay the rate of fruit maturity and therefore the degradation of methoxypyrazines.

#### 4.4 Influence of training systems on methoxypyrazines

As in the case of fruit exposure and basal leaf removal, certain training systems may enhance fruit environment and potentially lead to changes in methoxypyrazine concentration. Nonetheless, Sala et al. (2004) found that gobelet- and bilateral-cordon-trained Cabernet Sauvignon vines did not produce fruit with different berry IBMP concentrations. However, IBMP concentration of the final wines was much higher in the cordon-trained vines.

#### 4.5 Influence of irrigation on methoxypyrazines

Few studies exist that have addressed irrigation effects on methoxypyrazines. Sala et al. (2005) found that irrigated vines and vines planted at a higher plantation density had significantly higher concentrations of IBMP in berry samples than non-irrigated and lower plantation density vines.

#### 4.6 Impact of pre-fermentation decisions on methoxypyrazines

Methoxypyrazines are very stable compounds due to their chemical nature and therefore are very difficult to remove or reduce in wines. The use of oak can reduce vegetal/herbaceous aromas/flavours in wines with high methoxypyrazine concentrations by a straight masking effect. Aiken and Noble (1984) found that "vegetal" characteristics decreased in Cabernet Sauvignon wines after oak aging. Pickering et al. (2006) found that treatment with oak chips reduced the intensity of "ladybug taint" (Pickering et al. 2004), which is a taint associated with high concentrations of IPMP. Methoxypyrazines are highly extractable in wines (Roujou de Boubée et al. 2002). Since a majority of methoxypyrazines are found in grape stems and skins, limited skin contact and exclusion of stems or leaves from the winemaking process can reduce the amount of methoxypyrazines extracted into the resultant wines. Lighter pressing regimes can also reduce methoxypyrazine concentrations. Press wines contain higher concentrations of methoxypyrazines (Roujou de Boubée et al. 2002). This suggests that a fraction of methoxypyrazines is extracted from the skins during rigorous pressing. Settling and clarifying white wine must decreases methoxypyrazine concentration in half (Roujou de Boubée et al. 2002). They demonstrated that thermovinification could also be useful by reducing IBMP from 29 to 67%. However, heating wines is not widely practised in premium wine production. Since it can be shown that winemaking procedures probably have a minimal impact on pyrazine concentrations, it is most appropriate to focus on viticulture practices and methoxypyrazine management.

## 5. Norisoprenoids

Norisoprenoids are $C_{13}$ degradation products of carotenoids (b-carotene and lutein). The norisoprenoids accumulate during Stage III of berry growth much the same as the terpenes. Whereas the Muscat and floral cultivars owe their character to terpenes and the Bordeaux types to pyrazines, the norisoprenoids are more ubiquitous and add nuances to many cultivars across a wide range of regions. They are usually found as glucosides and hence represent a pool of flavour reserves in developing grapes. Typical compounds include -damascenone (megastigma-3,5,8-trien-7-one), vitispirane (6,9-epoxy-3,5(13)-megastigmadiene), 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), actinidol, vomifoliol, etc. Their aroma character varies from leafy, minty and fruity through various floral characters. Many of our common wine grapes such as Chardonnay owe much of their character to groups of norisoprenoids. The typical violet aroma of Syrah is due to specific norisoprenoid compounds.

The carotenoid pigments all have squalene as an intermediate, and hence these and the terpenes share a common pathway up as far as geranyl pyrophosphate. The carotenoids form by the condensation of two GPP molecules to form geranyl-geranyl pyrophosphate, which then goes through a few steps (prephytoene→lycopersene→phytoene, followed by cyclization→b-carotene).

### 5.1. Effects of fruit exposure on norisoprenoids

Enhanced light and temperature environments can lead to breakdown of carotenoid pigments and consequently higher concentrations of norisoprenoids (Martais et al. 1992). However, in many cases the impact of light exposure and/or cluster temperature has been inconsistent at best. Razungles et al. (1998) were among the first to study light and temperature effects on $C_{13}$ norisoprenoids. Sunlight effects on the berry carotenoid and $C_{13}$ norisoprenoid composition were studied before and after veraison. Syrah berries that were sun-exposed before veraison were higher in carotenoids than shaded berries. However, after veraison, sunlight caused the degradation of these pigments. Sunlight modified the non-epoxyxanthophyll/epoxyxanthophyll ratios. Metabolic relationships between the glycosylated $C_{13}$ norisoprenoids and their potential precursors were tentatively established between certain $C_{13}$ norisoprenoids and carotenoids in specific sun-exposure treatments. In addition, the sunlight increased other glycosidically bound compounds such as monoterpenes and phenols.

Effects of the modification of whole vine or individual cluster light environment by shade cloth from berry set to maturity were studied by Bureau et al. (2000a) on the volatiles and glycoconjugates in Muscat of Frontignan berries. Whole vines were shaded with 50 and 70% shade cloth, while clusters were shaded with 90% shade cloth. The sun-exposed berries were chosen as control berries, and the berries naturally shaded under foliage were also studied. The natural shading of clusters under foliage did not decrease the concentrations of free and bound compounds compared to sun-exposed berries. The artificially shaded clusters had lower concentrations of $C_{13}$ norisoprenoids (as well as monoterpenes) than both sun-exposed berries and berries from naturally shaded clusters. Moreover, the effect of vine shading on the aroma composition was lower compared to artificial cluster shading. Decreasing the cluster number per vine did not influence the total amounts of glycosidically bound compounds, except for monoterpene glycoconjugates. However, the higher monoterpene glycoconjugates in these berries were likely related to their early maturity. Authors concluded that under their experimental conditions, berry aroma composition did not appear to be affected by foliage shade. In a related study using the same experimental protocol, Bureau et al. (2000b) looked at the effects of the modification of vine or cluster environment on glycoconjugates in Syrah berries. Vines were shaded from berry set to maturity, with black polyethylene nets of different mesh size to obtain 30 and 50% of the direct sunlight. Clusters were naturally shaded by the leaves or artificially with 90% shade bags. Sun-exposed berries were chosen as control berries. A decrease in glycoconjugates was observed in shaded clusters, particularly for phenolic and $C_{13}$-norisoprenoidic glycosides. In the same way, vine shading caused a decrease in the concentrations of glycosides of terpenols, phenols and $C_{13}$ norisoprenoids in berries, but the grape environment (microclimate) affected the berry composition more than the vine environment. A cluster thinning experiment confirmed the independence of grapes with regard to the plant for the biosynthesis of the $C_{13}$ -norisoprenoid glycosides.

The effect of cluster exposure on the grape carotenoid profile was also investigated by Oliveira et al. (2004) on several Portuguese cultivars. Grape cultivar, ripeness stage, sunlight and shade exposure, altitude and vegetative height were all the variables studied. Differences between cultivar were observed in eight different red wine grape cultivars: Touriga Brasileira, Tinta Barroca, Tinta Amarela, Souzao, Touriga Franca, Touriga Nacional, Tinta Roriz and Tinto Cao, from the Douro region. Tinta Amarela and Touriga Brasileira produced higher concentrations of carotenoids. Carotenoids decreased during ripening. Decreases of lutein were observed until 66% of the original concentration, whereas β-carotene slowly decreased in a constant manner until the harvest date. Carotenoids were consistently higher in shaded grapes than in those exposed to direct sunlight in two white grape cultivars, Maria Gomes and Loureiro.

Lee et al. (2007) assessed the effects of light exposure and vine microclimate on $C_{13}$-norisoprenoid concentrations in Cabernet Sauvignon grapes and wines by measuring the amounts of β-damascenone, TDN and vitispirane. The most exposed treatment (all lateral and primary leaves removed) had the highest light intensity and temperature and showed the highest concentrations of TDN and vitispirane. However, in the more shaded treatments, concentrations of all norisoprenoids were variable and dependent on the treatment conditions. When leaves were removed, $C_{13}$-norisoprenoid concentrations were linearly and positively correlated with increasing sunlight exposure. In contrast, in the most shaded treatments with no leaf removal, there were high concentrations of norisoprenoids. β-Damascenone concentrations were highest when no leaves were removed. Grapes and corresponding wines from the south side of the vine had higher norisoprenoids than those from the north side.

Ristic et al. (2007) enclosed clusters of Shiraz grapes prior to flowering in boxes designed to eliminate light without altering bunch temperature and humidity. This artificial shading had little effect on berry ripening and accumulation of %SS but at harvest, the shaded clusters had smaller berries, pH and TA. Analysis of potential flavour compounds indicated that the wines made from shaded fruit had decreased concentrations of glycosides of **β**-damascenone and TDN. Sensory analysis of the wines indicated no difference in aroma attributes, but the wines made from shaded fruit were rated lower for astringency, fruit flavour and flavour persistence in mouth.

### 5.2 Influence of irrigation on norisoprenoids

The limited information we have on the response of norisoprenoids to irrigation suggests that deficit irrigation might lead to increases in norisoprenoid concentration. The influence of irrigation strategy on grape berry carotenoids and $C_{13}$-norisoprenoid precursors was investigated for Cabernet Sauvignon by Bindon et al. (2007) in Australia. Two irrigation treatments were compared, one in which vines received reduced irrigation applied alternately to either side of the vine (partial rootzone drying, PRD) and a second control treatment in which water was applied to both sides of the vine. The PRD vines received on average 66% of the water applied to the controls. In both irrigation treatments, the most abundant grape berry carotenoids, β-carotene and lutein, decreased post-veraison, but as the fruit approached maturity, the concentration of these carotenoids increased in fruit of PRD-treated vines relative to the controls, particularly for lutein. Moreover, PRD caused increases in the concentration of hydrolytically released $C_{13}$-norisoprenoids β-damascenone, β-ionone and 1,1,6-trimethyl-1,2-dihydronaphthalene in fruit at harvest. During the second season of the experiment, the effect of the PRD treatment on $C_{13}$-norisoprenoids was greater, and there was an increase in total $C_{13}$-norisoprenoid content per berry, suggesting that increases in $C_{13}$-norisoprenoids in response to PRD were independent of water deficit–induced changes in berry size and concomitant altered berry surface-area-to-volume ratios.

Linsenmeier and Löhnerz (2007) measured $C_{13}$-norisoprenoids in Riesling wines produced from the 1996, 1997 and 2003 vintages within the scope of a long-term nitrogen fertilization experiment (0, 60 and 150 kg N/ha). N fertilization led to lower TDN concentrations, whereas the trend was for actinidol and β-damascenone to increase with increasing fertilization, and for vitispirane to be unaffected by fertilization. Yield, which was affected by fertilization, showed negative correlations with norisoprenoids. Vitispirane, actinidol and TDN increased with storage time. The coolest year studied, which had fewest sunshine hours, resulted in the highest concentrations of β-damascenone and the lowest concentrations of vitispirane, actinidol and TDN.

## 6. Volatile Thiols

Volatile thiol compounds are currently one of the most active areas of wine flavour research. They are found in small quantities in grapes, and most certainly their precursors might be influenced by viticultural practices. They are among the most potent aroma compounds found in wine. These sulphur-containing compounds have extremely low perception thresholds, ranging from 0.8–1500 ng/kg (Tominaga et al. 2000). Currently, five highly aromatic volatile thiols—4-mercapto-4-methylpentan-2-one (4MMP), 3-mercaptohexyl acetate (A3MH), 4-mercapto-4-methylpentan-2-ol (4MMPOH), 3-mercaptohexan-1-ol (3MH) and 3-mercapto-3-methylbutan-1-ol (3MMB)—have been identified in grapes (Darriet et al. 1995, and Tominaga et al. 1998a, b, 2000). The compounds 4MMP, 4MMPOH and 3MH have aromas characteristic of box tree/broom, citrus zest and grapefruit/passion fruit respectively (Tominaga et al. 1998b, 2000). Moreover, 3MMB and A3MH are responsible for the overtones of cooked leeks and passion fruit respectively (Tominaga et al. 2000). These compounds impact varietal character in certain grapes and wine. For example, they play a major role in the aroma of Sauvignon Blanc (Darriet et al. 1995, and Tominaga et al. 1998b, 2000), Cabernet Sauvignon (Tominaga et al. 1998a) and Merlot (Murat et al. 2001a) wines. Furthermore, Tominaga et al. (2000) found that these aroma compounds are also responsible for nuances of several other *V. vinifera* cultivars including Semillon, Gewürztraminer, Riesling and Colombard.

Darriet et al. (1995) showed that 4MMP and 3MH exist in grapes but in the form of non-volatile, cysteine-bound conjugates. Peyrot des Gachons (2000) found that the cysteinylated precursors for 4MMP and 4MMPOH are located mainly in grape juice, while the precursor for 3MH is distributed equally between berry skin and juice. Their location does not depend on the stage of berry development (Peyrot des Gachons 2000). The biosynthesis of these S-cysteine conjugates in grapevines is virtually unknown. It is thought that these compounds are intermediate products in the detoxification system of living organisms and are formed from the breakdown of the corresponding S-glutathione conjugates (Peyrot des Gachons 2000). Although volatile thiols are almost non-existent in grapes and must (Peyrot des Gachons et al. 2000, 2005), they are released into wine from their corresponding precursors during alcoholic fermentation (Howell et al. 2004, and Murat et al. 2001b).

Since volatile thiols are a new area of research and have only recently been identified and quantified, there is little research on how these compounds can be altered in the vineyard or winery. Peyrot des Gachons et al. (2005) found that volatile thiol precursors were highest in vines with moderate nitrogen supply, whereas nitrogen deficiency seemed to limit aroma potential. Furthermore, they found that volatile thiol precursors were highest in vines under mild water deficit, whereas severe water deficit stress seemed to limit aroma potential. Therefore, highest aroma potential can be achieved under mild water stress and when nitrogen status is non-limited (Peyrot des Gachons et al. 2005). Powdery mildew infection on berries decreases the concentration of 3MH (Calonnec et al. 2004).

As mentioned, 4MMP and 3MH (and perhaps other volatile thiols) exist in grapes in the form of non-volatile, cysteine-bound conjugates. It is suggested that the amplification of these aromas during fermentation occurs through the action of yeast carbon-sulphur lyases (Tominaga et al. 1998a, b, and Howell et al. 2004). The compounds 4MMP, 4MMPOH and 3MH are released into wine from their grape-derived cysteinylated precursors during alcoholic fermentation (Darriet et al. 1995, Tominaga et al. 1998a, b, and Peyrot des Gachons et al. 2000, 2005). Therefore, it appears that volatile thiols might be manipulated easily in the winery by release of these thiol compounds from their precursors. Various yeast strains can produce varying amounts of each of the compounds (Murat et al. 2001b, and Howell et al. 2004). Fermentation temperature also has an influence on the release of thiol compounds. Howell et al. (2004) found that at 28°C some yeast strains released 100-fold more 4MMP than at 18°C. Although only small amounts (1.4 to 4.2%) of aroma precursors are transformed during alcoholic fermentation (Peyrot des Gachons et al. 2000, and Howell et al. 2004), these concentrations of volatile thiols still contribute significantly to the overall sensory profile of the resultant wine.

Following primary fermentation and malolactic fermentation, thiol concentrations can be considerable (relatively speaking). However, thiols can be easily oxidized after certain winemaking procedures such as racking or aging, so these compounds can decrease significantly. Blanchard et al. (2004) found a substantial decrease in 3MH in wines with the presence of oxygen and catechin. However, sulphur dioxide reduced these effects considerably. Anthocyanins can help stabilize volatile thiols in wine (Blanchard et al. 2004). Oak aging can be used to manipulate the composition and amount of volatile thiols present in wine; 2-furanmethanethiol (2FM), a thiol with a strong roast coffee aroma, has been identified in certain red Bordeaux wines and has been found in toasted oak staves (Tominaga et al. 2000).

Only recently have volatile thiols been a key area of research in wine flavour chemistry. Research thus far has focused mainly on method development for identification and quantification of these compounds and their contribution in certain key cultivars such as Sauvignon Blanc and Cabernet Sauvignon. There are ongoing studies looking at ways to release volatile thiols from their cysteinylated precursors by yeast strains. Generally speaking in oenology and viticulture, any aspect concerning volatile thiols is a wide-open research topic. Research is currently ongoing in places such as New Zealand, where it is felt that these compounds contribute more than just nuances to their wines. In the case that volatile thiols are in fact the most potent compounds in wines, practices in the vineyard can be further explored to maximize aroma potential from these compounds.

## 7. Effects of Viticultural Practices on Phenolic Analytes

### 7.1 Fruit exposure effects on phenolic analytes

There is considerable evidence that anthocyanin production is positively correlated with light levels, both on the canopy in general (Kliewer 1970, 1977; Keller and Hrazdina 1998; and Spayd et al. 2002) and on the fruit specifically (Spayd et al. 2002). Other results have been either more complicated or entirely contrary to these results, suggesting that there may be varietal differences in the synthesis of anthocyanins. Among those who have conducted studies using artificial shading, Kataoka et al. (1984) found that complete shading (in an aluminum foil bag) of Kyoho clusters during ripening resulted in inhibited phenylammonium lyase (PAL) activity and no anthocyanin accumulation in the berries. The same experiment found that complete shading of Super Hamburg berries had little or no effect on PAL activity nor on anthocyanin synthesis. Likewise, Downey et al. (2004) found that shading clusters inside opaque boxes had no effect on anthocyanin accumulation in Shiraz berries in two of three years. Differences occurred between shade and exposed fruit in the relative accumulation of different anthocyanins; shaded fruit contained relatively more peonidin and cyanidin glucosides.

Condensed tannins were not affected by shading, but flavonols were reduced by shading. Similarly, Yamakawa et al. (1983) found considerable anthocyanin accumulation in *Vitis* cell suspension in the absence of light, but light irradiation enhanced anthocyanin accumulation.

Cortell and Kennedy (2006) examined changes in flavonols, proanthocyanidins and anthocyanins in Pinot Noir in Oregon in shaded and exposed treatments. Light exclusion boxes were installed on pairs of clusters on the same shoot (shaded treatment), while a second set of clusters on an adjacent shoot were considered as the exposed treatment. Cluster shading resulted in a substantial decrease in flavonols and skin proanthocyanidins and minimal differences in anthocyanins. Shaded and exposed treatments were similar at veraison in terms of seed proanthocyanidins; however, by harvest, the shaded treatment had higher extension and terminal subunits compared to the exposed treatment. Shaded fruit was lower for all skin proanthocyanidins at both veraison and harvest. Shading caused an increase in the proportion of (-)-epicatechin and a decrease in (-)-epigallocatechin at harvest in berry skins. Seed proanthocyanidins in shaded fruit contained a lower proportion of (+)-catechin and a higher proportion of (-)-epicatechin-3-O-gallate and a lower proportion of (+)- catechin and (-)-epicatechin-3-O-gallate and a higher proportion of (-)- epicatechin. For anthocyanins, the shaded treatment had proportional reductions in delphinidin, cyanidin, petunidin and malvidin and a large increase in peonidin glucosides. Model wine extractions from the two treatments paralleled differences in the fruit with a lower concentration of flavonols, anthocyanins and proanthocyanidins in the shaded treatment, while the skin proanthocyanidin percent extraction was 17% higher in the exposed model extraction than the shaded treatment.

Among those assessing natural canopy sun and shade conditions, Crippen and Morrison (1986b) found differences between sun-exposed and shaded Cabernet Sauvignon fruit in terms of soluble phenol content per berry and in both anthocyanin concentration and total content per berry pre-harvest. There were no differences at harvest, however. Total soluble phenols increased until veraison and then decreased from veraison to harvest. The percentage of polymerized phenols decreased during early berry growth and then increased from veraison to harvest.

Temperature has also been found to affect anthocyanin production in grape berries. In general, high temperatures (above 35ºC) have been inhibitory to anthocyanin synthesis (Kliewer 1970, 1977; Kliewer and Torres 1972; Kataoka et al. 1984; and Spayd et al. 2002). Kliewer and Torres (1972) also determined that diurnal flux in temperature also affects fruit colouration. Day-night temperature differences of greater than 10ºC were generally found to be inhibitory to fruit colouration, beyond the detrimental effects of high temperature on colouration.

Bergqvist et al. (2001) assessed the effects of sunlight exposure and related temperature effects on phenolic analytes in addition to berry growth and composition of Cabernet Sauvignon and Grenache. Anthocyanins increased linearly as sunlight exposure on the north side of the canopy increased, but declined when cluster exposure on the south exceeded 100 mmol $m^{-2}$ $sec^{-1}$. Total phenols generally followed a similar pattern. The results suggest that the effects of light on fruit composition, including phenolic analytes, are heavily dependent on the extent to which berry temperature is elevated because of increased sunlight exposure. The authors concluded that prolonged exposure of clusters to direct sunlight should be avoided for maximum berry colour in the central San Joaquin Valley and other warm regions.

In Piedmont, Italy, Chorti et al. (2007) assessed five fruit exposure levels to Nebbiolo: vines exposed to normal light conditions throughout the season; fruit-zone shaded vines from fruit-set to veraison; fruit-zone shaded vines from fruit-set to harvest; fruit-zone shaded vines from veraison to harvest; and fruit-zone leaf-removed vines at fruit-set. Fruit-zone shading during the initial phase of berry development decreased berry size and consequently impacted concentration of many analytes. Fruit-zone shading delayed accumulation of %SS, and when applied from veraison to harvest, reduced total anthocyanin concentration, but it did not affect total flavonoid accumulation. Cluster-zone leaf removal notably increased cluster exposure and decreased total anthocyanin and total flavonoid accumulation.

Spayd et al. (2002) succeeded in identifying how light and temperature affect phenolic analytes in different ways. Anthocyanin and phenolic profiles of berry skins from Merlot in the Yakima Valley of Washington were examined in terms of the individual influences of sun exposure and temperature. West-exposed clusters were cooled to the temperature of shaded clusters, and shaded clusters were heated to the temperature of west-exposed clusters. Berry temperature was increased as much as 13ºC above ambient and shaded cluster temperatures when clusters were exposed to sunlight, regardless of aspect. However, maximum fruit temperatures were higher for clusters on the west side of the canopy (often > 40ºC) because ambient temperatures were higher after noon. East-exposed clusters had higher total skin monomeric anthocyanin (TSMA) concentrations than west-exposed or shaded clusters. Exposure to sunlight increased TSMA concentrations regardless of temperature. Cooling sun-exposed clusters increased TSMA concentrations, while heating shaded clusters decreased TSMA in warm seasons, but had no effect in cooler ripening seasons. Ultraviolet (UV) light barriers did not influence either cluster temperature or TSMA concentrations, and lower TSMA concentrations in berry skins from west-exposed clusters were due to temperature and not to UV radiation. Exposure to solar radiation increased concentrations of the 3-glycosides of quercetin, kaempferol and myricetin. Sun-exposed clusters, regardless of aspect, had almost 10 times greater concentrations of total flavonols than shaded clusters. UV-light barriers reduced individual and total flavonol concentrations, suggesting that their synthesis might be partly light driven; temperature had little to no effect on their concentrations.

Few of the aforementioned studies have investigated the impact of vine shading on the sensory attributes of the resultant wine. A study in the Sunraysia region, Victoria, Australia examined the effects of canopy exposure levels on phenolic composition plus aroma, flavour and mouthfeel aspects in Cabernet Sauvignon and Shiraz wines whose source vines were subjected to different levels of canopy exposure (Joscelyne et al., 2007). Canopy exposure treatments included a control (standard vineyard practice), exposed (achieved with a foliage wire 600 mm above the top cordon), highly exposed (using a foliage wire with leaf plucking in the fruit zone) and shaded treatment (using 70% shade cloth). Spectral and descriptive analyses showed that anthocyanins, other phenolics and perceived astringency were lower in wines made from shaded fruit; however, the reverse was generally not observed in wines of exposed and highly exposed fruit. Descriptive analysis also showed wines from the shaded fruit were different from other treatments for a number of flavour and aroma characters.

Another aspect given little attention in the literature is that of spatial variability in fruit exposure resulting from different vine vigour levels, and the relationship between vigour and phenolic analytes. Cortell et al. (2007) examined fruit exposure effects on Pinot Noir anthocyanins from a standpoint of vigour-induced fruit exposure effects. High vigour zones in two vineyards had lower %SS and higher TA, and there was a trend for lower anthocyanin concentration in the high vigour zones. In one year, there was a higher proportion of malvidin-3-*O*-glucoside and lower proportions of the other four anthocyanins (delphinidin-, cyanidin-, petunidin- and peonidin-3-*O*-glucosides) commonly found in Pinot Noir. In both years studied, one site had proportionally higher peonidin-3-*O*-glucoside and lower malvidin-3-*O*-glucoside than the other site. Authors opined that some of these differences might have been related to the higher exposure and temperatures found in Site B compared to Site A, which were found also in the low vigour zones.

It is reasonable to assume that significant enzymes responsible for anthocyanin and phenol synthesis are up-regulated by enhanced light and temperature environments. Downey et al. (2004) focused exclusively on light effects on the synthesis of phenolic analytes and the genes encoding those enzymes responsible. Opaque boxes were applied to clusters of Shiraz grapes prior to flowering to determine the effect of sunlight on berry development and accumulation of flavonoids. The boxes were designed in particular to exclude light and to minimize changes in temperature and humidity. There was no effect of shading on sugar accumulation and berry weight. Chlorophyll concentration was lower in the shaded fruit, which appeared pale yellow until veraison. The fruit coloured normally in the shaded clusters, and in two of three seasons there was no change in anthocyanin concentration. Anthocyanin composition was altered in the shaded fruit, which had a greater proportion of the anthocyanins cyanidin and peonidin glucosides. Shading had no effect on the concentrations of condensed tannins in the skin or seeds of ripe fruit, but it reduced flavonols in the skin. In the exposed fruit, flavonol concentration was highest around flowering then declined as the berries grew, but there was an increase in flavonols per berry during ripening. When the boxes were applied before flowering, shaded fruit had much lower concentrations of flavonols throughout berry development, and at harvest the flavonols were < 10% that in exposed fruit. The authors mentioned two key genes that appeared to be up-regulated during berry development: Expression of the gene encoding UDP-glucose flavonoid-3-O-glucosyl transferase, a key gene in anthocyanin synthesis, increased after veraison and was similar in both shaded and exposed fruit. Secondly, a gene encoding flavonol synthase was expressed at flowering and during ripening in exposed grapes, but its expression was reduced in shaded fruit. The authors suggested that shading had little effect on berry development and ripening, including accumulation of anthocyanins and tannins, but as Spayd et al. (2002) indicated, shade substantially decreased flavonol synthesis.

### 7.2 Growing season canopy manipulation

Many studies have also been done on the effects of canopy manipulation on red winegrape composition (Smith et al. 1988 and Reynolds et al. 1994c, 1995b, 1996c). Among research into growing season canopy manipulation, a New Zealand study unequivocally found that basal leaf removal (either 50% or 100% of leaves in fruit zone) increased both total phenol and anthocyanin concentrations in Cabernet Sauvignon, with the greatest increase (≈50% over control) occurring when the treatment was done five weeks after flowering (Smith et al. 1988). Mazza et al. (1999) also found that leaf removal resulted in higher phenolic concentration and colour density over the control. Conversely, Iacono et al. (1994) found that when 40% of basal leaves were removed from around the grape clusters, there was a reduction in %SS, no change in malic or tartaric acid and no change in anthocyanins, even though available radiation to the clusters was dramatically increased above the control. Shading of the entire canopy (with a 50% shade cloth), on the other hand, lowered the anthocyanin concentration across all canopy treatments (cluster thin and leaf removal). Leaf removal reduced the shading of clusters but also reduced photosynthetic capacity, resulting in lower sugar concentrations and no differences in anthocyanins or acidity. Among canopy manipulation resulting in negative consequences, late-season and/or severe hedging was shown to reduce anthocyanins in De Chaunac grapes in British Columbia (Reynolds and Wardle 1989c).

### 7.3 Crop control

Many studies have also been done on the effects of crop reduction on red winegrape composition (Weaver et al. 1961, Kliewer and Weaver 1971, Ough and Nagaoka 1984, and Reynolds et al. 1994c). A few studies (Iacono et al. 1994, Reynolds et al. 1994c, and Mazza et al 1999) have also been done on the effects of both canopy and crop manipulation on red winegrape maturity in terms of %SS, TA, pH and aroma and colour compounds. Studies with *V. vinifera* red wine cultivars in which the crop was adjusted by means of cluster thinning alone have found that crop reduction results in increased %SS, anthocyanins, total phenols and colour intensity (Reynolds et al. 1994c, Mazza et al. 1999, and Guidoni et al. 2002). The effectiveness of crop reduction has not always been consistent. Some have found no differences in must composition when crops were thinned (Ough and Nagaoka 1984) whereas others have found specific fruit composition attributes to be more affected than others. Crop reduction may also have beneficial effects on individual phenolic analytes as well (Di Profio et al. 2011).

One study with Pinot Noir conducted in British Columbia and Oregon involving three canopy management treatments (vertical shoot positioning with either 10 or 20 shoots/m row, Scott Henry [vertical canopy division] with 10 shoots/m canopy, 15 shoots/m row in Oregon only) in combination with two crop levels (full crop, half crop) found that by increasing the crop level, %SS were reduced substantially but colour was reduced to a lesser degree than by increasing canopy density (Reynolds et al. 1994c). In Scott Henry wines, TA and pH were reduced and ethanol and anthocyanins were increased. Ethanol and wine TA increased and pH decreased with increasing shoot density. Reducing crop level increased ethanol and anthocyanins. Clove, bell pepper and grassy aromas were least in 10 shoots/m and Scott Henry treatments. Reducing crop level increased colour, currant aroma, astringency and intensity of finish independent of canopy treatment (Reynolds et al. 1996b).

Reynolds et al. (2005) explored delayed shoot thinning as an alternative to canopy division in vigorous canopies. Pinot Noir and Cabernet Franc vines in Ontario were subjected to six different shoot-thinning timings between Eichhorn and Lorenz phenological stages 9 to 31. An additional treatment (double pruning retaining two disposable canes) was imposed on Cabernet Franc. Many of these treatments resulted in improved canopy microclimate, e.g., treatments imposed after bloom plus the double-pruned treatment produced lower leaf layer numbers and better leaf and cluster exposure than did the control and early shoot thinning in Cabernet Franc, while early shoot-thinning treatments induced higher leaf areas in both cultivars compared to later treatments. Early shoot thinning on Pinot Noir increased TA and %SS in berries and must. Early shoot thinning on Cabernet Franc increased colour intensity in berries and colour intensity, total phenolics and total anthocyanins in wines. The double-prune treatment was characterized by higher %SS, hue/tint, colour intensity and total phenolics overall.

### 7.4 Training systems and vine spacing

As with growing season canopy management, choice of a training system may have a substantial impact on leaf and cluster microclimate, and consequently, fruit composition and wine quality. These effects on red winegrape cultivars extend to phenolic analytes. Smart (1982) and Smart et al. (1985b) described the effects of GDC training on reduction of canopy shading in Shiraz vines, and ultimately the enhancement of ionized anthocyanins and total phenols. Carbonneau and Huglin (1982) and Carbonneau et al. (1978) described the positive impacts of the various iterations of the Lyre divided canopies on berry skin anthocyanins and total polyphenols in Cabernet Sauvignon, along with corresponding enhancements in wine quality.

Reynolds et al. (1994c) tested Scott Henry training against vertically shoot-positioned canopies (10 and 20 shoots per m row) on Pinot Noir vines in British Columbia. Scott Henry–trained vines had the lowest weight of cane prunings and highest crop loads, and also had lowest %SS, pH, anthocyanins and juice colour, but also had lowest berry and juice TA. The reduction in colour was unexpected, since Scott Henry treatments had the least dense canopies with the lowest percentage of shaded clusters. In a follow-up study, Reynolds et al. (1996c) found that wine TA and pH were reduced and anthocyanins were increased by Scott Henry training, in both British Columbia and Oregon. Means of five vintages from both wine regions indicated that clove, bell pepper and grassy aromas were lower in Scott Henry canopy treatments. Cherry and anise aromas were also higher in Scott Henry wines, but many flavours, colours and finishes were not increased by vertical canopy division.

Peterlunger et al. (2002) examined the effect of four training systems for Pinot Noir in the Friuli Hills (northeastern Italy). Simple Guyot, double Guyot, horizontal spurred cordon and vertical spurred cordon were assessed. As has often been the case with variations to non-divided systems, the training systems affected yield but showed little or no impact on grape and wine composition, including grape and wine phenolics. Sensory analysis could not show relevant differences among training systems.

Among work with French–American hybrids, horizontally divided canopies such as GDC and Y-trellises were tested by Reynolds et al. (1995c, 2004a) on Chancellor grapevines in British Columbia. Yields of divided canopies (GDC and Y) averaged 42% higher than non-divided systems, but cluster weights and berries per cluster tended to be lower in the divided canopies. Crop loads (ratio of yield: weight of cane prunings) of divided canopies exceeded the currently accepted level (10 to 12) beyond which wine quality could be compromised. The GDC system produced fruit with the lowest %SS, but also with the lowest TA and pH and the highest anthocyanin concentration. Increased vine spacing led to smaller vine size (per m row), lower cane weights and occasionally lower %SS. Chancellor GDC wines had highest berry flavour.

### 7.5 Irrigation

The positive effects of limited irrigation (i.e., regulated deficit irrigation) on phenolic analytes may invariably be a consequence of reduced vine vigour and improved fruit exposure. Generally, improvements in fruit exposure occur in situations where mild water deficits have been imposed (Balint and Reynolds 2010).

The influence of irrigation on grape and wine composition was investigated for Agiorgitiko in the Nemea appellation area in southern Greece by Koundouras et al. (2006). Three non-irrigated plots were studied during vintages that were very hot and devoid of summer rainfall. Vines were subjected to different water regimens as a result of the variation of soil water-holding capacity and evaporative demand. Water deficit accelerated sugar accumulation and malic acid reduction in the juice. Early water deficits during the growth period had beneficial effects on the concentration of anthocyanins and total phenolics in berry skins. A similar pattern was observed for the phenolic concentration of wines as well. Limited water availability also seemed to increase glycoconjugates in the main aromatic components of grapes. Wines produced from grapes of stressed vineyards were also preferred in tasting trials.

Chaves et al. (2007) assessed three irrigation strategies (deficit irrigation, partial root drying [PRD], fully irrigated) on two grapevine cultivars (Moscatel and Castelao) in Portugal. They indicated that the amount of water could be decreased by 50% (as in the case of PRD) without compromise to fruit composition. Non-irrigated and PRD vines exhibited higher concentrations of berry skin anthocyanins and total phenols than those in deficit-irrigated and fully irrigated vines. These effects on potential quality were mediated by a reduction in vigour, leading to an increase on light interception in the cluster zone. Because plant water status on most dates during the season was not different between PRD and deficit-irrigated (when different, PRD even exhibited a higher leaf water potential than deficit-irrigated vines), the authors concluded that the growth inhibition in PRD was not a result of diminished water status.

Anthocyanin biosynthesis is strongly up-regulated in ripening fruit of grapevines grown under drought conditions. Castellarin et al. (2007) investigated the effects of long-term water deficit on the expression of genes coding for flavonoid and anthocyanin biosynthetic enzymes and related transcription factors, genes sensitive to endogenous (sugars, abscisic acid) and environmental (light) stimuli connected to drought stress, and genes developmentally regulated in ripening berries. Total anthocyanin concentration increased at harvest in water-stressed fruits by 37 to 57% in two consecutive years. At least 84% of the total variation in anthocyanin concentration was explained by a linear relationship between the integral of mRNA accumulation of the specific anthocyanin biosynthetic gene, UDP-glucose: flavonoid 3-O-glucosyltransferase and metabolite concentration during a time series from veraison through ripening. Chalcone synthase and flavanone-3-hydroxylase (F3H) genes of the flavonoid pathway showed high correlation as well. Genes coding for flavonoid 3′,5′-hydroxylase and O-methyltransferase were also up-regulated in berries from dehydrated plants in which anthocyanin composition was enriched in more hydroxylated and more methoxylated derivatives such as malvidin and peonidin. The induction in water-stressed plants of structural and regulatory genes of the flavonoid pathway suggested that interrelationships between developmental and environmental signalling pathways were magnified by water deficits, which actively promoted fruit maturation and, in this context, anthocyanin biosynthesis. It is also worthy of note that Downey et al. (2004) likewise found an up-regulation of UDP-glucose flavonoid-3-O-glucosyl transferase in exposed fruit post-veraison, and an enhancement in the activity of a gene encoding flavonol synthase in exposed fruit.

## 8. Exogenous Abscisic Acid Applications

Abscisic acid (ABA) is a hormone that plays a major role in plant adaptation to abiotic environmental stresses (drought, cold and salinity), growth control, seed development and germination. ABA is involved in the signalling chain of water stress in plants (Antolin et al. 2008, and Jiang and Hartung 2008). ABA also has a major role in the fruit maturation process in grapes (Hale and Coombe 1974), where it is associated with the main molecular processes (Jeong et al. 2004). Transcripts and proteins linked to ABA biosynthesis have been found in berries during the maturation process (Castellarin et al. 2007). ABA has been described as a mediator, which at certain concentrations, stimulates the expression of genes encoding invertase or controlling phenol and anthocyanin biosynthesis (Gagné et al. 2006, Gambetta et al. 2010, and Koyama et al. 2010). Exogenous ABA treatments at veraison have enhanced several processes involved in grape berry maturation, such as the accumulation of %SS, the decrease in the concentration of organic acids and the accumulation of anthocyanin (Coombe and Hale 1973, Gambetta et al. 2010, and Yamane et al. 2006).

ABA is synthesized in roots and leaves, and transported to grape clusters via the phloem (Shiozaki et al. 1999). Grape berry ABA concentration increases just before veraison (Hale and Coombe 1974). Besides its genetic control, ABA concentration and its effects are moderated by environmental conditions such as temperature (Koshita et al. 2007), light (Jeong et al. 2004) and water stress (Antolín et al. 2008). Increased concentrations of ABA during the growing season are associated with growth restriction resulting from water stress, which is postulated to be an adaptation mechanism to the adverse conditions imposed by water stress (Jiang and Hartung 2008). In *Vitis vinifera*, periods of moderate water deficit (predawn leaf water potential of ≈ -0.8 MPa) during ripening enhanced the polyphenol and anthocyanin concentrations in the berries (Matthews and Anderson 1988).

Increases in cluster weight and yield per vine were found in ABA-treated vines of Cabernet Sauvignon in Mendoza, Argentina (Quiroga et al. 2009). Vines were subjected to weekly ABA applications, from budburst through harvest. They concluded that yield increased due to increased berry set or reduced early berry abortion brought about by ABA. Others (Giribaldi et al. 2009) have shown that ABA treatment is most effective in enhancing ripening when it is applied pre-veraison and not later (50% or more berry colouration). ABA was more effective than ethephon at improving the colour of Crimson Seedless table grapes (Cantin et al. 2007). The ABA treatments were not accompanied by any premature berry abscission, as was expected. Crimson Seedless table grapes treated with 300 µL/L ABA had their harvest date advanced by 10 to 30 days compared with non-treated vines (Cantin et al. 2007). Exogenous applications of ABA increased the anthocyanin concentration in skins of the table grape cultivar Redglobe (Peppi et al. 2007). However, no linear relationship was found between grape colour variables and anthocyanins, although grapes with high skin anthocyanins appeared darker and more red-coloured than grapes with low anthocyanin concentration (Peppi et al. 2007). Exogenous ABA applied to Cabernet Sauvignon clusters led to rapid accumulation of anthocyanins and flavonols, as well as enhanced gene activity of the phenylpropanoid and flavonoid pathways (Koyama et al. 2010). In the San Joaquin Valley in California, Gu et al. (2011) applied ABA to either clusters or leaves of Cabernet Sauvignon and found that berry anthocyanins increased only when ABA was applied to the clusters. From the winemaking point of view, anthocyanin composition affects colour stability. Cyanidin, delphinidin and petunidin have ortho-diphenolic groups that enhance susceptibility to oxidation, while methoxylated anthocyanins such as peonidin and malvidin are more stable.

In the Niagara Peninsula, Balint and Reynolds (2013) conducted two experiments in a Cabernet Sauvignon block. Both experimental years (2008, 2009) were characterized by higher rainfall and lower temperatures than average. The first experiment initiated one week pre-veraison consisted of four treatments (untreated control, 300 mg ABA/L applied to the full canopy, clusters only, leaves only) applied three times at two-week intervals. The second experiment had three treatments (0 [control] 150, 300 mg ABA/L applied to clusters only). In both years, the control still had clusters with 20% green berries two to four weeks after experiment initiation. Following treatment, berries had a lower ABA uptake rate than leaves. Both ABA rates hastened the onset of veraison. In both years the transpiration rate, leaf $\psi$ and fruit composition were most affected in the leaves-only and whole canopy treatments. At harvest, Brix was higher and the berry weight was lower in the ABA treatments than in the control. Total anthocyanins and total phenols also increased in most ABA treatments. Berries from clusters treated with the highest ABA rate showed a higher red-blue colour intensity and also had highest anthocyanins and phenols compared to berries from other treatments. The treated vines showed enhancement in individual anthocyanins and acetylated anthocyanins, with significant changes in the ratios of cyanidin, petunidin and malvidin occurring among the treatments. Exogenous ABA was effective in accelerating onset of veraison and improving the grape composition of Cabernet Sauvignon.

Exogenous ABA (or products with similar effects) could provide considerable benefits to the wine industry in terms of grape composition, wine style and winery scheduling, particularly in wet and cool years. It would be necessary to understand and assess the long-term effects of using exogenous ABA not only on general vine metabolism and physiology, but also on wine organoleptic characteristics. It is still not clear if ABA acts alone or in combination with other hormones on different processes during the ripening period. However, ABA could be successfully introduced as an alternative cultural practice, particularly in cool years, and also in regions and growing seasons when there is a high chance of early frost to occur, and a concomitant potential of premature loss of foliage. The temporal advancement of ripening through hormonal control might be an asset because earlier fruit maturation is a distinct advantage in cooler areas or areas with a high risk of early frost occurrence, and where an early end of the growing season might prevent adequate fruit maturation.

## 9. Conclusions

The four basic pillars of the Cool Climate Paradigm of winegrowing are

1. Keep the fruit warm
2. Keep the leaves exposed to sunlight
3. Achieve vine balance
4. Minimize water stress

Practices that are particularly relevant to these basic pillars include hedging and basal leaf removal, training systems, vine spacing, crop control and shoot density, vineyard floor management and irrigation. The results of many of our experiments suggest that fruit exposure, canopy manipulation, pre-fermentation practices and vineyard site may influence monoterpene concentration of berries and juices of several *V. vinifera* cultivars. These differences can sometimes be confirmed organoleptically in wines. A failure to find good agreement between analytical and sensory results may be due to variability among judges, but may also be ascribed in part to the confounding taster response to non-floral monoterpenes such as α-terpineol. This underscores the need to follow up work of this nature with gas chromatographic analyses of wines, to overcome problems of this nature. Our work and that of others have demonstrated that not only do vineyard and cellar practices often affect aroma compound concentration in berries and juices, but very often organoleptic evaluation may confirm these analytical results. Our specific conclusions to date are (1) PVT are more responsive to viticultural and oenological practices than FVT; (2) FVT and PVT are rarely correlated with %SS, TA or pH, thus cannot be predicted by standard harvest indices; (3) losses in FVT and PVT can occur between the berry and juice stages, hence the desirability of skin contact; and (4) FVT and PVT concentrations can, in some cases, be related to wine tasting results.

## Acknowledgement

The author would like to acknowledge the contribution of Gabriel Balint to this paper.

## References

Abbott, N. A., P. J. Williams, and B. G. Coombe. 1993. Measure of potential wine quality by analysis of grape glycosides. *Proceedings of the Eighth Australian Wine Industry Technical Conference*. Stockley, C. S., R.S. Johnstone, et al. (Eds.). Winetitles, Adelaide, SA. 72-75.

Aiken, J. W., and A. C. Noble. 1984. Comparison of the aromas of oak and glass aged wines. *Amer. J. Enol. Vitic.* 35:196-199.

Allen, M.S., M. J. Lacey, and S. Boyd S. 1995. Methoxypyrazines in red wines: occurrence of 2-methoxy-3-(1-methylethyl) pyrazine. *J. Agric. Food Chem*. 43: 769-772.

Allen, M. S., M. J. Lacey, R. L. N. Harris, and W. V. Brown. 1991. Contribution of methoxypyrazines to Sauvignon blanc wine aroma. *Amer. J. Enol. Vitic.* 42:109-112.

Antolín, M. C., H. Santesteban, E. Santa Maria, J. Aguirreolea, and M. Sanchez-Diaz. 2008. Involvement of abscisic acid and polyamines in berry ripening of *Vitis vinifera* (L.) subjected to water deficit irrigation. *Austral. J. Grape and Wine Res*. 14:123-133.

Arnold, R. A., and A. M. Bledsoe. 1990. The effect of various leaf removal treatments on the aroma and flavor of Sauvignon blanc wine. *Amer. J. Enol. Vitic.* 41:74-76.

Augustyn, O. P. H., A. Rapp, and J. Wyk. 1982. Some volatile aroma components of *Vitis vinifera* L. cv. Sauvignon blanc. *S. Afr. J.Enol. Vitic.* 3:53-60.

Austerweil, G. l946. Quelques observations sur les parfums des vins. *Ind. Parfum.* 1:195-199.

Balint, G., and A. G. Reynolds. 2010. Effect of different irrigation strategies on vine physiology and grape quality of Cabernet Sauvignon and Sauvignon blanc in a cool-climate area. *Progrès Agricole et Viticole.* 127(11):232-41.

Balint, G., and A. G. Reynolds. 2013. Impact of exogenous ABA on vine physiology and grape composition of Cabernet Sauvignon. *Amer. J. Enol. Vitic*. 64:74-87.

Bayonove, C. L. and R. E. Cordonnier. 1970a. Recherches sur l'arôme du muscat. I. Évolution des constituants volatils au cours de la maturation du Muscat d'Alexandrie. *Ann. Technol. Agric.* 10:79-93.

Bayonove, C. L. and R. E. Cordonnier. 1970b. Recherches sur l'arôme du muscat. II. Profils aromatiques de cépages muscat et non muscat. Importance du linalol chez les muscats. *Ann. Technol. Agric*. 19:95-105.

Bayonove, C. L. and R. E. Cordonnier. 1971a. Recherches sur l'arôme du muscat. III. Étude de la fraction terpénique. *Ann. Technol. Agric*. 20:347-355.

Bayonove, C. L. and R. E. Cordonnier. 1971b. Le linalol, constituant important mais non spécifique de l'arôme des muscats. *C.R. Acad. Agric. France*. 57:1374-1378.

Bayonove, C. L., R. E. Cordonnier, P. Benard, and R. Ratier R. 1976. L'extraction des composés de l'arôme du muscat dans la phase préfermentaire de la vinification. *C.R. Acad. Agric. France.* 62:734-750.

Bayonove, C. L., R. E. Cordonnier, and P. Dubois. 1975. Étude d'une fraction caractéristique de l'arôme du raisin de la variété Cabernet Sauvignon, mise en évidence de la 2-métoxy-3-isobutylpyrazine. *C.R. Acad. Sci. Series D.* 281:75-81.

Belancic, A., E. Agosin, A. Ibacache, E. Bordeu, R. Baumes, A. J. Razungles, and C. L. Bayonove. 1997. Influence of sun exposure on the aromatic composition of Chilean Muscat grape cultivars Moscatel de Alejandría and Moscatel rosada. *Amer. J. Enol. Vitic.* 48:181-186.

Bergqvist, J., N. Dokoozlian, and N. Ebisuda. 2001. Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the central San Joaquin Valley of California. *Amer. J. Enol. Vitic.* 52:1-7.

Bindon, K. A., P. R. Dry, and B. R. Loveys. 2007. Influence of plant water status on the production of C-13-nor-isoprenoid precursors in *Vitis vinifera* L. cv. Cabernet Sauvignon grape berries. *J. Agric. Food Chem.* 55:4493-4500.

Blanchard, L., P. Darriet, and D. Dubourdieu. 2004. Reactivity of 3-mercaptohexanol in red wine: Impact of oxygen, phenolic fraction and sulfur dioxide. *Amer. J. Enol. Vitic.* 55:115-120.

Bledsoe, A. M., W. M. Kliewer, and J. J. Marois. 1988. Effects of timing and severity of leaf removal on yield and fruit composition of Sauvignon blanc grapevines. *Amer. J. Enol. Vitic*. 39:49-54.

Bureau, S. M., R. L. Baumes, and A. J. Razungles. 2000a. Effects of vine or bunch shading on the glycosylated flavor precursors in grapes of *Vitis vinifera* L. cv. Syrah. *J. Agric. Food Chem.* 48:1290-1297.

Bureau, S. M., A. J. Razungles, and R. L. Baumes. 2000b. The aroma of Muscat of Frontignan grapes: effect of the light environment of vine or bunch on volatiles and glycoconjugates. *J. Science Food Agric*. 80:2012-2020.

Buttery, R. G., R. M. Seifert, R. E. Lundin, D. G. Guadagni, and L. C. Ling. 1969a. Characterization of an important aroma component of bell peppers. *Chem. Ind.* 4:490-491.

Buttery, R. G., R. M. Seifert, R. E. Lundin, D. G. Guadagni, and L. C. Ling. 1969b. Characterization of some volatile constituents of bell peppers. *J. Agric. Food Chem.* 17:1322-1327.

Calonnec, A., P. Cartolaro, C. Poupot, D. Dubourdieu, and P. Darriet. 2004. Effects of *Uncinula necator* on the yield and quality of grapes (*Vitis vinifera*) and wine. *Plant Pathology.* 53:434-445.

Cantín, C. M. , M. W. Fidelibus, and C. H. Crisosto. 2007. Application of abscisic acid (ABA) at veraison advanced red color development and maintained postharvest quality of Crimson Seedless grapes. *Postharvest Biol. Technol*. 46:237-241.

Carbonneau, A., P. Casteran, and P. Leclair. 1978. Essai de détermination en biologie de la plante entière, de relations essentielles entre le bioclimat naturel, la physiologie de la vigne et la composition du raisin. *Ann. Amélior. Plantes*. 28:195-221.

Carbonneau, A. and P. Huglin. 1982. Adaptation of training systems to French regions. *Proceedings of the Grape and Wine Centennial Symposium*. Webb AD (Ed.), University of California Press, Berkeley. 376-385.

Castellarin, S. D., M. A. Matthews, G. Di Gaspero, and G. A. Gambetta. 2007. Water deficits accelerate ripening and induce changes in gene expression regulating flavonoid biosynthesis in grape berries. *Planta*. 227:101–112.

Castellarin, S. D., A. Pfeiffer, P. Sivilotti, M. Degan, E. Peterlunger, and G. Di Gaspero. 2007. Transcriptional regulation of anthocyanin biosynthesis in ripening fruits of grapevine under seasonal water deficit. *Plant Cell and Environment*. 30:1381-1399.

Chapman, D. M., M. A. Matthews, and J. X. Guinard. 2004a. Sensory attributes of Cabernet Sauvignon wines made from vines with different crop yields. *Amer. J. Enol. Vitic.* 55:325–334.

Chapman, D. M., J. H. Thorngate, M. A. Matthews, J. X. Guinard, and S. E. Ebeler. 2004b. Yield effects on 2-methoxy-3-isobutylpyrazine concentration in Cabernet Sauvignon using a solid phase microextraction gas chromatography/mass spectrometry method. *J. Agric. Food Chem.* 52:5431–5435.

Chaves, M. M., T. P. Santos, C. R. Souza, M. F. Ortuno, M. L. Rodrigues, C. M. Lopes, J. P. Maroco, and J. S. Pereira. 2007. Deficit irrigation in grapevine improves water-use efficiency while controlling vigour and production quality. *Annals of Applied Biology.* 150:237-252.

Chorti, E., S. Guidoni, A. Ferrandino, L. Gangemi, and V. Novello. 2007. Ombreggiamento della fascia produttiva in *Vitis vinifera* L. cv. Nebbiolo: Effetti sulla composizione polifenolica delle bacche. Quaderni di Scienze Viticole ed Enologiche, Univ. Torino. 29:155-167.

Coombe, B. G., and C. R. Hale. 1973. The hormone content of ripening grape berries and the effects of growth substance treatments. *Plant Physiol.* 51:629-634.

Coombe, B.G. and P. G. Iland. 1987. Grape berry development. *Proceedings of the 6th Australian Wine Industry Technical Conference*. Lee T. (ed.), Australian Industrial Publishers, Adelaide, S. Australia. 50-54.

Cordonnier, R. E. 1955. Recherche de l'addition frauduleuse d'aromatisants aux vins naturels. Observations sur le parfum naturel de ces vins. *C.R. Acad. Agric. France.* 41:399-403.

Cordonnier, R.E. 1956. Recherches sur l'aromatisation et le parfum des vins doux naturels et des vins de liqueur. *Ann. Technol. Agric*. 5:75-110.

Cordonnier, R. E., and C. L. Bayonove. 1974. Mise en évidence dans la baie de raisin, variété Muscat d'Alexandrie, des monoterpènes liés révélables par une ou plusieurs enzymes de fruits. *C.R. Acad. Sci.Series D* 278:3387-3390.

Cordonnier, R. E., and C. L. Bayonove. 1979. Les composantes variétales et préfermentaires de l'arôme des vins. *Rev. Enol. Franc.* 16:79-90.

Cordonnier, R. E., and C. L. Bayonove. 1981. Étude de la phase préfermentaire de la vinification: Extraction et formation de certains composés de l'arôme; cas des terpenols, des aldehydes, et des alcools en C6. *Conn. Vigne Vin.* 15:269-86.

Corino, L., and R. Di Stefano. 1988. Comportamento del vitigno Moscato Bianco in relazione ad ambienti di coltivazione diversi e valutazione di sistemi di allevamento e potatura. *Riv. Vitic. Enol. Conegliano.* 41:72-85.

Cortell, J. M., M. Halbleib, A. V. Gallagher, T. L. Righetti, and J. A. Kennedy. 2007. Influence of vine vigor on grape (*Vitis vinifera* L. cv. Pinot noir) anthocyanins. 1. Anthocyanin concentration and composition in fruit. *J. Agric. Food Chem.* 55:6575-6584.

Cortell, J. M., and J. A. Kennedy. 2006. Effect of shading on accumulation of flavonoid compounds in (*Vitis vinifera* L.) Pinot noir fruit and extraction in a model system. *J. Agric. Food Chem.* 54:8510-8520.

Crippen, D. D., and J. C. Morrison. 1986a. The effects of sun exposure on the compositional development of Cabernet Sauvignon berries. *Amer. J. Enol. Vitic.* 37:235-242.

Crippen, D. D., and J. C. Morrison. 1986b. The effects of sun exposure on the phenolic content of Cabernet Sauvignon berries during development. *Amer. J. Enol. Vitic.* 37:243-247.

Darriet, P., T. Tominaga, V. Lavigne, J. Boidron, and D. Dubourdieu. 1995. Identification of a powerful aromatic compound of *Vitis vinifera* L. var. Sauvignon wines: 4-mercapto-4-methylpentan-2-one. *Flavour Fragrance J.* 10:385-392.

Dimitriadis, E., and P. J. Williams. 1984. The development and use of a rapid analytical technique for estimation of free and potentially volatile monoterpene flavorants of grapes. *Amer. J. Enol. Vitic.* 35:66-71.

Di Profio, F. D., A. G. Reynolds, and A. Kasimos. 2011. Canopy management and enzyme impacts on Merlot, Cabernet franc, and Cabernet Sauvignon. II. Wine composition and quality. *Amer. J. Enol. Vitic*. 62:152-168.

Di Stefano, R., and L. Corino. 1984. Valutazione comparative fra Moscato bianco e Moscato giallo con particolare riferimento alla componente terpenica. *Riv. Vitic. Enol. Conegliano*. 37:657-670.

Di Stefano, R., and L. Corino. 1986. Caratteristiche chimiche ed aromatiche di vini secchi prodotti con Moscato Bianco e Giallo di Chambave e con Moscato Bianco di Canelli. *Riv. Vitic. Enol. Conegliano*. 39:3-11.

Dos Santos, T. P., C. M. Lopes, M. L. Rodrigues, C. R. de Souza, J. M. Ricardo-da-Silva, J. P. Maroco, J. S. Pereira, and M.M. Chaves. 2007. Effects of deficit irrigation strategies on cluster microclimate for improving fruit composition of Moscatel field-grown grapevines. *Scientia Horticulturae*. 112:321-330

Downey, M. O., J. S. Harvey, and S. P. Robinson. 2004. The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. *Austral. J. Grape and Wine Research* 10:55-73.

Drawert, F., and A. H. Rapp. 1966. Über Inhaltstoffe von Mosten und Weinen. VII. Gaschromatographische Untersuchung der Aromastoffe des Wines und ihrer Biogenese. *Vitis*. 5:351-376.

Eschenbruch, R., R. E. Smart, B. M. Fisher, and J. G. Whittles. 1987. Influence of yield manipulations on the terpene content of juices and wines of Müller Thurgau. *Proceedings of the 6th Australian Wine Industry Technical Conference*. Lee, T. (ed.), Australian Industrial Publishers, Adelaide, S. Australia. 89-93.

Ewart, A. J. W. 1987. Influence of vineyard site and grape maturity on juice and wine quality of *Vitis vinifera* cv. Riesling. *Proceedings of the 6th Australian Wine Industry Technical Conference*. Lee, T. (ed.), Australian Industrial Publishers, Adelaide, S. Australia. 89-93.

Gagné, S., K. Esteve, C. Deytieux, C. Sauvier, and L. Gény. 2006. Influence of abscisic acid in triggering veraison in grape berry skins of *Vitis vinifera* L. cv. Cabernet Sauvignon. *Vitis*. 40:7-14.

Gambetta, G. A., M. A. Matthews, T. H. Shaghasi, A. J. McElrone, and S. D. Castellarin. 2010. Sugar and abscisic acid signaling orthologs are activated at the onset of ripening in grape. *Planta*. 232:219-234.

Gholami, M., Y. Hayasaka, B. G. Coombe, J. F. Jackson, S. P. Robinson, and P. J. Williams. 1995. Biosynthesis of flavour compounds in Muscat Gordo Blanco grape berries. *Austral. J. Grape and Wine Research*. 1:19-24.

Giribaldi, M., W. Hartung, and A. Schubert. 2009. The effects of abscisic acid on grape berry ripening are affected by the timing of treatment. *Int. J. Vine Wine Sci*. 43:1-7.

Gu, S., S. Jacobs, and G. Du. 2011. Efficacy, rate and timing of applications of abscisic acid to enhance fruit anthocyanin contents in 'Cabernet Sauvignon' grapes. *J. Hortic. Sci. Biotechnol*. 86:505-510.

Guidoni, S., P. Allara, and A. Schubert. 2002. Effect of cluster thinning on berry skin anthocyanin composition of *Vitis vinifera* cv. Nebbiolo. *Amer. J. Enol. Vitic*. 53:224-226.

Gunata, Y. Z., C. L. Bayonove, R. L. Baumes, and R. E. Cordonnier. 1985. The aroma of grapes. Localisation and evolution of free and bound fractions of some grape aroma components c.v. Muscat during first development and maturation. *J. Sci. Food Agric*. 36:857-862.

Hale, C. R., and B. G. Coombe. 1974. Abscisic acid – an effect on the ripening of grapes. *Bull. R. Soc. NZ*. 12:831-836.

Hardy, P. J. 1970. Changes in volatiles in muscat grapes during ripening. *Phytochemistry*. 9:709-715.

Hashizume, K. and T. Samuta. 1999. Grape maturity and light exposure affect berry methoxypyrazine concentration. *Amer. J. Enol. Vitic*. 50:194-198.

Howell, K. S., J. H. Swiegers, G. M. Elsey, T. E. Siebert, E. J. Bartowsky, G. H. Fleet, I. S. Pretorius, and M. A. de Barros Lopes. 2004. Variation in 4-mercapto-4-methylpentan-2-one release by *Saccharomyces cerevisiae* commercial wine strains. *FEMS Microbiol. Lett*. 240:125-129.

Iacono, F., M. Bertamini, F. Mattivi, and A. Scienza. 1994. Differential effects of canopy manipulation and shading of *Vitis vinifera* L. cv. Cabernet Sauvignon. I. Composition of grape berries. *Vitic. Enol. Sci.* 49:220-225.

Jeong, S. T., N. Goto-Yamamoto, S. Kobayashi, and M. Esaka. 2004. Effects of plant hormones and shading on the accumulation of anthocyanins and the expression of anthocyanin biosynthetic genes in grape berry skins. *Plant Sci*. 167:247-252.

Jiang, F., and W. Hartung. 2008. Long-distance signaling of abscisic acid (ABA): the factors regulating the intensity of the ABA signal. *J. Exp. Bot*. 59:37–43.

Joscelyne, V. L., M. O. Downey, M. Mazza, S. E. P. Bastian. 2007. Partial shading of Cabernet Sauvignon and Shiraz vines: altered wine color and mouthfeel attributes, but increased exposure had little impact. *J. Agric. and Food Chem.* 55:10888-10896.

Kataoka, I., Y. Kubo, A. Sugiura, and T. Tomana. 1984. Effects of temperature, cluster shading and some growth regulators on L-Phenylalanine ammonia lyase activity and anthocyanin accumulation in black grapes. *Mem. Coll. Agric.*, Kyoto Univ. 124:35-44.

Keller, M., and G. Hradzina. 1998. Interaction of nitrogen availability during bloom and light intensity during veraison. II. Effects on anthocyanin and phenolic development during grape ripening. *Amer. J. Enol. Vitic*. 49:341-349.

Kinzer, G. and P. Schreier. 1980. Influence of different pressing systems on the composition of volatile constituents in unfermented grape musts and wines. *Amer. J. Enol. Vitic*. 31:7-13.

Kliewer, W. M. 1970. Effect of day temperature and light intensity on coloration of *Vitis vinifera* L. grapes. *J. Amer. Soc. Hort. Sci*. 95:693-697.

Kliewer, W. M. 1977. Influence of temperature, solar radiation and nitrogen on coloration and composition of Emperor grapes. *Amer. J. Enol. Vitic*. 28:96-103.

Kliewer, W. M., and L. A. Lider. 1968. Influence of cluster exposure to the sun on the composition of Thompson Seedless fruit. *Amer. J. Enol. Vitic*. 19:175-184.

Kliewer, W. M., and R. E. Torres. 1972. Effect of controlled day and night temperatures on grape coloration. *Amer. J. Enol. Vitic*. 23:71-77.

Kliewer, W. M., and R. J. Weaver. 1971. Effect of crop level and leaf area on growth, composition, and coloration of 'Tokay' grapes. *Amer. J. Enol. Vitic*. 22:172-177.

Koblet, W., C. Zanier, H. Tanner, P. Vautier, J. L. Simon, and F. Gnägi. 1977. Reifverlauf von Sonnen- und Schattentrauben. *Schweiz. Z. Obst. - und Weinbau*. 113:558-567.

Koshita, Y., T. Asakura, H. Fukuda, and Y. Tsuchida. 2007. Night-time temperature treatment of fruit clusters of 'Aki Queen' grapes during maturation and its effect on the skin color and abscisic acid content. *Vitis*. 46:208-209.

Kotseridis, Y., A. Anocibar Beloqui, A. Bertrand, and J. P. Doazan. 1998. An analytical method for studying the volatile compounds of Merlot noir clone wines. *Amer. J. Enol. Vitic*. 49:44-48.

Kotseridis, Y., A. Anocibar Beloqui, C. L. Bayonove, R. L. Baumes, and A. Bertrand. 1999. Effects of selected viticultural and enological factors on levels of 2-methoxy-3-isobutylpyrazine in wines. *J. Int. des Sciences Vigne et du Vin*. 33:19-23.

Koundouras, S., V. Marinos, A. Gkoulioti, Y. Kotseridis, and C. van Leeuwen. 2006. Influence of vineyard location and vine water status on fruit maturation of nonirrigated cv. Agiorgitiko (*Vitis vinifera* L.). Effects on wine phenolic and aroma components. *J. Agric. Food Chem*. 54:5077-5086.

Koyama, K., K. Sadamatsu, and N. Goto-Yamamoto. 2010. Abscisic acid stimulated ripening and gene expression in berry skins of the Cabernet Sauvignon grape. *Functional and Integrative Genomics*. 10:367-381.

Lacey, M. J., M. S. Allen, R. L. N. Harris, and W. V. Brown. 1991. Methoxypyrazines in Sauvignon blanc grapes and wines. *Amer. J. Enol. Vitic*. 42:103-108.

Larrechi, M. S., J. Guasch, and F. X. Ruiz. 1988. The definition of two Catalan viticultural regions by classification methods. *Acta Alimentaria*. 17:177-182.

Larrechi, M. S., and F. X. Ruiz. 1987. Multivariate data analysis applied to the definition of two Catalan viticultural regions. I. Cluster analysis. *Z. Lebensm. Unters. Forsch*. 185:181-184.

Lee, S.-H., M.-J. Seo, M. Riu, J. P. Cotta, D. E. Block, N. K. Dokoozlian, and S. E. Ebeler. 2007. Vine microclimate and norisoprenoid concentration in Cabernet Sauvignon grapes and wines. *Amer. J. Enol. Vitic*. 58:291-300.

Linsenmeier, A. W., and O. Löhnertz. 2007. Changes in norisoprenoid levels with long-term nitrogen fertilisation in different vintages of *Vitis vinifera* var. Riesling wines. *S. Afr. J. Enol. Vitic*. 28:17-24.

Macaulay, L. E., and J. R. Morris. 1993. Influence of cluster exposure and winemaking processes on monoterpenes and wine olfactory evaluation of Golden Muscat. *Amer. J. Enol. Vitic*. 44:198-204.

Marais, J. 1987. Terpene concentrations and wine quality of *Vitis vinifera* L. cv. Gewurztraminer as affected by grape maturity and cellar practices. *Vitis*. 26:241-245.

Marais, J. 1994. Sauvignon blanc cultivar aroma – a review. *S. Afr. J. Enol. Vitic*. 15:41-45.

Marais, J., and A. Rapp. 1988. Effect of skin-contact time and temperature on juice and wine composition and quality. *S. Afr. J. Enol. Vitic.* 9:22-30.

Marais, J., and C. J. van Wyk. 1986. Effect of grape maturity and juice treatments on terpene concentrations and wine quality of *Vitis vinifera* L. cv. Weisser Riesling and Bukettraube. *S. Afr. J. Enol. Vitic.* 7:26-35.

Marais, J., C. J. van Wyk, and A. Rapp. 1992. Effect of sunlight and shade on norisoprenoid levels in maturing Weisser Riesling and Chenin blanc grapes and Weisser Riesling wines. *S. Afr. J. Enol. Vitic*. 13:23-32.

Matthews, M. A., and M. M. Anderson. 1988. Fruit ripening in *Vitis vinifera* L.: responses to seasonal water deficits. *Amer. J. Enol. Vitic*. 39:313-320.

Mazza, G., L. Fukumoto, P. Delaquis, B. Girard, and B. Ewart. 1999. Anthocyanins, phenolics, and color of Cabernet Franc, Merlot, and Pinot noir wines from British Columbia. *J. Agric. Food Chem*. 47:4009-4017.

McCarthy, M. G. 1986. *Influence of irrigation, crop thinning, and canopy manipulation on composition and aroma of Riesling grapes.* M.Ag.Sci. Thesis, The University of Adelaide, Adelaide, S. Australia.

McCarthy, M. G., R. M. Cirami, and D. G. Furkaliev. 1987. Effect of crop load and vegetative growth control on wine quality. *Proceedings of the 6th Australian Wine Industry Technical Conference*. Lee, T. (ed.), Australian Industrial Publishers, Adelaide, S. Australia. 75-77.

McCarthy, M. G., and B. G. Coombe. 1985. Water status and winegrape quality. *Acta Hortic.* 171:447-456.

Murat, M. L., T. Tominaga, and D. Dubourdieu. 2001a. Assessing the aromatic potential of Cabernet Sauvignon and Merlot musts used to produce rosé wine by assaying the cysteinylated precursor of 3-mercaptohexan-1-ol. *J. Agric. Food Chem*. 49:5412-5417.

Murat, M. L., I. Masneuf, P. Darriet, V. Lavigne, T. Tominga, and D. Dubourdieu. 2001b. Effect of *Saccharomyces cerevisiae* yeast strains on the liberation of volatile thiols in Sauvignon blanc wine. *Amer. J. Enol. Vitic.* 52:136-139.

Noble, A. C. 1979. Evaluation of Chardonnay wines obtained from sites with different soil compositions. *Amer. J. Enol. Vitic,* 30:214-217.

Oliveira, C., A. C. Ferreira, P. Costa, J. Guerra, and P. G. de Pinho. 2004. Effect of some viticultural parameters on the grape carotenoid profile. *J. Agric. and Food Chem.* 52:4178-4184.

Ough, C. S., and A. A. Bell. 1980. Effects of nitrogen fertilization of grapevines on amino acid metabolism and higher alcohol formation during grape juice fermentation. *Amer. J. Enol. Vitic*. 31:122-123.

Ough, C. S., and T. H. Lee. 1981. Effect of vineyard nitrogen fertilization level on the formation of some fermentation esters. *Amer. J. Enol. Vitic*. 32:125-127.

Ough, C. S., and R. Nagaoka. 1984. Effect of cluster thinning and vineyard yields on grape and wine composition and wine quality of Cabernet Sauvignon. *Amer. J. Enol. Vitic*. 35:30-34.

Peppi, M. C., M. W. Fidelibus, and N. Dokoozlian. 2007. Application timing and concentration of abscisic acid affect the quality of 'Redglobe' grapes. *J. Hortic. Sci. Biotech*. 82:304-310.

Peterlunger, E., E. Celotti, G. Da Dalt, S. Stefanelli, G. Gollino, and R. Zironi. 2002. Effect of training system on Pinot noir grape and wine composition. *Amer. J. Enol. Vitic*. 53:14-18.

Peyrot des Gachons, C. 2000. *Recherches sur le potentiel aromatique des raisins de* Vitis vinifera *L cv Sauvignon blanc.* Ph.D. Thesis, Université Victor Ségalen Bordeaux.

Peyrot des Gachons, C, T. Tominaga, and D. Dubourdieu. 2000. Measuring the aromatic potential of *Vitis vinifera* L. cv. Sauvignon blanc grapes by assaying S-cysteine conjugates, precursors of the volatile thiols responsible for their varietal aroma. *J. Agric. Food Chem*. 48:3387-3391.

Peyrot des Gachons, C., C. Van Leeuwen, T. Tominaga, J. P. Soyer, J. P. Gaudillère, and D. Dubourdieu. 2005. Influence of water and nitrogen deficit on fruit ripening and aroma potential of *Vitis vinifera* L cv Sauvignon blanc in field conditions. *J. Sci Food Agric.* 85:73-85.

Pickering, G. J., J. Lin, A. G. Reynolds, G. Soleas, and R. Riesen. 2006. The evaluation of remedial treatments for wine affected by *Harmonia axyridis*. *Int. J. Food Sci. Technol*. 41:77-86.

Pickering, G. J., J. Lin, R. Riesen, A. G. Reynolds, I. Brindle, and G. Soleas. 2004. Influence of *Harmonia axyridis* on the sensory properties of white and red wine. *Amer. J. Enol. Vitic*. 55:153-159.

Quiroga, A. M., F. J. Berli, D. Moreno, J. B. Cavagnaro, and R. Bottini. 2009. Abscisic acid sprays significantly increase yield per plant in vineyard-grown wine grape (*Vitis vinifera* L.) cv. Cabernet Sauvignon through increased berry set with no negative effects on anthocyanin content and total polyphenol index of both juice. *J. Plant Growth Reg*. 80:28-35.

Razungles, A. J., R. L. Baumes, C. Dufour, C. N. Sznaper, C. L. Bayonove. 1998. Effect of sun exposure on carotenoids and C-13-norisoprenoid glycosides in Syrah berries (*Vitis vinifera* L.). *Sciences Des Aliments*. 18:361-373.

Reynolds, A. G., C. G. Edwards, D. A. Wardle, D. R. Webster, and M. J. Dever. 1994a. Shoot density affects Riesling grapevines. I. Vine performance. *J. Amer. Soc. Hort. Sci.* 119:874-880.

Reynolds, A. G., C. G. Edwards, D. A. Wardle, D. R. Webster, and M. J. Dever. 1994b. Shoot density affects Riesling grapevines. II. Wine composition and sensory response. *J. Amer. Soc. Hort. Sci.* 119:880-892.

Reynolds, A. G., T. Molek, and C. de Savigny. 2005. Timing of shoot thinning in *Vitis vinifera*: Impacts on yield and fruit composition variables. *Amer. J. Enol. Vitic*. 56:343-356.

Reynolds, A. G., P. Parchomchuk, R. Berard, A. P. Naylor, and E. J. Hogue. 2006. Gewürztraminer vines respond to length of water stress duration. *Int. J. Fruit Sci*. 5(4):75-94.

Reynolds, A. G., R. M. Pool, and L. R. Mattick. 1986. Influence of cluster exposure on fruit composition and wine quality of Seyval blanc. *Vitis*. 25:85-95.

Reynolds, A. G., S. F. Price, D. A. Wardle, and B. T. Watson. 1994c. Fruit environment and crop level effects on Pinot noir. I. Vine performance and fruit composition. *Amer. J. Enol. Vitic*. 45:452-459.

Reynolds, A. G., J. W. Schlosser, R. Power, R. Roberts, and C. de Savigny. 2007a. Magnitude and interaction of viticultural and enological of effects. I. Impact of canopy management and yeast strain on sensory and chemical composition of Chardonnay musqué. *Amer. J. Enol. Vitic*. 58:12-24.

Reynolds, A. G., J. W. Schlosser, D. Sorokowsky, R. Roberts, and C. de Savigny. 2007b. Magnitude and interaction of viticultural and enological of effects. II. Relative impacts of cluster thinning and yeast strain on composition and sensory attributes of Chardonnay musqué. *Amer. J. Enol. Vitic*. 58:25-41.

Reynolds, A. G., I. Senchuk, and C. de Savigny. 2007c. Use of GPS and GIS for elucidation of the basis for terroir. Spatial variation in an Ontario Riesling vineyard. *Amer. J. Enol. Vitic*. 58:145-162.

Reynolds, A. G., and D. A. Wardle. 1989a. Impact of several canopy manipulation practices on growth, yield, fruit composition, and wine quality of Gewürztraminer. *Amer. J. Enol. Vitic*. 40:121-129.

Reynolds, A. G., and D. A. Wardle. 1989b. Influence of fruit microclimate on monoterpene levels of Gewürztraminer. *Amer. J. Enol. Vitic*. 40:149-154.

Reynolds, A. G., and D. A. Wardle. 1989c. Effects of timing and severity of summer hedging on growth, yield, fruit composition, and canopy characteristics of De Chaunac. II. Yield and fruit composition. *Amer. J. Enol. Vitic*. 40:299-308.

Reynolds, A. G., D. A. Wardle, M. A. Cliff, and M. J. King. 2004a. Impact of training system and vine spacing on vine performance, berry composition, and wine sensory attributes of Seyval and Chancellor. *Amer. J. Enol. Vitic*. 55:84-95.

Reynolds, A. G., D. A. Wardle, and M. J. Dever. 1993. Terpenes in berries and juices of *Vitis vinifera* in response to pressing, harvest date, and skin contact. *HortScience*. 28:920-924.

Reynolds, A. G., D. A. Wardle, and M. J. Dever. 1994d Shoot density effects on Riesling grapevines: interaction with cordon age. *Amer. J. Enol. Vitic*. 45:435-443.

Reynolds, A. G., D. A. Wardle, and M. J. Dever. 1996a. Vine performance, fruit composition, and wine sensory attributes of Gewürztraminer in response to vineyard location and canopy manipulation. *Amer. J. Enol. Vitic*. 47:77-92.

Reynolds, A. G., D. A. Wardle, J. W. Hall, and M. J. Dever. 1995a. Fruit maturation in four *Vitis vinifera* cultivars in response to vineyard location and basal leaf removal. *Amer. J. Enol. Vitic*. 46:542-558.

Reynolds, A. G., D. A. Wardle, and A. P. Naylor. 1995c. Impact of training system and vine spacing on vine performance and berry composition of Chancellor. *Amer. J. Enol. Vitic*. 46:88-97.

Reynolds, A. G., D. A. Wardle, and A. P. Naylor. 1996b. Impact of training system, vine spacing, and basal leaf removal on Riesling. Vine performance, berry composition, canopy microclimate, and vineyard labor requirements. *Amer. J. Enol. Vitic*. 47:63-76.

Reynolds, A.G., S. Yerle, B. T. Watson, S. F. Price, and D. A. Wardle. 1996c. Fruit environment and crop level effects on Pinot noir. III. Composition and descriptive analysis of Oregon and British Columbia wines. *Amer. J. Enol. Vitic.* 47:329-39.

Ribéreau-Gayon, P., J. N. Boidron, and A. Terrier. 1975. Aroma of muscat grape varieties. *J. Agric. Food Chem*. 23:1042-1047.

Ristic, R., M. O. Downey, P. G. Iland, K. Bindon, I. L. Francis, M. Herderich, S. P. Robinson. 2007. Exclusion of sunlight from Shiraz grapes alters wine colour, tannin and sensory properties. *Austral. J. Grape and Wine Research*. 13:53-65.

Rodopoulo, A. K., I. A. Egorov, A. A. Bezzubov, and K. P. Skuin. 1974. [Compounds responsible for the aroma of grapes and their role in formation of the bouquet of wine]. Russian. *Prikl. Biokhim. Mikrobiol*. 10:280-287.

Roujou de Boubée, D., A. M. Cumsille, M. Pons, and D. Dubourdieu. 2002. Location of 2-methoxy-3-isobutylpyrazine in Cabernet Sauvignon grape bunches and its extractability during vinification. *Amer. J. Enol. Vitic*. 53:1–5.

Roujou de Boubée, D., C. Van Leeuwen, and D. Dubordieu. 2000. Organoleptic impact of 2-methoxy-3-isobutylpyrazine on red Bordeaux and Loire wines. Effect of environmental conditions on concentrations in grapes during ripening. *J Agric Food Chem*. 48:4830–4834.

Sala, C., O. Busto, J. Guasch, and F. Zamora. 2004. Influence of vine training and sunlight exposure on the 3-alkyl-2-methoxypyrazines content in musts and wines from the *Vitis vinifera* variety Cabernet Sauvignon. *J. Agric. Food Chem.* 52:3492-3497.

Sala, C., O. Busto, J. Guasch, and F. Zamora. 2005. Contents of 3-alkyl-2-methoxypyrazines in musts and wines from *Vitis vinifera* variety Cabernet Sauvignon: influence of irrigation and plantation density. *J. Sci. Food Agric*. 85:1131-1136.

Sala, C., M. Mestres, M. P. Marti, O. Busto, and J. Guasch. 2000. Headspace solid-phase microextraction method for determining 3-alkyl-2-methoxypyrazines in musts by means of polydimethylsiloxane-divinylbenzene fibres. *J Chromatogr A*. 880:93–99.

Schreier, P., F. Drawert, and A. Junker A. 1976. Identification of volatile constituents from grapes. *J. Agric. Food Chem*. 24:331-336.

Shiozaki, S., Y. Kamata, T. Ogata, S. Horiuchi, and K. Kawase. 1999. Localisation of abscisic acid in grape berry by immunohistochemical techniques. *J. Japan. Soc. Hort. Sci*. 68:1-9.

Smart, R. E. 1982. Vine manipulation to improve wine grape quality. *Proceedings of the University of California, Davis, Grape and Wine Centennial Symposium*. Webb AD (Ed.), University of California Press, Berkeley. 362-375.

Smart, R. E., J. B. Robinson, G. Due, and C. J. Brien. 1985b. Canopy microclimate modification for the cultivar Shiraz. II. Effects on must and wine composition. *Vitis*. 24:119-128.

Smith, S., I. C. Codrington, M. Robertson, and R. E. Smart RE. 1988. Viticultural and oenological implications of leaf removal for New Zealand vineyards. *Proceedings of the 2nd International Symposium Cool Climate Vitic. Oenol*., 11-15 January, 1988, Auckland, New Zealand. Smart, R. E., R. J. Thornton, S. B. Rodriguez, and J. E. Young JE (eds.), New Zealand Society for Viticulture and Oenology, Auckland. 127-133.

Spayd, S. E., J. M. Tarara, D. L. Mee, J. C. Ferguson. 2002. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *Amer. J. Enol. Vitic*. 53:171-182.

Stevens, K. L., J. Bomben, A. Lee, and W. H. McFadden. 1966. Volatiles from grapes. Muscat of Alexandria. *J. Agric. Food Chem*. 14:249-252.

Strauss, C.R., B. Wilson, P. R. Gooley, and P. J. Williams. 1986. Role of monoterpenes in grape and wine flavor. *Amer. Chem. Soc. Symp.* 317:222-242.

Terrier, A., J.-N. Boidron, and P. Ribéreau-Gayon. 1972a. L'identification des composés terpéniques dans les raisins de *V. vinifera*. *C.R. Acad Sci. Ser. D*. 275:495-497.

Terrier, A., J.-N. Boidron, and P. Ribéreau-Gayon. 1972b Teneurs en composés terpéniques des raisins de *Vitis vinifera*. *C.R. Acad. Sci. Ser. D*. 275:941-944.

Tominaga, T., R. Baltenweck-Guyot, C. Peyrot des Gachons, and D. Dubourdieu. 2000. Contribution of volatile thiols to the aromas of white wine made from several *Vitis vinifera* grape varieties. *Amer. J. Enol. Vitic*. 51:178-181.

Tominaga, T., A. Furrer, R. Henry, and D. Dubourdieu. 1998a. Identification of new volatile thiols in the aroma of *Vitis vinifera* L. var. Sauvignon blanc wines. *Flavour Fragrance J*. 13:159-162.

Tominaga, T., C. Peyrots des Gachons, and D. Dubourdieu. 1998b. A new type of flavor precursors in *Vitis vinifera* L. cv. Sauvignon blanc: S-cysteine conjugates. *J. Agric. Food Chem*. 46:5215-5219.

Van Wyck, C. J., A. D. Webb, and R. E. Kepner. 1967. Some volatile constituents of *Vitis vinifera* variety White Riesling. I Grape juice. *J. Food Sci*. 32:660-664.

Weaver, R. J., S. B. McCune, and M. A. Amerine. 1961. Effect of level of crop on vine behavior and wine composition in Carignane and Grenache grapes. *Amer. J. Enol. Vitic*. 12:175-184.

Webb, A. D. and R. E. Kepner. 1957. Some volatile aroma constituents of *Vitis vinifera* var. Muscat of Alexandria. *Food Res*. 22:384-394.

Webb, A. D., R. E. Kepner, and L. Maggiora. 1966. Gas chromatographic comparison of volatile aroma materials extracted from eight different muscat flavored varieties of Vitis vinifera. *Amer. J. Enol. Vitic*. 17:247-254.

Webster, D., C. G. Edwards, S. E. Spayd, J. C. Peterson, and B. J. Seymour. 1993. Influence of nitrogen fertilization on the concentrations of monoterpenes, higher alcohols, and esters in aged White Riesling wines. *Amer. J. Enol. Vitic*. 44:275-84.

Winkler, A. J., J. A. Cook, W. M. Kliewer, and L. A. Lider. 1974. General Viticulture. Univ. of Calif. Press, Berkeley, Los Angeles, and London.

Wolf, T. K., R. M. Pool, and L. R. Mattick. 1986. Responses of young Chardonnay grapevines to shoot tipping, ethephon, and basal leaf removal. *Amer. J. Enol. Vitic*. 37:263-268.

Yamakawa, T., S. Kato, K. Ishida, T. Kodama, and Y. Minoda. 1983. Production of anthocyanins by *Vitis* cells in suspension culture. *Agric. Biol. Chem*. 47:2185-2191.

Yamane, T., S. T. Jeong, N. Goto-Yamamoto, Y. Koshita, and S. Kobayashi. 2006. Effects of temperature on anthocyanin biosynthesis in grape berry skins. *Amer. J. Enol. Vitic*. 57:54-59.

Zoecklein, B. W., T. K. Wolf, J. E. Marcy, and Y. Jasinski. 1998a. Effect of fruit zone leaf thinning on glycosides and selected aglycone concentrations of Riesling *(Vitis vinifera* L.) grapes. *Amer. J. Enol. Vitic*. 49:35-43.

Zoecklein, B. W., T. K. Wolf, S. E. Duncan, J. E. Marcy, and Y. Jasinski. 1998b. Effect of fruit zone leaf removal on total glucoconjugates and conjugate fraction concentrations of Riesling and Chardonnay *(Vitis vinifera* L.) grapes. *Amer. J. Enol. Vitic*. 49:259-265.

Zoecklein, B. W., T. K. Wolf, L. Pélanne, M. K. Miller, and S. S. Birkenmaier. 2008. Effect of vertical shoot-positioned, Smart-Dyson, and Geneva double-curtain training systems on Viognier grape and wine composition. *Amer. J. Enol. Vitic*. 59:11-21.

## List of Figures

# PRECISION IRRIGATION OF GRAPEVINES: METHODS, TOOLS AND STRATEGIES TO MAXIMIZE THE QUALITY AND YIELD OF THE HARVEST AND ENSURE WATER SAVINGS

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## Abstract

Vineyard irrigation has been used for a very long time in the so-called "new world vineyards" and is widely practiced. Its adoption in the French Mediterranean regions is much more recent and it is one of the first techniques adopted by the grapegrowers to combat the impact of climate change. Vineyard irrigation is a tool that makes it possible to maintain both the quality and the quantity of the harvest. Irrigation control is based on the characterization of vine water status and on the knowledge of plant response to water deficits according to the phenological stage. This knowledge helps define an irrigation strategy adapted to the vineyard objective, e.g., grape juice, white wines, red wines or wines for aging.

## 1. Vines and Water

Around the world, grapevines are grown in areas where the water regime is highly variable according to the climate (e.g., rainfall and evapotranspiration) and the type of soil (the water-holding capacity). In several wine-producing regions of countries such as Australia, Argentina, the United States (California) and Chile, irrigation is a cultivation technique like any other, increasingly used to manage the performance and quality of the grapes and wines. In all these "New World Wine" countries, the irrigated vineyard area is now 580,000 hectares, or about 83% of the total vineyard area. In Argentina, the entire winegrowing area is irrigated (205,000 ha).

In the south of France, vine irrigation has been a reality since the early 2000s. Languedoc-Roussillon is the main irrigated area in France, with 26,000 ha (11% of the vineyard area), followed by the Provence-Alpes-Côte d'Azur (PACA) administrative region with 10,000 ha of irrigated vines.[1] This area is increasing due to climate change and the current grapegrowing and winemaking crisis, which requires an evolutionary adaptation of agricultural techniques for Mediterranean vineyards. In fact, the rise in average temperatures, along with the accompanying significant increase in evapotranspiration, generates drought during the growing cycle, induced by a severe and early water deficit (figure 1).

Winegrowers in the region are increasingly confronted with the choice of accepting the consequences of severe water stress or irrigating to prevent the serious degradation of yields and crop quality. This has led the Languedoc-Roussillon region to carry out the Aqua Domitia project, co-financed mainly by the regional government in collaboration with the General Council of the Aude, which aims to expand the regional hydraulic network by linking

1 Source: Association des Irrigants des Régions Méditerranéennes Françaises (AIRMF)

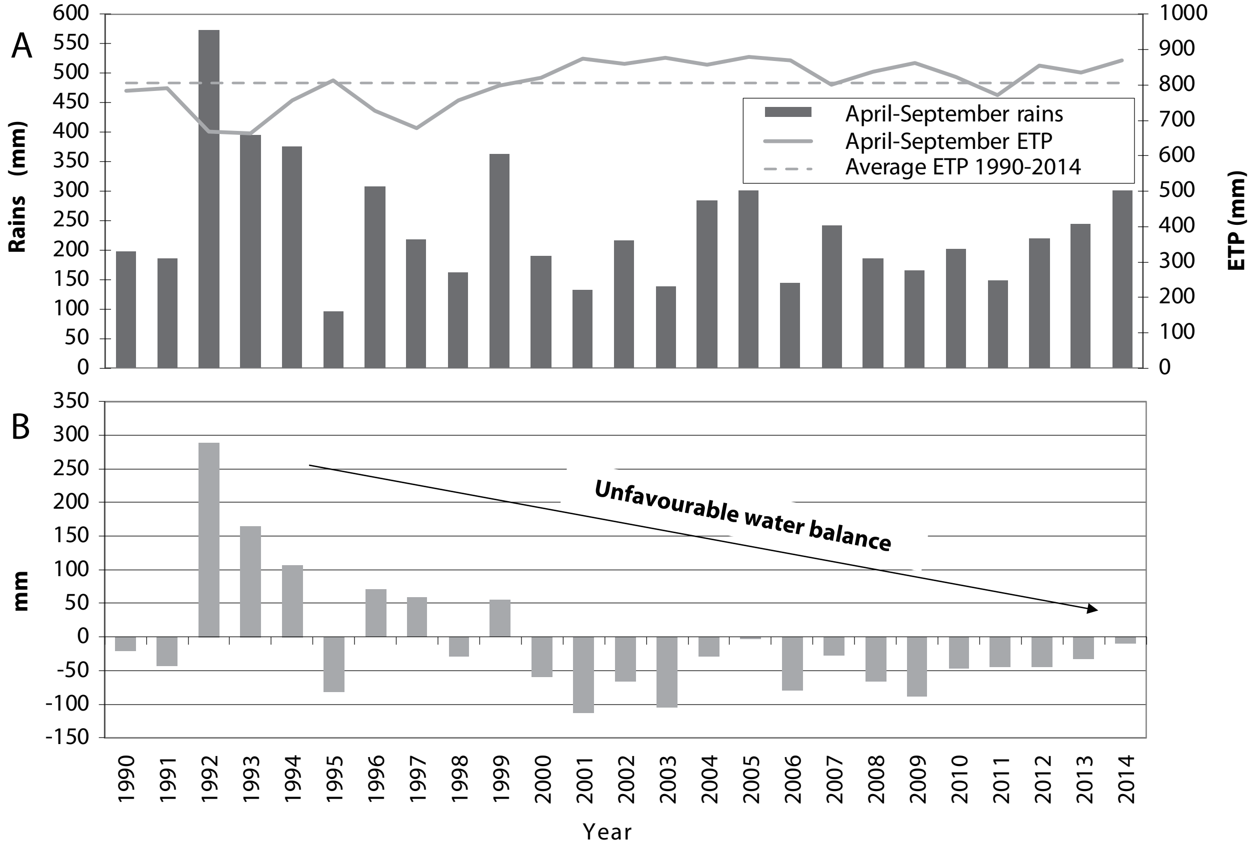


**Figure 1.** A: Rains and evapotranspiration (ETP) fluctuations; B: Dryness Index (DI)
Source: Tonietto and Carbonneau 2004, INRA, Unité Expérimentale de Pech Rouge, Gruissan, France

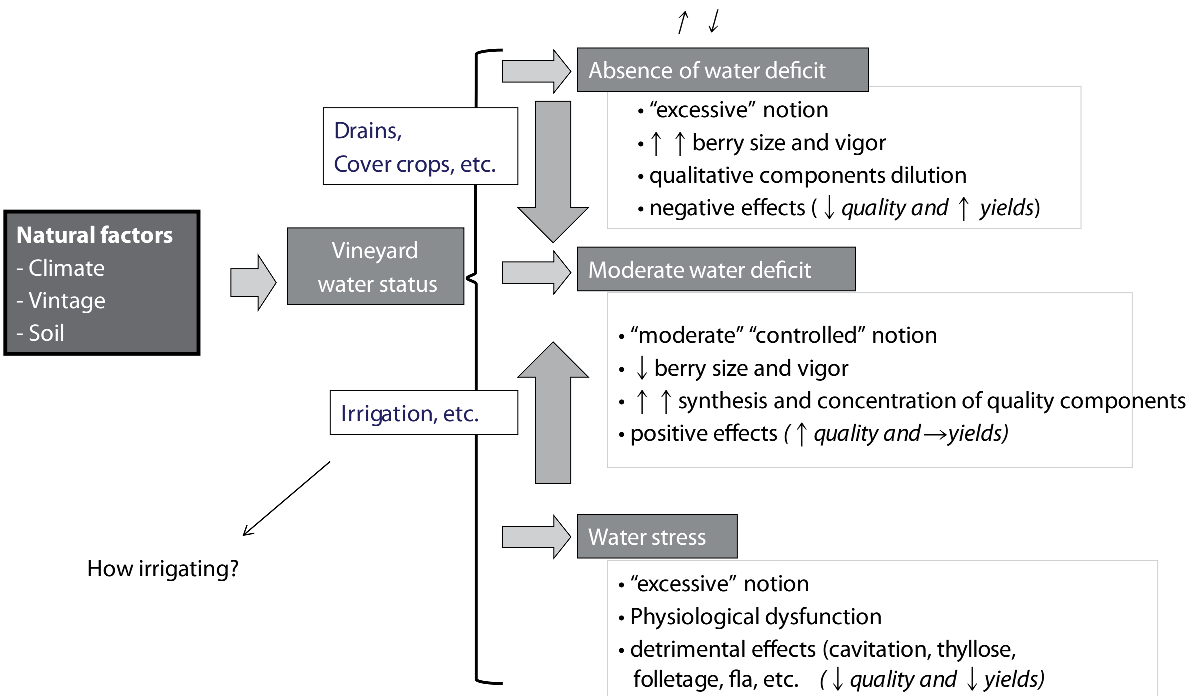


**Figure 2.** Different situations and possible consequences of vineyard water status according to the natural characteristics of the terroirs

waterways fed by the Rhône with those fed by the Orb, Hérault and Aude Rivers.[2]

In this context, responsible irrigation management should be based on the quantitative analysis of the need for water that takes into account the soil and climate characteristics of the plots and the irrigation strategies (the demand) in relation to the production target, with the possible water resources (the state of resources) and, in the longer term, to consider the evolution of this balance in a changing climate (figure 2).

Therefore, controlling irrigation to regulate the production of a quality harvest has become a constant concern of the winemaking professionals who demand more reliable and powerful tools to assist decision-making to manage the water status of the vines.

## 2. Irrigation Methods

Throughout the winegrowing world, the method of irrigation traditionally used for viticulture was gravity or flooding (figure 3). This limited the development of irrigation to those areas where it was possible to systematically distribute water, adequately prepare the site and have a significant quantity of water available, notably due to large losses of water through leaching (over 50%). The incorporation of localized drip irrigation systems has intensified in the winegrowing world since the early 1990s, mainly due to the system's ability to save water and the precision of its management. At present, localized drip irrigation is the most common irrigation method in the world.

When well-managed, drip irrigation technology allows better control of the vineyard water status and ensures good water management with preciseness, labour-saving automation and water savings. It is also possible to practice fertigation, i.e., the application of nutrients through irrigation. However, in some soils, the salinity of the root bulb must be constantly monitored. Furthermore, in some sandy soils prolonged irrigation system breakdown can cause the soil in contact with the root to dry out very rapidly, with catastrophic results for the vine.

2 http://www.reseau-hydraulique-regional.fr/presentation_du_proiet-72.html

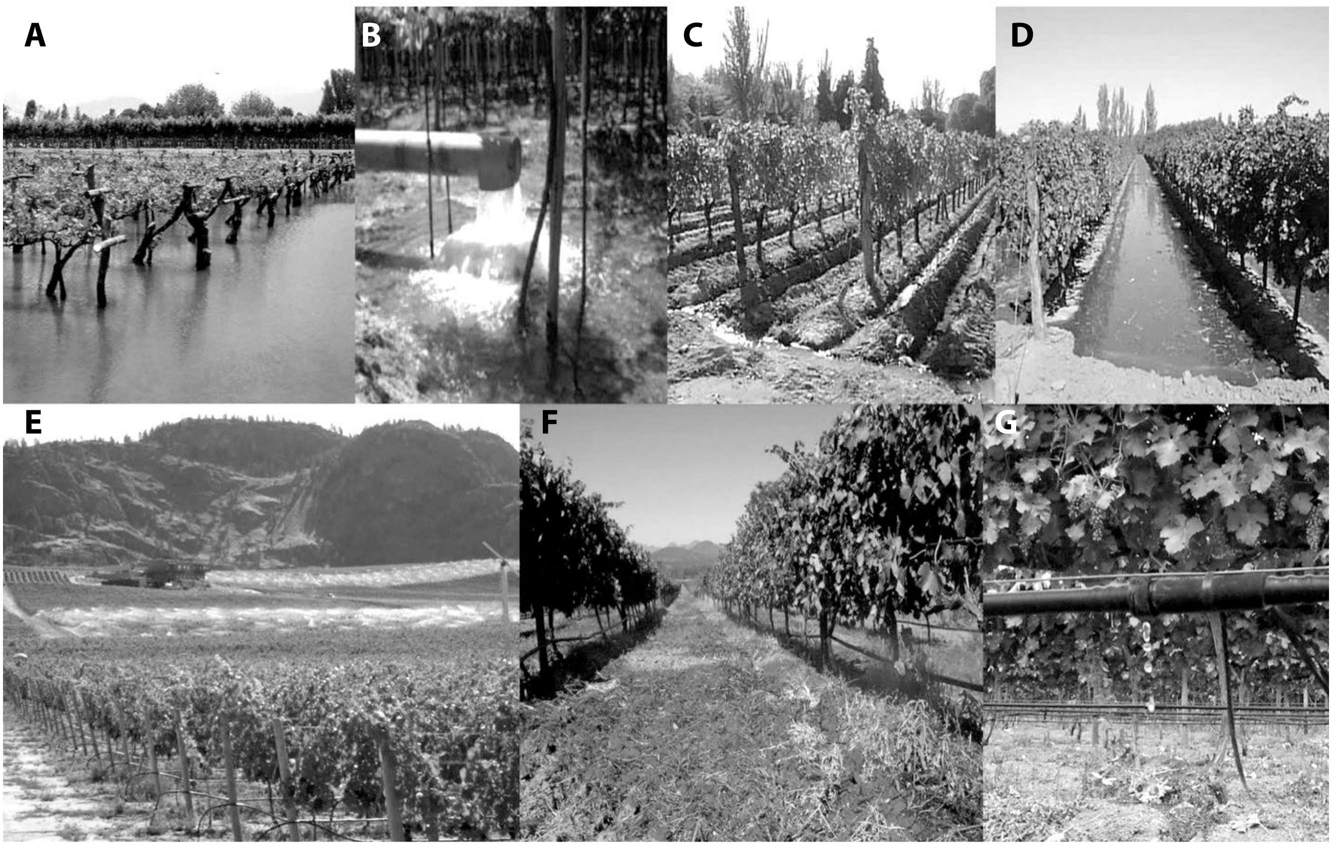


**Figure 3.** Different types of irrigation used for viticulture around the world. A: By submersion in pools (Ica, Peru); B: Californian flooding (Mendoza, Argentina); C: By gravity with grooves; D: By gravity with *melgas* (Mendoza, Argentina); E: Sprinkling (Osoyoos, Canada); F: Microsprinkling (Stellenbosch, South Africa); G: Drip (Mendoza, Argentina).

## 3. Decision-making Methods

Several direct and indirect techniques for estimating the plant water status or water resources available have been proposed for the vine. In general, they can be classified into two main categories.

**Methods based on measurements of the plant**: Stomatal conductance with potometers or gas exchange analyzers (Bravdo and Naor 1996, Flexas et al. 2002, Cifre et al. 2005, and Loveys et al. 2005); leaf water potential with the pressure chamber (McCutchan and Shackel 1992, Schultz 1996, Choné et al. 2001, Carbonneau et al. 2004, Girona et al. 2006, and Sibille et al. 2007); transpiration with sap flow sensors (Yunusa et al. 2000, Fernandez et al. 2001, Escalona et al. 2002, Cifre et al. 2005, and Saurin et al. 2011); estimate the water potential of the plant with the use of hygrometers on the stem (Dixon and Tyree 1984, and Hessdörfer et al. 2013); dendrometry to monitor trunk diameter fluctuations (Loveys et al. 2001, Naor and Cohen 2003, and Cifre et al. 2005); temperature measuring of leaf and canopy (Idso 1982, Sinclair et al. 1984, Jones 1999, and Jones et al. 2002); determination of the carbon isotopic ratio (13C/12C) on grapes (van Leeuwen et al. 2001, and Gaudillère et al. 2002).

**Methods *not* based on direct measurements of the plant**: Estimating crop evapotranspiration from climate data (Sammis et al. 1988, Allen et al. 1989, Pereira et al. 1999, and McCarthy 1997); availability of soil water (McCarthy 1997, Lebon et al. 2003, Peregrino et al. 2004 and 2006, and Loveys et al. 2005); the use of soil moisture sensors (tensiometers, electrical resistance, neutron probes, probes TDR and FDR, etc.) (Topp et al. 1980, Ortega-Farías and Acevedo 2004, and Loveys et al. 2005); or calculation of indices based on one or more methods (McCarthy 1997, Colaizzi et al. 2003, and Ortega-Farías et al. 2004).

Among all the possible tools and methods for estimating the plant water status and water resources available for the vine, it is advisable to favour those where the decision is based on measures interpreting of the physiological functioning of the plant, because they integrate all the parameters responsible for the water status of the vineyard (i.e., evapotranspiration [ETP], rainfall, soil type, viniculture practices, etc.).

By far the best method is still the leaf water potential (Carbonneau 1998, Choné et al. 2001, Ojeda et al. 2001, Williams and Araujo 2002, and Deloire et al. 2004), which was gradually adopted by wine companies as a method


**Figure 4.** Determining vine water status with the pressure chamber

to support decision-making regarding irrigation (Figure 4). Leaf water potential, which is determined with a pressure chamber (Scholander et al., 1965), led to the establishment of solid reference values, valid worldwide and for different agro-climatic conditions, particularly for predawn leaf water potential values.

Other techniques may be more relevant in a specific context, more economical or easier to implement. They can be useful and precise provided they are close to the reference values obtained through the leaf water potential.

## 4. Water Status and Its Effect on the Vine

Irrigation is a major tool for controlling the vineyard water status according to the characteristics of the particular plot and to the production goals. For proper management, the grapegrower must understand how the plant responds to water stress according to the different phenological stages.

During the period from budding to flowering, shoot growth is a priority. Vegetative growth is very sensitive to water deficits. Shoot growth decreases or stops at relatively low stress levels, which do not affect such physiological parameters as photosynthesis and transpiration (Williams et al. 1994). Consequently, during this period the vine must not be affected by a major water deficit if shoot growth is to occur normally – leaf area develops through an adequate supply of water to the roots.

Between fruit-set and veraison, the water status has a strong influence on yield by affecting berry size (Becker and Zimmermann 1984, McCarthy 1997, and Ojeda et al. 2001). The controlled reduction of berry size can be a quality target knowing that berry size determines the surface-to-volume ratio and subsequently the dilution of the specific constituents of the skin, including phenols and aroma precursors, in the volume of must or wine (Singleton 1972, Cordonnier 1976, and Ojeda et al. 2002). Nutrient uptake may also be affected if the drought is major during this period. This can only be done correctly if minerals are diluted in a water solution easily available to the roots (Keller 2005), and for a period of maximum consumption of nitrogen, potassium, phosphorus and calcium that is mostly between fruit-set and veraison (Fregoni 1985).

The absence of any water deficit during the stage of grape ripening between veraison and harvest generally promotes high yields. Qualitative components, such as polyphenols and sugars, are diluted by the effect of increasing berry size (Ojeda et al. 2002). Therefore, this situation should be avoided for the production of quality wines, but is suitable for other production targets, like concentrated must or grape juice.

A progressive water deficit during the ripening period is conducive to reduced berry size, and therefore reduced yield, which tends to concentrate phenolic compounds, predominantly the anthocyanins (Ojeda et al. 2002). This is also associated with stimulation of the secondary metabolism. However, the optimal water status thresholds are likely to be different when it comes to promoting phenols or aroma precursors. As aroma precursors are more susceptible than phenols to high water deficit (Peyrot des Gachons et al. 2005, and Tejerina et al. 2013), it is important to avoid high water deficits when the aromas are a priority, especially for white wines.

Vineyard water status during the veraison and harvest period determines in large part the type of wine produced (Deloire et al. 2005). When there is no water deficit at all, herbaceous, diluted and acid wines are produced. In the case of very severe water stress, red wines are overly tannic, hard, astringent and high alcohol, while whites lose much of their aroma.

After harvest, it is important that the vine return to an unconstrained water status, in order not to disturb the vine's metabolism, which is active during this period. Indeed, the vine, now free of grape bunches, directs photosynthesis to the areas for reserves, i.e., the roots, trunk and branches (Champagnol 1984), increases the absorption of minerals (Conradie 2005) and, in some cases, resumes the production of new roots (Freeman and Smart 1976, and van Zyl 1984).

In summary, during the period of grape ripening between veraison and harvest, as the water deficit levels rise, the levels of quality-related components (e.g., phenols, aroma precursors, sugars, etc.) also rise, despite the reduction in yield, notably by reducing berry size (figure 5). Nevertheless, beyond a certain water deficit threshold that can be considered optimal, the grapes produce no more so-called "qualitative" components, while the physiological (i.e., photosynthesis, stomatal conductance and transpiration) and quantitative (e.g., vegetative growth, yield, etc.) parameters continue to decrease. This optimum water deficit threshold varies according to the desired quality parameter, such as phenolic components in red wines or aroma precursors in white wines. More severe water deficit levels strongly reduce all parameters (qualitative, quantitative and physiological) and lead to a serious weakening of plants that can cause survival problems for some varietals if the situation persists for several successive vintages.

These results justify the interest in expanding research to establish the optimum water status level for each situation, varietal, water delivery system and training system/terroir combination, to obtain the best quality/yield ratio. In parallel, we must continue studying the physiological aspects that explain the adaptation mechanisms of different varietals to the hydric characteristics of different soils in the context of climate change. Important contributions are already available to establish the physiological response curves (e.g., photosynthesis, transpiration, stomatal conductance, etc.) in relation to different water deficit levels (Prieto 2012).

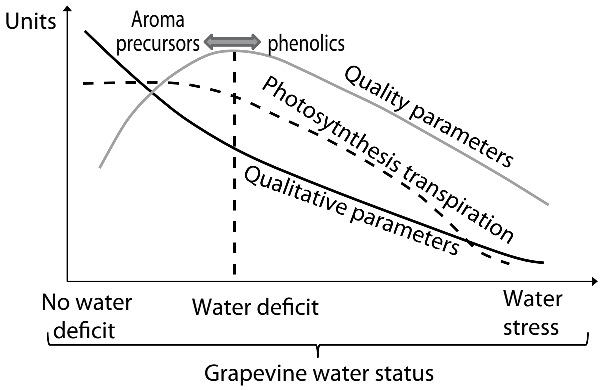


**Figure 5.** Influence of the water status of the vineyard on qualitative, quantitative and physiological parameters

## 5. Irrigation Strategies

Models for irrigation management in accordance with the optimum level of vine water status for each stage in the growing cycle have been proposed (Ojeda 2007, 2008). On this basis, irrigation strategies began to be applied in different wine regions of the world, and several companies are starting to offer services and tools to help and support grape producers in the implementation of these strategies[3, 4, 5]. We can foresee some different alternatives possible for an irrigation strategy based on the vineyard objective, the growing season and the water deficit level (Figure 6).

Thus, for a vineyard oriented toward the production of grape juice with the aim of high yields per hectare, the irrigation strategy to follow would be to avoid water stress during the entire growing season (Figure 6A) to promote high yields through most of its components. This strategy is also generally advisable for the production of table wines or for young vineyards.

For a vineyard where the goal is to produce aromatic white wine or fruity red wine, the irrigation should be controlled to ensure a slight and gradual water deficit toward the end

3 http://itkweb.com/solutions/itk-vigne

4 http://www.fruitionsciences.com/fr/login/irrigation

5 http://www.viverys.com/vivelys-system-vigne.html

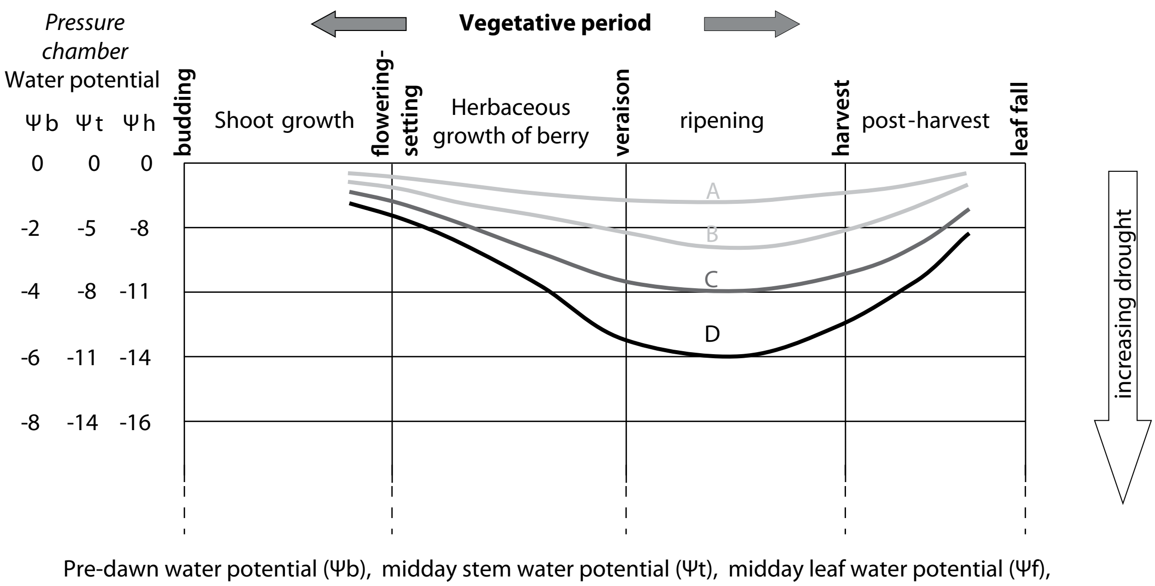


**Figure 6.** Possible different irrigation strategies to control the vineyard water status according to the growing season and the type of desired product:

A: Concentrated musts, grape juice, table wines and young vineyards in formation

B: White wines, light red and fruity wines

C: Quality wines, but well equilibrated with a predominance of the fruit to the structure, limit values for white wines

D: Quality wines, concentrated, balanced and suitable for aging.

of the veraison–maturity period (Figure 6B) in order not to significantly affect berry size and photosynthesis, and to favour the accumulation of sugars, and especially flavour precursors, while controlling vegetative growth.

For more concentrated wines, one would seek a moderate and progressive water deficit during the ripening period to facilitate a reduction in berry size, and consequently the yield, and to promote the concentration and synthesis of phenolic compounds, notably anthocyanins (Figure 6C).

Another option for red wines is to reach a high water deficit level (Figure 6D), to ensure greater control over berry size, and a significant increase in the concentration of phenols (more colour and structure) despite some reduction of aromatic intensity. This strategy is very suitable for red wines for aging but is not recommended for white wines, where the aromatic component is preferred.

Controlling water in relation to the chosen water status model can be very difficult in certain extreme situations. In zones with deep soils, clay, high nitrogen content and poor drainage, the strategy must be directed towards the control of excess water retention through soil management, using cover crops and canopy management. When the soil is sandy or sandy loam with good drainage, the risk of water stress is high; therefore, it is necessary to regularly monitor the water status of the vineyard to prevent this risk.

Depending on the strategy, keep the vineyard at appropriate levels close to the optimum water status level during the entire growing season to ensure maximum benefits and to stave off problems caused by excess water or drought. (Fig. 6)

## References


Allen, R. G., M. E. Jensen, J. L. Wright, and R. D. Burman. 1989. Operational Estimates of Reference Evapotranspiration. *Agronomy Journal*. 81:650-662.

Becker, N., and H. Zimmermann. 1984. Influence de divers apports d'eau sur des vignes en pots, sur la maturation des sarments, le développement des baies et la qualité du vin. *Bull. O. I. V*. 573-583.

Bravdo, B., and A, Naor. 1996. Effects of water regime on productivity and quality of fruit and wine. Proceedings of the Workshop Strategies to Optimize Wine Grape Quality. *Acta Hort. (ISHS)*. 427:15-26.

Carbonneau, A., 1998. *Irrigation, vignoble et produit de la vigne*. In Traité d'Irrigation. J.-R. Tiercelin, coord. Tec & Doc.Lavoisier. Paris. 257-298.

Carbonneau, A., A. Deloire, and P. Constanza. 2004. Leaf water potential meaning of different modalities of measurements. *J. Int. Sci. Vigne Vin*. 38:15-19.

Champagnol, F. 1984. *Eléments de physiologie de la vigne et de viticulture générale*. Montpellier.

Choné, X., C. van Leeuwen, D. Dubourdieu, and J. P. Gaudillère. 2001. Stem water potential is a sensitive indicator of grapevine water status. *Annals of Botany*. 97(4):477-483.

Cifre, J., J. Bota, J. M. Escalona, H. Medrano, and J. Flexas. 2005. Physiological tools for irrigation scheduling in grapevines (*Vitis vinifera* L.). An open gate to improve water-use efficiency? *Agriculture, Ecosystems and Environment*. 106(2-3):159-170.

Colaizzi, P. D., E. M. Barnes, T. R. Clarke, C. Y. Choi, and P. M. Waller. 2003. Estimating soil moisture under low frequency surface irrigation using crop water stress index. *Journal of Irrigation and Drainage Engineering*. 129:27-35.

Conradie, W. J. 2005. *Partitioning of mineral nutrients and timing of fertilizer applications for optimum efficiency*. Proceedings of the Soil Environment and Vine Mineral Nutrition Symposium. P. Christensen and D. R. Smart, eds. 69-81. American Society for Enology and Viticulture, Davis, CA.

Cordonnier, R. 1976. Qualité de la vendange et méthodologie de la sélection viticole. *Le Progrès Agricole et Viticole*. 93(24):760-762.

Deloire, A., H. Ojeda, O. Zebic, N. Bernard, J.-J. Hunter, and A. Carbonneau. 2005. Influence of grapevine water status on the style of wine. *Le Progrès Agricole et Viticole*. 122(21):455-462.

Dixon, M. A., and M. T. Tyree. 1984. A new stem hygrometer, corrected for temperature gradients and calibrated against the pressure bomb. *Plant, Cell and Environment*. 7:693-697.

Escalona, J. M., J. Flexas, and H. Medrano. 2002. Drought effects on water flow, photosynthesis and growth of potted grapevines. *Vitis*. 41:57-62.

Fernández, J. E., M. J. Palomo, A. Díaz-Espejo, B. E. Clothier, S. R. Green, I. F. Girón, and F. Moreno. 2001. Heat-pulse measurements of sap flow in olives for automating irrigation: tests, root flow and diagnostics of water stress. Agricultural Water Management. 51:99-123.

Flexas, J., J. Bota, J. M. Escalona, B. Sampol, and H. Medrano. 2002. Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. *Funct. Plant Biol*. 29:461-471.

Freeman, B. M., and R. E. Smart. 1976. A root observation laboratory for studies with grapevines. *Amer. J. Enol. Vitic*. 27(1):36-39.

Fregoni, M. 1999. *Viticoltura di qualitá*. Edizioni l'Informatore Agrario S.R.L. Verona, Italy.

Gaudillère, J. P., C. van Leeuwen, and N. Ollat. 2002. Carbon isotope composition of sugars in grapevines, an integrated indicator of vineyard water status. *Journal of Experimental Botany*. 53(369):757-763.

Girona, J., M. Mata, J. Del Campo, A. Arbonés, E. Bartra, and J. Marsal. 2006. The use of midday leaf water potential for scheduling deficit irrigation in vineyards. *Irrig. Sci.* 24:115-117.

Hessdörfer, D., A. B. Schwab, and B. R. Gruber. 2013. *Continuous measurements of grapevine water status in an irrigated vineyard under changing weather conditions*. Proceedings of the 18th International Symposium Giesco, Porto, Portugal.

Idso, S. B. 1982. Non-water-stressed baselines: a key to measuring and interpreting plant water stress. *Agricultural Meteorology*. 27:59-70.

Jones, H. G. 1999. Use of thermography for quantitative studies of spatial and temporal variation of stomatal conductance over leaf surfaces. *Plant, Cell and Environment.* 22:1043-1055.

Jones, H. G., M. Stoll, T. Santos, C. De Sousa, M. Chavez, and O. M. Grant. 2002. Use of infrared thermography for monitoring stomatal closure in the field: application to grapevine. *Journal of Experimental Botany*. 53:2249-2260.

Keller, M. 2005. Deficit irrigation and vine mineral nutrition. *Am. J. Enol. Vitic*. 56(3):267-283.

Lebon, E., V. Dumas, P. Pieri, and H. R. Schultz. 2003. Modelling the seasonal dynamics of the soil water balance of vineyards. *Funct. Plant. Biol.* 30:699-710.

Loveys, B. R., M. Stoll, P. R. Dry, and M. G. McCarthy. 2001. Using plant physiology to improve the water use efficiency of horticultural crops. *Acta Hort. (ISHS)*. 537:187-197.

Loveys, B. R., M. McCarthy, H. G. Jones, J. Theobold, and A. Skinner. 2005. *When to water? Assessment of plant-based measurements to indicate irrigation requirements*. Final Report to grape and wine research & development corporation. CSIRO Plant Industry. 111.

McCarthy, M. G. 1997. The effect of transient water deficit on berry development of cv. Shiraz (*Vitis vinifera* L.). *Australian Journal of Grape and Wine Research*. 3:102-108.

McCutchan, H., and K. A. Shackel. 1992. Stem-water potential as a sensitive indicator of water stress in prune trees (*Prunus domestica* L. cv. French). *J. Amer. Soc. Hort. Sci*. 117:607-611.

Naor, A., and S. Cohen. 2003. Sensitivity and variability of maximum trunk shrinkage, midday stem water potential, and transpiration rate in response to withholding irrigation from field-grown apple trees. *HortScience*. 38:547-551.

Ojeda, H., A. Deloire, and A. Carbonneau. 2001. Influence of water deficits on grape berry growth. *Vitis*. 40(3): 141-145.

Ojeda, H., C. Andary, E. Kraeva, A. Carbonneau, and A. Deloire. 2002. Influence of pre- and postveraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* L., cv. Shiraz. *Am. J. of Enol. Vitic*. 53(4):261-267.

Ojeda, H. 2007. Irrigation qualitative de précision de la vigne. *Le Progrès Agricole et Viticole*. 7:133-141.

Ojeda, H. 2008. Stratégies d'irrigation en fonction des particularités et les objectifs du vignoble. Cahier Technique, *Revue Française d'œnologie*. 229.

Ortega-Farías, S., and C. Acevedo. 2004. Irrigation Scheduling in Vineyards (VIIth Region of Chile) by Using Time Domain Reflectometry. *Acta Hort (ISHS)*. 646:115-119.

Ortega-Farías, S., M. Duarte, C. Acevedo, Y. Moreno, and F. Córdova. 2004. Effect of four levels of water application on grape composition and midday stem water potential of *Vitis vinifera* L. cv. Cabernet Sauvignon. *Acta Hort. (ISHS)*. 664:491-497.

Pellegrino, A., E. Lebon, M. Voltz, and J. Wery. 2004. Relationships between plant and soil water status in vine (*Vitis vinifera* L.). *Plant and Soil*. 266:129-142.

Pellegrino, A., E. Gozé, E. Lebon, and J. Wery. 2006. A model-based diagnosis tool to evaluate the water stress experienced by grapevines in a network of farmers' fields. *European Journal of Agronomy*. 25:49-59.

Pereira ,L. S., A. Perrier, R. G. Allen, and I. Alves. 1999. Evapotranspiration: review of concepts and future trends. *Journal of Irrigation and Drainage Engineering ASCE*. 125:45-51.

Peyrot des Gachons, C., T. Tominaga, and D. Dubourdieu. 2002b. Sulfur aroma precursor present in S-glutathione conjugate form: Identification of S-3-(hexan-1-ol)-glutathione in must from *Vitis vinifera* L. cv. Sauvignon Blanc. *J. Agric. Food Chem*. 50:4076-4079.

Prieto, J., G. Louarn, J. Perez Peña, H. Ojeda, T. Simonneau, and E. Lebon. 2012. A leaf exchange model that accounts for intra-canopy variability by considering leaf nitrogen content and local acclimation to radiation in grapevine (*Vitis vinifera* L.). *Plant Cell & Environment*. 35(7):1313-1328.

Sammis, T. W., W. R. Rirey, and D. G. Lugg. 1988. Crop water stress index of pecan. *Applied Engineering in Agriculture*. 4:39-45.

Saurin, N., H. Ojeda, E. Lucero, R. Mondjou, and T. Scholasch. 2011. New approach for grapevine irrigation scheduling using sap flow sensors: relationship with predawn leaf water potential. *American Journal of Enology and Viticulture*. 62(3):411A.

Schultz, H. R. 1996. Water relations and photosynthetic responses of two grapevine cultivars of different geographical origin during water stress. *Acta Hort. (ISHS)*. 427:251-266.

Sibille, I., H. Ojeda, J. Prieto, S. Maldonado, J.-N. Lacapere, and A. Carbonneau. 2007. *Relation between the values of three pressure chamber modalities (midday leaf, midday stem and predawn water potential) of 4 grapevine cultivars in drought situation in the south of France*. Applications for the irrigation control. Proceedings XV International Symposium GESCO. Porec, Croatia. 685-695

Sinclair, T. R., C. B. Tanner, and J. M. Bennett. 1984. Water-use efficiency in crop production. *Bioscience*. 34:36-40.

Singleton, V. L. 1972. Effects on red wine quality of removing juice before fermentation to simulate variation in berry size. *Amer. J. Enol. Vitic*. 23(3):106-113.

Scholander, P. F., H. T. Hammel, E. T. Brandstreet, and E. A. Hemmingsen. 1965. Sap pressure in vascular plants. *Science*. 148:339-346.

Tejerina, M., D. Velado, E. Hernandez, M. Puxeu, and H. Ojeda. 2013. *Influence of water deficit on plant physiology, grape aromatic precursors and wine quality of White Grenache*. Proceedings of the 18th International Symposium GIESCO, Porto, Portugal. 500-504.

Topp, G. C., J. L. Davis, and A. P. Annan. 1980. Electromagnetic determination of soil water content: Measurement in coaxial transmission lines. *Water Resources Res*. 16:574-582.

TONIETTO, J.; CARBONNEAU, A. 2004 A multicriteria climatic classification system for grape-growing regions worlwide. Agricultural and Forest Meteorology, Amsterdam, v.124, p.81-97, 2004.

van Leeuwen, C., J. P. Gaudillère, and O. Tregoat. 2001. The assessment of vine water uptake conditions by C-13/C-12 discrimination in grape sugar. *J. Int. Sci. Vigne Vin*. 35:195-205.

van Zyl, J. K. 1984. Response of Colombar grapevines to irrigation as regards quality aspects and growth. *S. Afr. Enol. Vitic*. 5(1):19-28.

Williams, L. E., and F. J. Araujo. 2002. Correlation among predawn leaf, midday leaf, and midday stem water potential and their correlations with other measures of soil and plant water status in *Vitis vinifera*. *J. Amer. Soc. Hort. Sci*. 127(3):448-454.

Williams, L. E., N. K. Dokoozlian, and R. Wample. 1994. *Grape*. In B. Shaffer and P. Andersen, eds. Handbook of Environmental Physiology of Fruit Crops. Vol. I. Temperature crops. CRC Press, Inc. Florida, U.S.A. 85-133.

Yunusa, I. A. M., R. R. Walker, B. R. Loveys, and D. H. Blackmore. 2000. Determination of transpiration in irrigated grapevines: comparison of the heat-pulse technique with gravimetric and micrometeorological method. *Irrigation Science*. 20:1-8.

# EVIDENCE OF COGNITIVE IMPACT OF ODOUR RECOGNITION TRAINING: INTERMODALITY BY LEARNING WINE AROMAS

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Recent evidence of the impact of olfactory sensory training reveals how circumscribed it is, particularly in connoisseurs (Tempere, Cuzange, Bougeant, de Revel & Sicard 2012). Nonetheless, studies have shown that *learning* to tell aromas apart is rather a multisensory cognitive task (Booth, Kendal-Reed & Freeman 2010) involving visual and tactile perceptions (Sugiyama et al. 2006). To investigate these ideas, we conducted an experimental study combining sensorial and psychological data in order to assess whether odour recognition training in the wine matrix promotes multisensory cognition, i.e., intermodality.

A total of 18 laypersons (housewives aged 35 to 45 [?◊? = 39.2] with technical-level education and no previous sensory training or wine sensory education) were selected to be trained according to the ISO 8586-1 standard for wine aroma sensory evaluation. They were trained using state-of-the-art descriptive analysis methodologies (Murray et al. 2001) to identify 15 sensory attributes (Table 1). Prior to and after the 12 training sessions, the participants were asked to verbally describe various images, from clearly defined pictures to abstract illustrations (Figure 1). A second, untrained group was assessed in exactly the same fashion to serve as the control group. Results showed that sensory training increased sensory skills (i.e., accuracy and repeatability) as well as the ability to discriminate consistently between wine aroma profiles over several samples. The RANOVA from the formal QDA results for 6 wines and 3 replicates showed that 14 out of 15 attributes were discriminant, i.e., p<0,05 (Figure 2). In cognitive terms, trained participants increased the total answer time (t(107)=-2.971; p=.004), number of topics mentioned (t(107)=-3.733; p=.000) and intermodality level (t(107)=-1.926; p=.050) of their verbal descriptions for abstract images though not for well-defined pictures (Figure 3). In both cases, the control group showed no differences. Furthermore, intermodality was positively correlated to sensory triangular recognition performance (r=.58, p=.029) and showed an association trend with ranking recognition (r = .51, p = .068).

## Significance of the study

These multilevel results lead us to think that olfactory training not only teaches people to recognize wine aromas, but also has a broader impact on domains supposedly unrelated to olfaction, e.g., how to describe visual stimuli. These results suggest new frontiers in sensory science, particularly new opportunities in wine sensory education for professionals and consumers alike. Sensory science can play an active role in cognitive development among the general public, and the wine industry can help to face this challenge.

## References

Murray, J. M., C. M. Delahunty, I. A. Baxter. 2001. Descriptive sensory analysis: past, present and future. *Food Research International*. 34:461–471

Booth, D. A., M. S. Kendal-Reed, R. P. J. Freeman. 2010. A strawberry by any other name would smell as sweet, green, fruity and buttery. Multisensory cognition of a food aroma. *Appetite*. 55:738-741

Sugiyama, H., S. Ayabe-Kanamura, T. Kikuchi. 2006. Are olfactory images sensory in nature? *Perception*. 35:1699-1708.

Tempère, S., E. Cuzange, G. de Revel, G. Sicard. 2012. Explicit sensory training improves the olfactory sensitivities of wine experts. Chemosensory *Percept*. 5:205–213 DOI: 10.1007/s12078-012-9120-1.

International Organization for Standardization. 1993. International Standard – ISO 8586-1: Sensory Analysis – General Guidance for the Selection, Training and Monitoring of Assessors – Part 1: Selected Assessors. Copenhagen: Dansk Standard.

**Table 1.** Sensory descriptors and aromatic standards employed

| Descriptor | Standard used |
|---|---|
| *Prune* | 100 grams of dry prune, macerated for 24 hours in a hydro-alcoholic solution |
| *Mushroom* | 1-Octen-3-ol; 0.0398 ppm |
| *Coffee* | 5 grams of instant coffee in 250 ml of hydro-alcoholic solution |
| *Cloves* | Eugenol; 0.4 ppm |
| *Raspberry* | Raspberry, whole fresh fruit |
| *Eucalyptus* | 5 drops of eucalyptus essence in 250 mL of hydro-alcoholic solution |
| *Chocolate* | 10 grams of bitter chocolate in 250 mL of hydro-alcoholic solution |
| *Bell pepper* | 2-isobutil-3-metoxipyrazine; 0.8 ppm |
| *Pepper* | 5 grams of black pepper in 250 mL of hydro-alcoholic solution |
| *Vanilla* | Vainillin; 30.4 ppm |
| *Coconut* | Whiskey lactone; 0.2 ppm |
| *Asparagus* | 100 grams of asparagus macerated 24 hours in hydro-alcoholic solution |
| *Tobacco* | 10 grams of tobacco in 250 hydro-alcoholic solution |
| *Woody* | 25 grams of wood chips, macerated 24 hours in hydro-alcoholic solution |
| *Smoky* | 5 drops of methyl guaiacol in 250 ml of hydro-alcoholic solution |


**Figure 1.** Examples of verbal descriptions of images, pre and post workshop interviews, from clearly defined pictures to abstract illustrations.

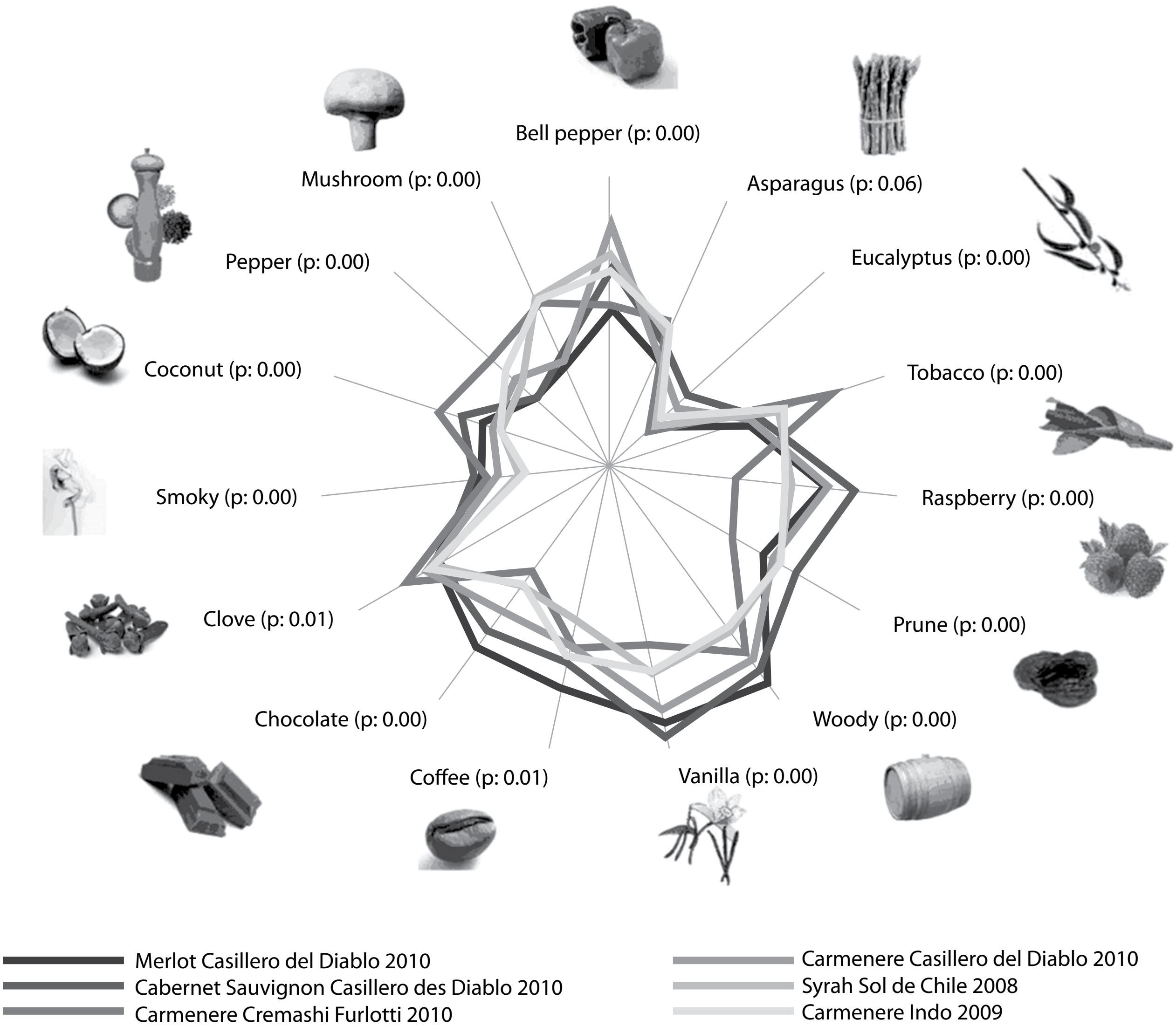


**Figure 2.** Quantitative descriptive analysis from trained panel data (6 red wines, 3 replicates) showing training process success through discriminant ability for 14 out of 15 attributes (RANOVA, p<0,05)

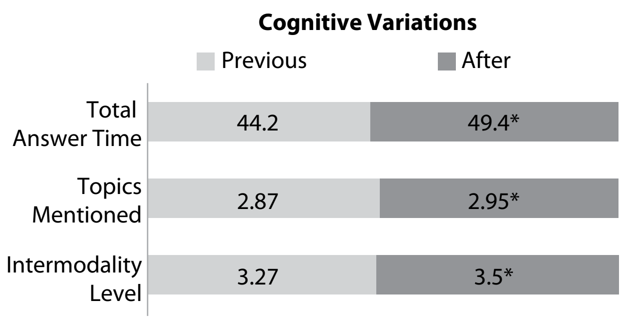


**Figure 3.** Cognitive variables measured prior to and after training that show significant differences, 18 panelists (total answer time t(107)=-2.971; p=.004), number of topics mentioned (t(107)=-3.733; p=.000) and intermodality level (t(107)=-1.926; p=.050).

# PHENOLIC MATURITY: CONCEPT, METHODOLOGY AND OENOLOGICAL IMPLICATIONS

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The concept of "phenolic maturity" is not new. In fact, it has been a subject of great interest for winemakers over the past two decades. There is no doubt that the concentration and extractability of anthocyanins in the grape skin, as well as the proportion of seed tannin, are some of the main factors affecting the future quality of red wine (Ribéreau-Gayon et al. 1999). For this reason, in recent years there has been talk – and we will continue talking – about the need for effective methods to determine the actual level of phenolic maturity in the grapes, to provide more appropriate criteria for deciding the optimum harvest date (Glories and Agustin 1993, Lamadon 1995, Izcara and González 2001, and Zamora 2002).

To illustrate these concepts, it is necessary to show the evolution of phenolic compounds from grapes throughout the ripening process (figure 1).

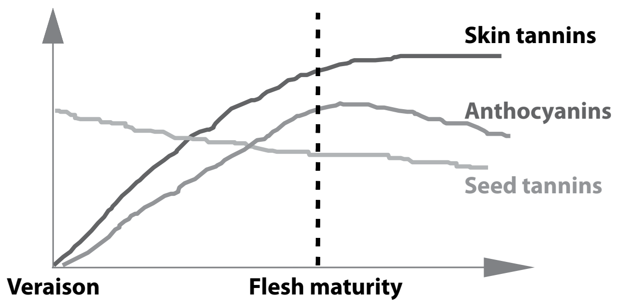


**Figure 1.** Evolution of phenolic compounds throughout the maturation process

The concentration of anthocyanins can be seen to increase during the maturation process up to maximum value, followed by a slight decrease. Meanwhile skin tannins increase during the ripening process whereas seed tannins decrease (Ribéreau-Gayon et al. 1999).

As seen in Figure 2, the astringency of skin tannins tends to decrease, while that of seed tannins is kept constant throughout the maturation process. In general, unripe grapes have fewer tannins than well-ripened grapes, but the contribution of ripe grapes to astringency is tought to be greater as they can release more seed tannins (Delteil 1998, and Ribéreau-Gayon et al. 1999).

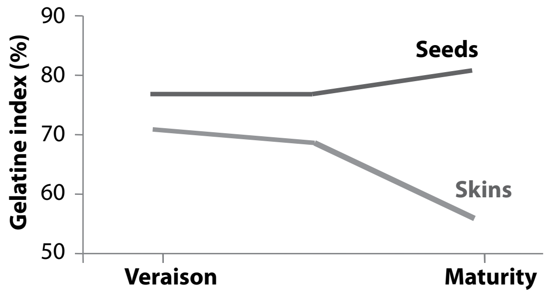


**Figure 2.** Evolution of the astringency of the tannins throughout the maturation process

The main reason why seed tannins are more astringent than skin tannins is related to the fact that the tannins of the seeds and skins do not have the same composition, as shown in figure 3. Basically, the tannins from the skins are rich in prodelphinidin and have a small proportion of gallate units, while the seeds are particularly rich in epicatechin gallate, which confers higher astringency on them (Cheynier 2002).

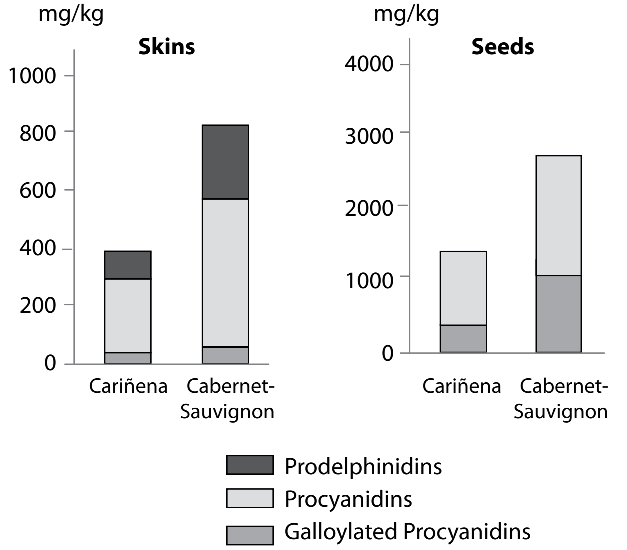


**Figure 3.** Distribution of grape proanthocyanidins

In summary, unripe grapes have a low concentration of anthocyanins, which are also less extractable. Similarly, unripe grapes have a high concentration of tannins from seeds, so if you force maceration in order to extract enough colour, herbaceous and astringent tannins will be also extracted. In contrast, well-ripened grapes have a high concentration of easily extractable anthocyanins and will produce wines with a soft body and tannins.

The degree of the phenolic maturity of the grapes is clearly a key factor for red wine quality, and therefore the harvest date should be determined using this criterion.

In recent years, several methodologies for the determination of phenolic maturity have appeared in the literature (Dupuch 1993, Lamadon 1995, Venencie et al. 1997, Izcara and González 2001, Dubernet et al. 2000, and Celotti et al. 2007), but probably the most used is the methodology described by Professor Yves Glories (Glories and Agustin, 1993). Overall, the methods for measuring phenolic maturity can be classified into three groups:

- Methods based on tasting berries
- Methods based on physical measurements of berries
- Methods based on the preparation of an extract that reproduces the wine, and later analysis.

## 1. Methods based on tasting berries

Methods based on tasting berries are very useful, because we obtain a good idea of the real maturity of grapes by tasting them. However, these methods require a great deal of experience and laborious sampling. They are applicable mainly in small wineries with their own vineyards or at least very controlled vineyards. It is particularly difficult to establish parameters with these methods, but all grapegrowers and winemakers know that observing the colour of seeds is probably the best way to determine if the grapes are really ripe. Figure 4 shows the changes in seed colour throughout ripening.

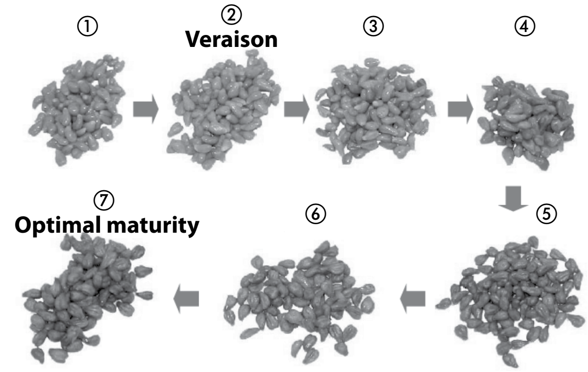


**Figure 4.** Evolution of seed colour throughout the maturation process

It is clear that when seeds have a dark brown colour, the grapes are ready to be harvested.

## 2. Methods based on physical measurements of berries

This group includes several methods based on different physical properties of grapes, such as texture and colour. Methods based on texture (Rio Segade et al. 2008) obtain good correlations between grape maturity and the texture properties of grapes. However, applying this procedure to determine the harvest date is not imaginable, because it is a slow and laborious process requiring very specific equipment. Methods based on colour measurements are faster, but only give an idea of skin maturity and not of seed maturity.

## 3. Methods based on the preparation of an extract which reproduces the wine, and later analysis

Methods based on the preparation of an extract are currently the most used.

However, all these methods are weighty in their implementation, and the results obtained do not always reflect what is happening in the tank during winemaking. In fact, all these methods consist of three steps:

**Extraction phase** Should quickly and efficiently reproduce the release of phenolic compounds that occurs during winemaking.

**Analytical phase** Should try to determine the parameters of interest for the future wine quality.

**Interpretation of results** Should allow the following:

Table 1. The existing methods

| Method | Extraction Phase | Analysis | Time/Difficulty |
|---|---|---|---|
| Glories and Agustin,1993 | Grinding with mixer Maceration for 4 hours with two solutions at pH = 1 and pH = 3.2 | Extractable anthocyanins | +++++ |
| ITV (Dupuch 1993) | Grinding with mixer Maceration for 1 hour with HCl/ethanol solution | A520 A280 | +++ |
| Cromoenos (Gracia) | Grinding with mixer Maceration at high temperature with a special equipement and with commercial solutions | Probable colour Phenolic maturity index | + |
| The AWRI method | Grinding with mixer Maceration for 1 hour with ethanol 50% | Anthacyanins (A520) TPI (A280) and Tannins | ++++ |
| Dubernet et al. 2000 | Grinding with mixer and centrifugation | FTIR Multiple parameters | +++++ |

- Identification of the optimal time of harvest
- Sorting of the grapes according to their actual quality
- Adaptation to winemaking to the phenolic maturity level of the grapes.

Each of the steps must be differentiated, because they are independent and largely determine the reliability and robustness of the method.

The extraction phase is key, as it is involved in an accelerated process of solubilization of phenolic compounds that will take place later in the tank. This is probably the determining factor in all the methodologies, as it is not extracting anything that contains grape, but rather solubilizing what the future will have.

The analytical phase analyzes the parameters considered most suitable from the extract obtained above. It is important to consider that if the extraction is not representative, nothing we analyze will be useful. The objective is not to know the composition of the extract, but to know what these grapes are going to generate. Therefore, the reliability of all the methods is largely determined by the extraction procedure.

Table 1 summarizes the characteristics of the methods currently used to determine the phenolic maturity of the grapes.

As shown in this table, there are several possible methods, but the most widely used to date is probably the one designed by Professor Yves Glories. Figure 5 illustrates this procedure schematically.

The anthocyanins in the extract at pH 1.0 are considered to be the total anthocyanins present in grapes, while the anthocyanins in the extract at pH 3.2 are considered to

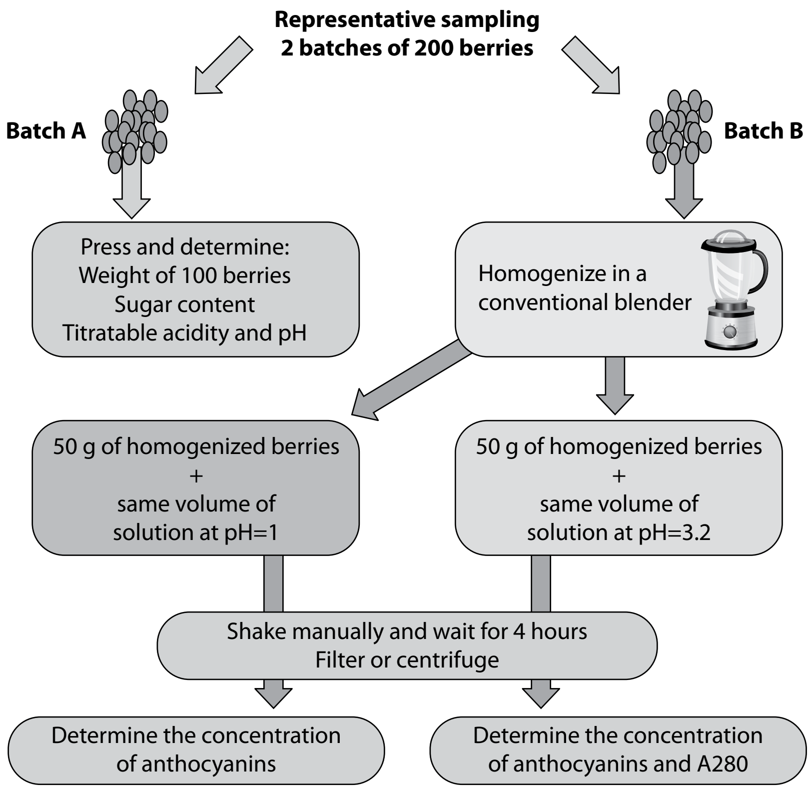


**Figure 5.** The Glories Method (1993)

be those which will be extracted during winemaking. This method also allows us to obtain two other parameters (Figure 6).

**Potential Anthocyanins = ApH1**

**Extractable Anthocyanins = ApH3.2**

**Anthocyanins Extractibility**

$$EA\% = \frac{ApH1 - ApH3.2}{ApH1} \times 100$$

**Seed Tannin Contribution**

$$MP\% = \frac{A280 - (ApH3.2 \times 40)}{A280} \times 100$$

**Figure 6.** Analytical parameters of the Glories Method (1993)

EA% (percentage of extractable anthocyanins) represents the percentage of anthocyanins that will be extracted, and MP% represents the percentage of tannin released from seeds.

In theory, the potential anthocyanins should increase during the ripening process to reach a maximum value. The extractable anthocyanins should also increase. The EA% and MP% should decrease as the level of maturity of the grapes increases. The EA generally ranges between 70% and 20%, while the MP usually ranges between 60% and 10%. The optimal harvest date should, according to Professor Glories, be when the potential anthocyanins reach very high values (more than 1000 mg/L), and when the levels of EA% and MP% are very low (below 30% in both cases).

The methodology described by Professor Glories is laborious and difficult to apply in wineries. For this reason, other approaches have emerged to try to simplify the process. Such is the case of the ITV method, which is faster, and especially the recent Cromoenos method, which permits rapid determination (under 10 minutes).

However, the main problem with all three methods is knowing their degree of reliability. Most research is limited to comparing the methods, not trying to verify whether

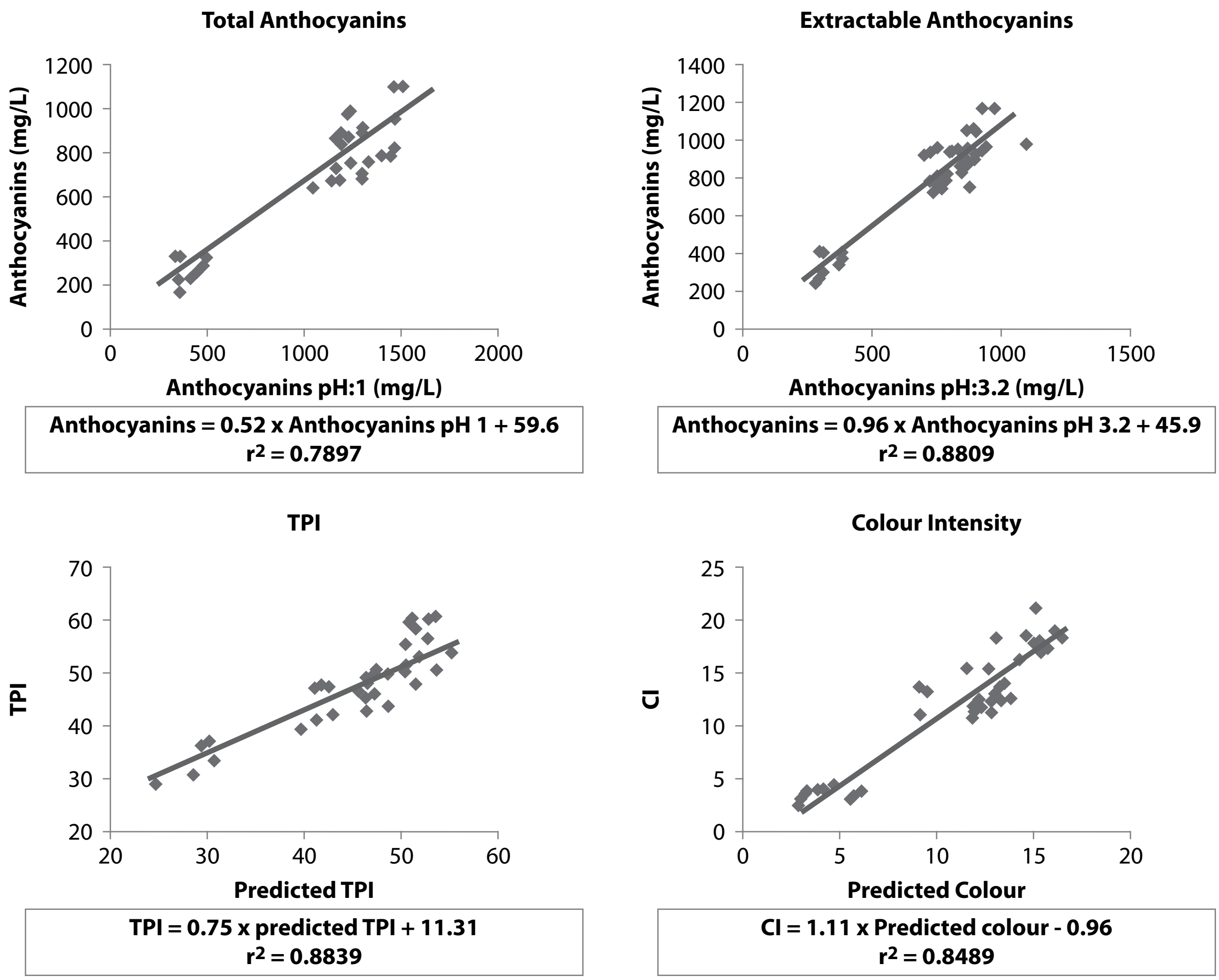


**Figure 7.** Predictive effectiveness of the Glories method

they are truly effective for predicting the colour and/or phenolic composition of future wines. For this reason, our research group considered it necessary to perform a study of the real effectiveness of each of these methods.

To do this, four cultivars (Tempranillo, Garnacha [Grenache], Merlot and Cabernet Sauvignon) were harvested in 2007 at three maturity levels (three, five and seven weeks after veraison). In all cases, winemaking was performed in triplicate, and the wine colour and composition were compared to that predicted by the Glories, ITV and Cromoenos methods (Kontoudakis et al. 2010). Figure 7 show the results obtained by the Glories method.

As can be seen, the Glories method provides reasonably good results in terms of the relationship between extractable anthocyanins and the real anthocyanin concentration in wine. But this is not true for the total polyphenol index (TPI). Figure 8 show the results obtained by the ITV method.

The ITV method results were somewhat disappointing because the correlation coefficients were very low. Figure 9 (next page) shows the results obtained with the Cromoenos method.

With the Cromoenos method, the correlation was much better than in the previous case. It can be concluded that among these three methods, the Glories method generates the best correlation coefficient between the measured and predicted anthocyanin concentration in wine. However, it is laborious and difficult to apply in the winery. The ITV method does not seem to be appropriate, because it creates a very low correlation coefficient and therefore does not correctly predict the colour intensity of future wines. The Cromoenos method presents a reasonable correlation coefficient between the predicted colour and that obtained in the wine, and also has the advantage of being very quick.

It should be noted that the implementation of a harvest date decision-making system based on phenolic maturity is very complicated. Practical experience in the application of this methodology indicates significant variability in the results. In fact, many wineries have tried it with inconsistent results. The problem is that this methodology, as described, must be applied with great rigour. If not, it can lead to erratic results that mislead the winemaker.

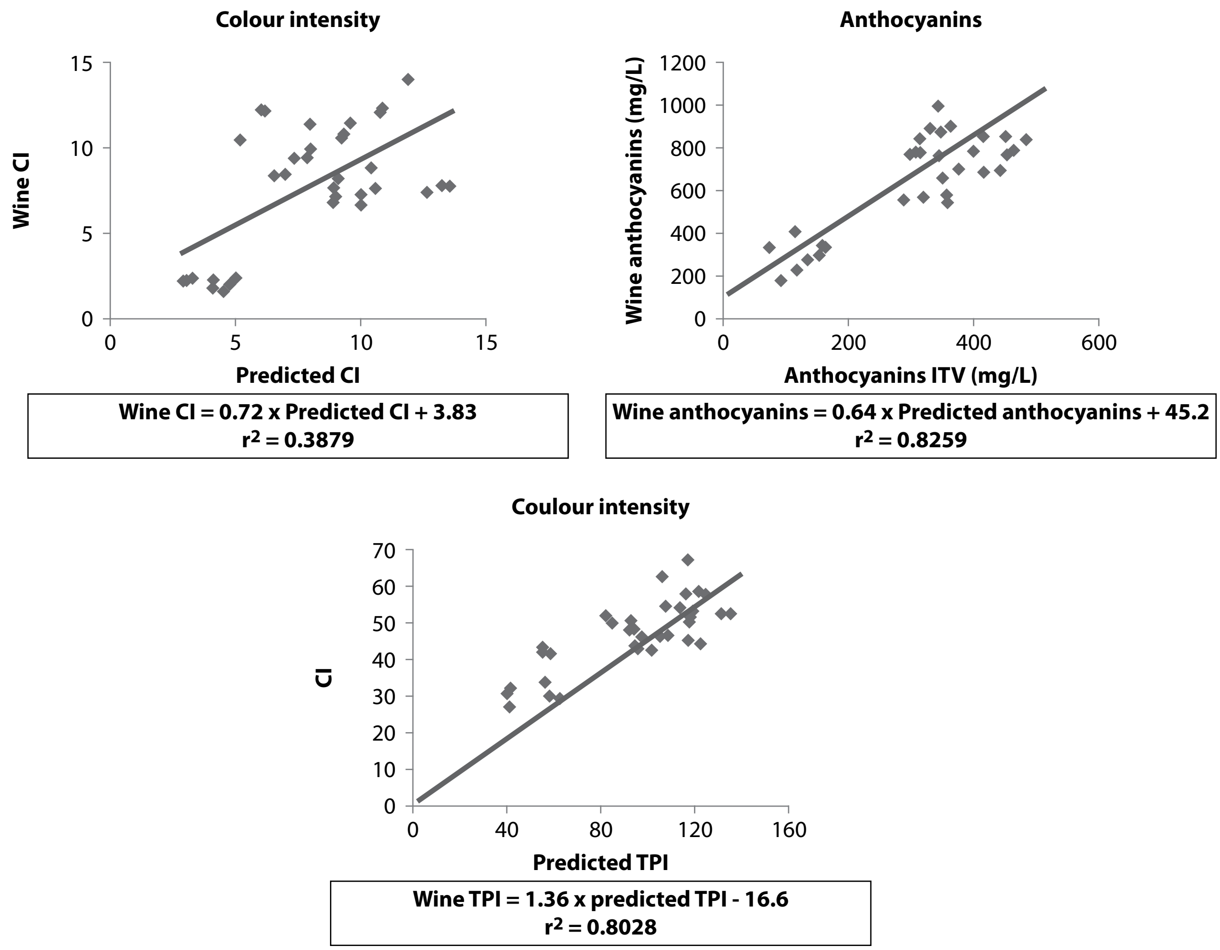


**Figure 8.** Predictive effectiveness of the ITV method

The subject is therefore still up for debate. Existing methods for determining the degree of phenolic maturity must be optimized first. On the one hand, the conditions in which the sample is prepared must be specified in greater detail, and on the other hand, systems need to be designed to automate the process to make it faster and more efficient.

Realistically, another necessary consideration is that using phenolic maturity as a tool for deciding the harvest date is, in many cases, utopian. The harvest, as we all know, is conditioned by many factors, including the weather, the health status of the grapes, the capacity of the winery, and so on, which, in the end, are unfortunately more important than the maturity of the grapes. Only wineries that use grapes from their own vineyards could afford to use this criterion.

Nevertheless, determining of phenolic ripeness can be very useful as a monitoring tool at the winery gate. The grapes could be classified according to their degree of phenolic maturity, which would serve as a criterion for separating qualities and would set a price for the grapes according to their actual quality. In addition, the winemaker, knowing the actual level of phenolic maturity, could apply different strategies to obtain the most suitable degree of extraction (Delteil 1995, and Ribéreau-Gayon et al. 1999).

## References

Celotti, E.,S. Dell'oste, P. Fiorini, and G. Carceri. 2007. Une nouvelle méthode pour l'évaluation des polyphénols des raisins rouges. Rev. Œnol. 125:23-27.

Cheynier, V. (2002). Oxygen in wine and its role in phenolic reactions during aging. *Uses of gases in winemaking*. Eds: M. Allen, S. Bell, N. Rowe and G. Wall. Proceedings of the Australian Society of Viticulture and Oenology Seminar, October 2002, Adelaide.ASVO, Adelaide.

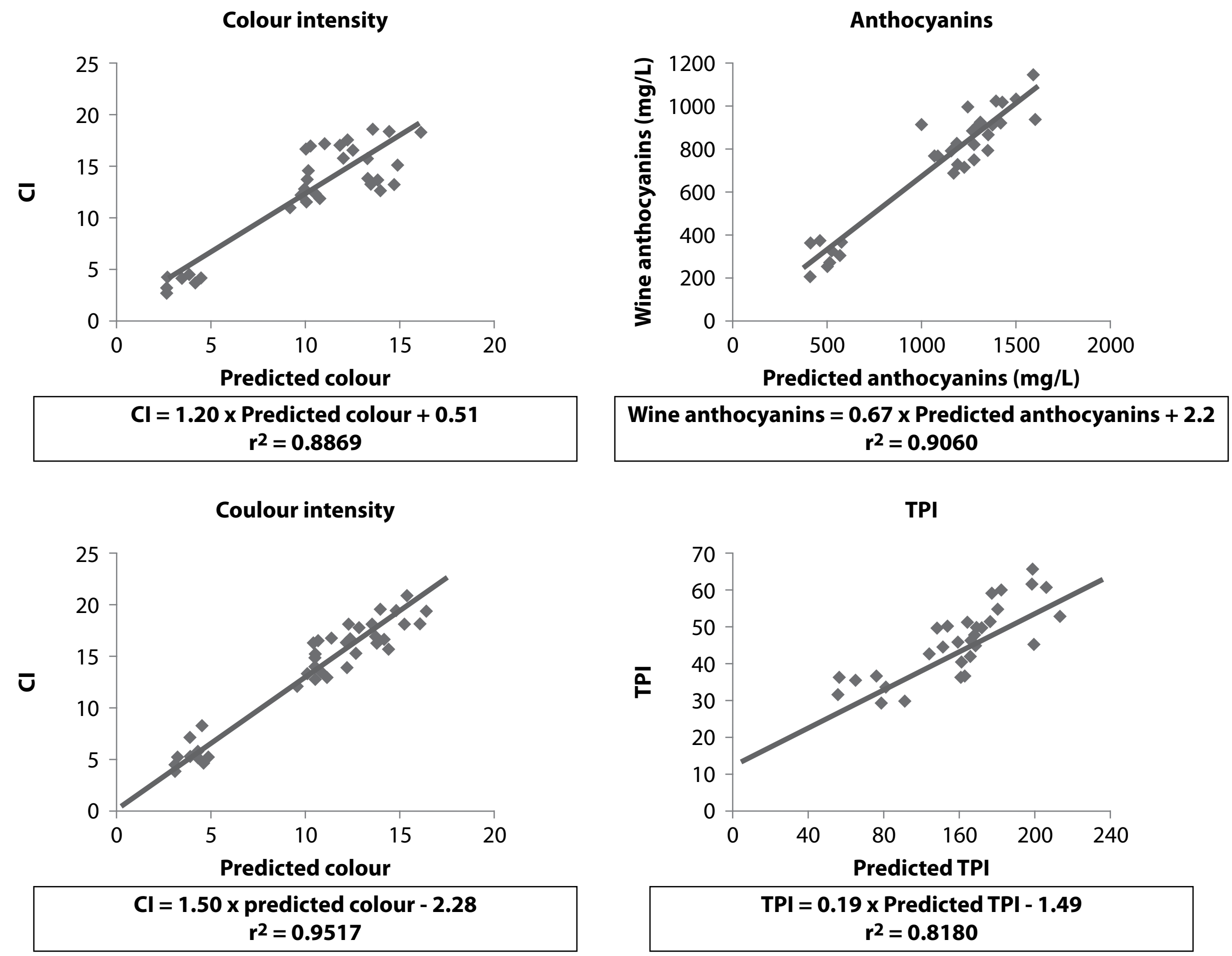


**FIGURE 9.** Predictive effectiveness of the Cromoenos method

Delteil, D. 1995. Les macérations en rouge: L'art du détail. *Rev. Œnol*. 77:23-25.

Dubernet, M., V. Dubernet, M. Lerch, S. Coulomb, and I. Traineau. 2000. Analyse objective de la qualité des vendanges par spectroscopie infrarouge à transformée de Fourier et réseaux de neurones. *Rev. Fran. Œnol*. 185:18-21.

Dupuch, V. 1993. *Appréciation de la matière phénolique des vins rouges ; application à la détermination de la date de récolte*. Actes du Colloque Journée technique du CIVB. Bordeaux. 62-69.

Glories, Y., and M. Agustin. 1993. *Maturité phénolique du raisin, conséquences technologiques; application aux millésimes 1991 et 1992*. Actes du Colloque Journée technique du CIVB. Bordeaux. 56-61.

Izcara, E., and M. L. González. 2001. Análisis de métodos rápidos de extracción para seguir la maduración fenólica de la uva. *Enólogos*. 14:14-18.

Kontoudakis, N., M. Esteruelas, F. Fort, J. M. Canals, and F. Zamora. 2010. Comparison of methods for estimating phenolic maturity in grapes: Correlation between predicted and obtained parameters. *Analytica Chimica Acta*. 660:127-133.

Lamadon, F. 1995. Protocole pour l'évaluation de la richesse polyphénolique des raisins. *Rev. Œnolog. Techniq. Vitvinic. Œnol*. 76:37-38.

Ribéreau-Gayon, P., Y. Glories, A. Maujean, and D. Dubourdieu. 1999. Phenolic Compounds. Handbook of Enology, Vol. 2. The Chemistry of Wine Stabilization and Treatments. John Wiley & Sons, Ltd, Chichester. 129-186.

Rio Segade S., E. Soto Vázquez and E. Díaz Losada, 2008. Influence of ripeness grade on accumulation and extractability of grape skin anthocyanins in different cultivars. *J. Food Comp. Anal*., 21, 599-607.

Venencie, C., M. N. Uveira, and S. Guiet. 1997. Maturité polyphénolique du raisin ; mise en place d'une méthode d'analyse de routine. *Rev. Fran. Œnol.* 167:36-41.

Zamora, F. 2002. La madurez fenólica; Un tema abierto. *Enólogos*. 18:24-28.

# IMPACT ON AGRONOMIC PARAMETERS IN VINES AND WINE QUALITY OF FOLIAR TREATMENTS WITH SPECIFIC FRACTIONS OF YEAST DERIVATIVES

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## 1. Introduction

Winemakers and grape growers are constantly looking for opportunities to improve wine quality. Producing high quality fruit for wine production is challenging and dependent on many factors, including the regional climate, seasonal variations in precipitation and the temperatures in the growing season. As a result, wineries are often required to work with unbalanced grapes, overcoming such obstacles as poorly ripened fruit, high pH, low acidity and wines that will lose quality quickly. High quality wine production in the cellar starts with high quality grape production in the vineyard.

Many producers would like to get grapes with moderate sugar content in the pulp, good acidity, low pH and good skin maturity, skin that is both phenolic and aromatic and, if possible, mature seeds.

To try to meet these challenges to wine quality, Lallemand has developed a series of products that stimulate phenolic maturity and increase the synthesis of grape aroma precursors through the foliar application of yeast derivatives. These yeast derivatives are designed for use as per WO/2014/024039 patent-pending foliar application technology.

Two different yeast derivative compositions, RD-LM for red grape varietals and RD-LA for white grape varietals, were tested to evaluate their ability to stimulate phenolic maturity and enhance the synthesis of grape aroma precursors during ripening. Evaluation of wine tasting was done by a trained expert tasting panel.

The concept of phenolic maturity in grapes is linked to the concentration of anthocyanins in the skin, their extractability and the tannin content, as well as the concentration of condensed tannins in the seeds.

Compounds of a phenolic nature greatly impact the colour, aroma, taste and organoleptic characteristics of the must, and therefore, of the wine (de la Fuente et al. 2007). Anthocyanins and tannins are the main representatives of the phenolic compounds present in grapes and red wines. Anthocyanins are the main colour components in red wines (Jackson and Lombard 1993), and tannins impart bitterness and astringency (Harborne 1984). The union of anthocyanins and tannins forms polymers that provide stable pigments necessary to maintain the stability of the colour of red wines over the long term.

During the early stages of berry ripening, the amount of anthocyanins increases. At ripening, the accumulated amount of anthocyanins reaches a maximum then remains relatively constant or declines. Several studies have shown a decrease in anthocyanin content in the later stages of ripening (e.g., Somers 1976, Keller and Hrazdina 1998, and Holt et al. 2010).

Meanwhile, it appears that tannin synthesis occurs early in berry development (Downey et al. 2003). Studies by Bogs et al. (2005) suggest that tannin is being synthesized even before the berry has set. Tannins increase at a steady rate from fruitset to a peak around veraison. Some studies show a decrease – for example for Shiraz, a 60% decrease over a range of 6° to 30°Brix (Downey et al. 2003) – while others have shown that tannin content remained essentially constant from veraison to harvest, for Cabernet Sauvignon and Pinot Noir, for example, which are essentially constant from veraison to approximately 24°Brix (Harbertson et al. 2002). In addition, cultural practices and climatic conditions are likely to influence the metabolism of tannins in skins (Iland et al. 2011).

From this, we can take away that the harvest moment is a key factor in the degree of phenolic maturity. Grapes that have not reached phenolic maturity will produce wines that are not so balanced and have a strong sensation of astringency.

Regarding aroma, clearly aroma is one of the factors that most determines the quality of a wine. Aroma compounds, which come from the secondary metabolism of the vine, include terpenes, norisoprenoids, thiol precursors and methoxypyrazines. These compounds contribute to the varietal character that typifies the wine. This varietal character comprises, on one hand, the free volatile compounds of direct aromatic expression, which are few in number, and on the other hand, the aroma precursors, which are non-volatile components freed during aging or thanks to the metabolic activity of yeasts (Jackson 2008). Some research shows that glycosidic aroma precursors are not present in all grape varieties, and when they are present they are not in the same concentrations (Reyero et al. 2000, Garcia-Moruno et al. 2000, and Reynolds and Wardle 1997).

During the period of berry formation, the concentration of the free monoterpenes and most of the bound ones fell to low levels. From veraison onwards, only the linalool, diendiol and the pyran ring linalool oxides accumulated to any significant extent. These continued to a maximum of about 24°Brix, and then declined.

In grape berries, norisoprenoid levels are low prior to veraison and increase after veraison (Razungles et al. 1993 and 1996, Marais et al. 1991, Baumes et al. 2002, Bindon 2004, and Bindon et al. 2007). The pattern of change in these compounds and carotenoids led researchers to propose that the formation of norisoprenoids is linked to the degradation of carotenoids.

Methoxypyrazine content increases during berry formation, reaches a peak around veraison, then decreases during berry ripening (Boss et al. 2008, Ryona et al. 2008, and Reynolds 2010). Typically, levels of methoxypyrazines are higher in berries from vines grown in cool climates than those from warm and hot climates (Boss et al. 2008).

There are many factors that affect phenol synthesis and aroma precursors in the vine, including light, temperature, altitude, soil type, water, nutritional state, microbial interactions, pathogens, growth regulators and defoliation. Accordingly, many techniques have been applied to improve the content of these factors in the berries. The most common techniques are related to agricultural techniques, as thorough studies have been conducted on the impact of such factors as the optimal exposure of clusters (Carbonneau and Costanza 2004), the canopy microclimate (Jackson and Lombard 1993), the optimization of leaf surface areas (Bonnisseau and Dufourcq 2004), moderate hydric stress (Seguin 1975, Bravdo et al. 1985, and Carbonneau 1987) and the effect of nitrogen fertilizer (Bell and Henschke 2005).

In an attempt to improve the phenolic content in the grape, other mechanisms have recently garnered attention, such as the use of elicitors – phytochemicals that do not kill pathogens per se but rather trigger mechanisms that improve plant resistance to pathogens, including an increase in phenolic compound levels (Vitalini et al. 2011).

The concentration and quality of phenolic compounds, as well as aroma precursors, are parameters that depend on the variety being studied. Therefore, these trials have been done on two common viticultural varieties with great oenological potential: Shiraz and Sauvignon Blanc.

Our studies have focused on trying to determine the behaviour and response mechanisms of vines to the application of yeast derivatives, in order to verify and quantify agronomical and oenological interest, showing that the studied applications have an impact on the quality of the final wine. This project aimed to study the effect of yeast derivatives on the vines – evaluating responses in varieties with great oenological potential, verifying the effect of the yeast derivative composition, doses and timing, studying the relationship between the response and the applied dose – then come to a conclusion regarding cause–effect relationships. We also verified whether the effect of yeast derivative products on the grapes and wine is manifested according to the time of harvest and/or the ripeness of the grapes.

Yeast derivative applications tested in the vineyard were effective for defining different wine qualities, without affecting yield or other agronomical parameters. The wine descriptors were evaluated as more important than the control plots.

## 2. Material and methods

### 2.1 Vineyard Location. Plant Material and Experimental Design

This experiment was performed in Finca Constancia (Toledo, Spain) in 2013. The area presents a warm climate with marked seasonal variation and a period of noticeable summertime drought. In 2013, there were 2,135 growing degree days (GDD) and 384 mm of rain. Vineyard soils are inceptisols and alfisols, according to the USDA.

The study was carried out on grapevines planted in 2002, with a distance of 2.4 m between rows and 1.2 m between plants within the row. The rows are orientated North–South, and the trellis system is Vertical Shoot Positioned with bilateral cordon and spur pruning.

The plant material used to perform the trials was made up of vines from two different cultivars, the Shiraz clone 470 grafted on 1103-P and the Sauvignon Blanc clone 700 grafted on 110-R.

All treatments were applied in four repetitions arranged in a completely randomized design. Vines were sprayed with a water solution of yeast derivatives at the beginning of veraison (5 to 10% of veraison then again 10 days later). The solutions applied were the RD-LM product at a rate of 1 kg/ha on Shiraz vines, and the RD-LA product at 3 kg/ha on Sauvignon Blanc. In both cases, 300 L/ha was applied with a 16-litre capacity Matabi model E+ electric motor sprayer, which ensures constant pressure.

### 2.2 Measurements

**Vegetative Growth**

*Pruning Weight and Shoot Weight*

The pruning weight (PW) was measured in five plants of average vigour at each repetition. The number of shoots per vine was counted, and the shoots removed were weighed with a 5 kg maximum weight and 50 g precision Pesola model 8.004 dynamometer. Two average-sized shoots were taken in each repetition, then weighed on a scale with a sensitivity of 0.01 g (OHAUS Adventurer™ Pro model AP AV412) and were kept in separate labeled bags. These bags were placed on a stove at 70°C until a constant weight was reached.

**Vegetative/Productive Balance**

*Dry Weight*

Two representative vine shoots of average vigour were chosen from each repetition in each of the experimental plots for a total of eight per treatment. Stems, leaves and bunches were weighed on a scale with a sensitivity of 0.01 g (OHAUS Adventurer™ Pro model AP AV412), to obtain their fresh weight. Each part was then kept in a separate labeled bag and placed on a stove at 70°C with forced ventilation until a constant weight was reached. Once dry, each sample was weighed to obtain the dry weight and percentage of water.

The humidity level of the stems and bunches was applied to all stems and bunches in the repetition.

**Physiology**

To determine the effect of the experimental products on the physiological activity of the plant, foliar water potential ($\psi f$) and the exchange of gasses in the atmosphere were measured on individual leaves.

The measurements took place at two moments of the day, at optimal environmental conditions for photosynthetic activity and at solar noon. Optimal conditions for photosynthesis were determined according to Sánchez-de-Miguel (2007).

These measurements were taken twice during the cycle. At pre-veraison five leaves of each treatment were sampled and at full maturity five leaves of each treatment were sampled.

The foliar water potential was evaluated using a Scholander pressure chamber (Model 3000 Soil Moisture Equipment Corp., Santa Barbara, CA, USA) with a reading resolution of 0.01 MPa.

Via an open system of infrared gas exchange measurement (IRGA) (LI-6400 portable photosynthesis system from LI-COR®, Lincoln, Nebraska, USA), the instantaneous rate of $CO_2$ (An) assimilation in µmol $CO_2/m^2$ leaf and stomatal conductance (gs) in mol $H_2O/m^2$ leaf was obtained. This measurement was taken only on the Shiraz cultivar.

**Yield Components (Three Harvests)**

*Harvest Weight*

The harvest weight was determined at three different moments, defined as the average °Brix of the different repetitions from the same cultivar. The °Brix intervals that were set to decide the times of harvest were equivalent to an early harvest, an industrial harvest and an overripe harvest.

Harvest weight was measured in six plants by experimental plot and time of harvest. Shoots and bunches were counted for each vine, and the bunches from the six plants were weighed on a 30 kg maximum 5 g precision electronic scale (Calitrol Control Gram model PM-30, Barcelona, Spain). Using these data and the average weight of the berries, the following yield components were determined: berry weight, kg/vine, number of bunches per shoot and bunch weight.

**Evolution of the Berry Composition During the Ripening Period**

*Technological Maturity*

Weekly samplings were performed of 100 berries per elemental plot from pre-veraison to time of harvest. Each sample was weighed on a scale with a sensitivity of 0.01 g (OHAUS brand Adventurer Pro Model AP AV412) to determine average berry weight. The must from these 100 berries was extracted with a strainer and, after centrifugation, the supernatant material was collected for analysis of total soluble solids (°Brix), pH and total titratable acidity.

The total soluble solids were measured using a portable digital refractometer (PALETTE WM-7, ATAGO Inc., Kirkland, Washington, USA), with results shown in °Brix; total titratable acidity was measured using an automatic titrator (736 GP Titrino, METROHM AG, Herisau, Switzerland), with the values shown in g tartaric acid/L; and pH was determined using a pH meter (micropH 2001, CRISON, Barcelona, Spain) that had been previously calibrated.

*a. Phenolic Maturity*

Samples were taken of 150 berries during each of the three harvests from every one of the experimental Shiraz plots and then triturated in a blender (Royal Blender Turbo 10-speed, Princess).

The extraction method proposed by Glories and Augustin (1993) was used to calculate the total polyphenol index (TPI) and the concentration of total and extractable anthocyanins. Two standards were applied: one at pH 1 (HCl 0.1 N) for extracting all anthocyanins and another at pH 3.2 (5 g/L tartaric acid solution) for extracting the extractable anthocyanins. Absorbency measurements were taken at 280 nm and at 520 nm with a spectrophotometer *(J.P. Selecta*, SPECTROPHOTOMETER UV-2005). Tannins were extracted from the berry using methodology from the Standard Methods of the Australian Wine Research Institute (AWRI) (Iland et al. 2004).

Quantification of the tannins was performed using the method of Methyl Cellulose Precipitation (MCP) (Sarneckis et al. 2006, and Mercurio and Smith 2008). This reading was obtained by using a spectrophotometer (J.P. Selecta, SPECTROPHOTOMETER UV-2005).

#### 1.1.1 Fermentations

Separate fermentations were conducted for the 16 repetitions during the three specified harvests, resulting in a total of 48 micro-fermentations. Fermentations were achieved by following the micro-fermentation method described by Sampaio et al. (2007). The fermentation micro-deposit consisted of a glass jug (1 gallon) with a cap for alimentary use perforated in the centre, through which passed the fermentation value, which was thermosealed with food-grade silicone.

#### 1.1.2 Sensory Analysis

The wines from the two experiments were subjected to sensory evaluation. These were performed by a group of 11 professional tasters made up of professional oenologists and researchers from the viticulture research group at the Universidad Politécnica de Madrid.

The method of analysis involved triangle taste tests that showed whether or not the tasters were able to perceive a difference in treatments. This procedure allows information to be collected on sensory differences and similarities for a wide variety of products (Blancher et al. 2007 and Cartier et al. 2006).

The results of the triangle taste tests were calculated statistically according to the tables of Roessler et al. (1948) for levels of significance of 5%, 1% and 0.1%.

After the triangular tests, treatment wines that were significantly different according to the aforementioned underwent a descriptive test to determine which wine the tasters preferred.

#### 1.1.3 Analysis of Results

Due to its experimental design, statistical analysis of a large part of the results was performed through variance analysis. The significance of the variance analysis was determined for probability levels $p<0.05$ (*), $p<0.01$ (**) y $p<0.001$ (***). Whenever this showed significant effects of treatment, the averages were compared using Duncan's multiple range test for a probability level of $p<0.05$. The program SPSS, version 18.0 (SPSS Inc. Chicago, Illinois) was used for all statistical analyses.

## 2. RESULTS AND DISCUSSION

All media data obtained in the field and laboratory during the campaign was reported and compared with data obtained by other authors, with particular attention paid to differences possibly attributable to different yeast derivative treatments. See table 1.

### 2.1 Vegetative Growth

The most reliable estimator of plant vigour is pruning weight (Huglin 1986). It gives the best estimation of plant yield, growth and development, and thus the productive potential of the vine for given conditions (Yuste 1995).

The limits of shoot weight cited in the literature are 20-40 g/shoot. Sauvignon data showed low shoot vigour due to high crop load. Shiraz shoot weight was within normal values mentioned in the literature.

None of the treatments with yeast derivatives affected the vegetative growth of Shiraz or Sauvignon Blanc – neither

**Table 1.** RD-LM treatment effect on Shiraz and RD-LA treatment effect on Sauvignon Blanc re pruning weight (kg/m²), shoot weight (g) and % of shoot humidity. Statistical significance: *, **, ***, ns: significant differences for p≤0.05, 0.01, 0.001, or not significant, respectively.

| Variety | Treatment | PW (kg/m2) | Shoot weight (g) | % Hum shoot |
|---|---|---|---|---|
| Shiraz | Control | 0.23 | 30.18 | 55.9 |
| | RD-LM | 0.25 | 30.43 | 55.8 |
| | Sig. | ns | ns | ns |
| **Variety** | **Treatment** | **PW (kg/m2)** | **Shoot weight (g)** | **% Hum shoot** |
| Sauvignon Blanc | Control | 0.17 | 16.80 | 60.0 |
| | RD-LA | 0.15 | 15.28 | 61.9 |
| | Sig. | ns | ns | ns |
| **Variety** | **Treatment** | **PW (kg/m2)** | **Shoot weight (g)** | **% Hum shoot** |
| Shiraz | Control | 0.23 | 30.18 | 55.9 |
| | RD-LM | 0.25 | 30.43 | 55.8 |
| | Sig. | ns | ns | ns |
| **Variety** | **Treatment** | **PW (kg/m2)** | **Shoot weight (g)** | **% Hum shoot** |
| Sauvignon Blanc | Control | 0.17 | 16.80 | 60.0 |
| | RD-LA | 0.15 | 15.28 | 61.9 |
| | Sig. | ns | ns | ns |
| Sauvignon Blanc | Control | 0.17 | 16.80 | 60.0 |
| | RD-LT | 0.14 | 14.40 | 63.6 |
| | Sig. | ns | ns | ns |

the vigour estimated by the shoot weight nor the vine's vegetative expression as evidenced by the pruning weight. This was to be expected, since the yeast derivatives were applied after vegetative growth was well underway and in some aspects (such as on the stems) almost complete. (The conditions of the shoots, like humidity content, were not affected during the ripening period.)

### 2.2 Vegetative/Productive Balance

Dry Weight

Knowing that dry weight production indicates a vineyard's capacity or potential (Carbonneau and Casteran 1986) and that dry weight accumulated on renewable parts provides a good approximation of the overall productivity of the vine by assuming 88–93% of the dry weight produced annually (Williams 1996), we can conclude from treatments that there was no effect on vineyard capacity or potential.

Like with vegetative growth, the experimental treatments had no effect on the humidity conditions of leaves and bunches, which change during the ripening period.

Dry Weight Distribution

According to the dry weight distribution data obtained by various authors (Fregoni 1980, Fernandez et al. 1997, Williams and Grimes 1987, and Williams and Biscay 1991), our trial showed a lower % of dry weight accumulated in stems and a higher % in leaves. The % in bunches was similar to that obtained by these authors. This indicates the low vigour of the shoots, especially in Sauvignon Blanc, due to the high number of shoots per plant and the water stress present.

Dry weight distribution between leaves, stems and bunches did not have a significant effect, and where grape harvests varied the values were within normal ranges (between 54% and 64% of dry weight corresponding to renewable parts).

**Table 2.** RD-LM treatment effect on Shiraz and RD-LA treatment effect on Sauvignon Blanc re dry weight and % of humidity in leaves (LDW and %LH) and dry weight and % of humidity in the bunches (BDW and %BH). Statistical significance: *, **, ***, ns: significant differences for p≤0.05, 0.01, 0.001, or not significant, respectively.

| | | LEAVES | | BUNCHES | | | | LEAVES | | BUNCHES | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variety** | **Treatm.** | **LDW** | **%LH** | **BDW** | **%BH** | **Variety** | **Treatm.** | **LDW** | **%LH** | **BDW** | **%BH** |
| Shiraz | Control | 55.22 | 63.6 | 80.20 | 70.1 | Sauvignon Blanc | Control | 36.04 | 61.0 | 47.94 | 72.1 |
| | RD-LM | 51.14 | 62.2 | 68.39 | 71.2 | | RD-LA | 33.03 | 61.2 | 58.71 | 72.2 |
| | Sig. | ns | ns | ns | ns | | Sig. | ns | ns | ns | ns |

**Table 3.** RD-LM treatment effect on dry weight distribution in the renewable parts of Shiraz vines, and RD-LA treatment effect on dry weight distribution in the renewable parts of Sauvignon Blanc vines. Statistical significance: *, **, ***, ns: significant differences for p≤0.05, 0.01, 0.001, or not significant, respectively.

| Variety | Treatm. | % stems | % leaves | % bunches | Variety | Treatm. | % stems | % leaves | % bunches |
|---|---|---|---|---|---|---|---|---|---|
| Shiraz | Control | 12.14 | 32.03 | 55.84 | Sauvignon Blanc | Control | 11.44 | 31.31 | 57.25 |
| | RD-LM | 14.02 | 31.08 | 54.90 | | RD-LA | 9.53 | 26.67 | 63.80 |
| | Sig. | ns | ns | ns | | Sig. | ns | ns | ns |

**Physiology**

*Leaf Water Potential*

The leaf water potential measured at noon (ψ mid) decreased as the cycle progressed, even under conditions of sufficient moisture (Williams and Matthews, 1990), remaining relatively stable after veraison (Williams and Grimes, 1987). In Table 4 we see that in maturation, the ψ mid decreased with respect to pre-veraison values.

Furthermore, according to Williams and Mathews (1990), leaf water potential measured at noon must be lower than -1.3 MPa for premature leaf abscission to occur, and Kriedman and Smart (1971) observed that leaf water potential measured at 12 p.m. began to limit photosynthesis from -1.2 MPa. According to the values obtained by these authors, Shiraz and Sauvignon Blanc vines in our trial experienced since pre-veraison limiting values for photosynthesis, and explain the senescence of basal leaves already present on this period.OK

According to Vallone et al. (1997), leaf water potential at noon in the ripening period has an effect on yield components.

The leaf water potential although in some cases showed significant differences only in the LA treatment on Sauvignon Blanc is worthy of considerationt. For LA treatment, the leaf water potential were significantly reduced compared to the control during the ripening period.

*Gas Exchange*

According to the schema by Medrano et al. (2002) on vine photosynthetic response to drought with stomatal conductance used as a reference parameter, we see that the values at the time of maximum photosynthetic activity suggest moderate drought. The stomatal effects are predominant, and photosynthesis is restored once the leaf is rehydrated (Flexas et al., 1999), and no stomatal effects are detectable (Naor et al., 1994, Flexas et al., 2002, and Maroco et al ., 2002). For measurements performed at noon, the values show severe water stress with predominance of non-stomatal effects and no recovery of photosynthesis after the leaf is rehydrated (Dühring 1988).

Unlike leaf water potential, physiological measurements at the leaf level in Shiraz were not significantly affected either at maximum photosynthesis or at noon by the application of LM. We consider the significant reduction in the net assimilation rate at midday with respect to maximum photosynthesis noteworthy, as values at maturation are much lower than pre-veraison values, which shows that the effects of water status are more limiting at noon and when the ripening period has advanced.

### 2.4 Yield Components

Yield components are already determined at the time when the yeast derivatives are applied, except berry weight, which tends to double during ripening. As might

**Table 4.** RD-LM treatment effect on Shiraz and RD-LA treatment effect on Sauvignon Blanc leaf water potential (MPa) at maximum photosynthesis (ψ max) and at midday (ψ mid). Statistical significance: *, **, ***, ns: significant differences for $p \leq 0.05$, 0.01, 0.001, or not significant, respectively.

| Variety | Date | Treatment | Ψmax | Ψmid | Variety | Date | Treatment | Ψmax | Ψmid |
|---|---|---|---|---|---|---|---|---|---|
| Shiraz | Pre-veraison | Control | -1.50 | -1.44 | Sauvignon Blanc | Pre-veraison | Control | -1.26 | -1.35 |
| | | RD-LM | -1.33 | -1.38 | | | RD-LA | -1.22 | -1.39 |
| | | Sig. | ** | ns | | | Sig. | ns | ns |
| | Maturation | Control | -1.60 | -1.67 | | Maturation | Control | -1.22 | -1.58 |
| | | RD-LM | -1.46 | -1.62 | | | RD-LA | -1.64 | -1.72 |
| | | Sig. | * | ns | | | Sig. | ** | * |

**Table 5.** RD-LM treatment effect on Shiraz re net photosynthesis rate (µmol CO2/m2 leaves) and stomata conductance (mol $H_2O$/m2 leaves). Statistical significance: *, **, ***, ns: significant differences for $p \leq 0.05$, 0.01, 0.001, or not significant, respectively.

| Variety | Date | s.h. | Treatm. | Photos. | Cond. | Date | s.h. | Treatm. | Photos. | Cond. |
|---|---|---|---|---|---|---|---|---|---|---|
| Shiraz | Pre-veraison | maximum | Control | 7.48 | 0.0876 | Maturation | maximum | Control | 7.70 | 0.1948 |
| | | | RD-LM | 8.39 | 0.1002 | | | RD- LM | 6.71 | 0.1638 |
| | | | Sig. | ns | ns | | | Sig. | ns | ns |
| | | midday | Control | 3.11 | 0.0454 | | midday | Control | 1.57 | 0.0248 |
| | | | RD-LM | 3.37 | 0.0311 | | | RD-LM | 1.43 | 0.0206 |
| | | | Sig. | ns | ns | | | Sig. | ns | ns |
| | | s.h. *applic. | Sig. | ns | ns | | s.h. *applic. | Sig. | ns | ns |

be expected, yield components were not significantly affected in any of the treatments or varieties. Overall values for fertility (number of clusters/shoots), bunch weight and berry weight were quantitatively small, probably due to the high number of shoots present in the vineyard and the very limited water availability.

### 2.5 Evolution of Berry Composition during Ripening

*a. Berry Weight*

Berry weight is a basic estimator of growth and the quality/quantity of the harvest.

Significant differences were found only in the sampling from August 29, which coincided with the first harvest, showing greater berry weight vs. the control in the case of RD-LA treatment, as we saw earlier in the yield components.

*b. Total Soluble Solids (°Brix)*

During ripening, grapes are a major sink for carbohydrates from the active leaves and reserve structures (Roubelakis-Angelakis 2009). We can see in figure 2 (next page) that treatments did not have any impact on carbohydrate accumulation during the ripening period.

*c. Total Acidity*

Evolution of the total titratable acidity (expressed as g/L of tartaric acid) was characterized by an increase in the herbaceous phase followed by a decrease throughout maturation, coinciding with the increase in sugar content and increased berry weight (Esteban et al. 1999 and Hrazdina et al. 1984). In figure 3 (next page) we see that treatments did not affect the degradation of acids during ripening.

*d. pH*

The pH of the must depends on the balance between concentrations of free organic acids and their salts, with potassium as the primary cation (Smart and Coombe, 1983). In figure 4 we see that treatments did not affect this balance.

During the ripening period, the technological maturity of the pulp did not undergo significant changes in sugar con-

**Table 6.** RD-LM treatment effect on Shiraz and RD-LA treatment effect on Sauvignon Blanc yield components. Statistical significance: *, **, ***, ns: significant differences for $p \leq 0.05, 0.01, 0.001$, or not significant, respectively.

| Variety | Date | Treatm. | Bunch Weight (g) | Kg /vine | Berry Weight (g) | Num. Bunches / Shoot | Variety | Date | Treatm. | Bunch Weight (g) | Kg /vine | Berry Weight (g) | Num. Bunches / Shoot |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Shiraz | 1st Harvest | Control | 94.21 | 3.30 | 0.96 | 1.39 | Sauvignon Blanc | 1st Harvest | Control | 81.63 | 3.23 | 0.78 | 1.38 |
| | 09/03/2013 | RD-LM | 96.37 | 3.43 | 0.98 | 1.38 | | 08/29/2013 | RD-LA | 83.13 | 3.92 | 0.86 | 1.61 |
| | 2nd Harvest | Control | 100.50 | 3.54 | 0.98 | 1.49 | | 2nd Harvest | Control | 75.39 | 3.52 | 0.78 | 1.41 |
| | 09/18/2013 | RD-LM | 94.45 | 3.37 | 0.94 | 1.40 | | 09/10/2013 | RD-LA | 83.62 | 3.82 | 0.82 | 1.39 |
| | 3rd Harvest | Control | 96.30 | 3.18 | 0.93 | 1.35 | | 3rd Harvest | Control | 67.67 | 2.91 | 0.78 | 1.41 |
| | 09/24/2013 | RD-LM | 80.65 | 2.85 | 0.85 | 1.37 | | 09/18/2013 | RD-LA | 72.37 | 3.21 | 0.86 | 1.40 |
| | Harvest | Sig. | ns | ns | ns | ns | | Harvest | Sig. | ns | ns | ns | ns |
| | Application | Sig. | ns | ns | ns | ns | | Application | Sig | ns | ns | ns | ns |
| | Harv. * Applic. | Sig. | ns | ns | ns | ns | | Harv. * Applic. | Sig. | ns | ns | ns | ns |

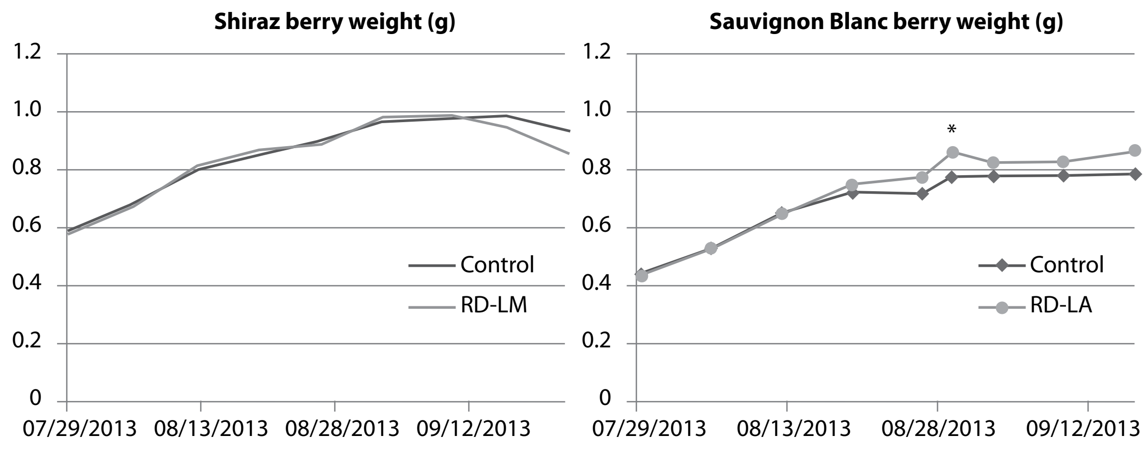



**Figure 1.** RD-LM treatment effect on Shiraz and RD-LA treatment effect on Sauvignon Blanc berry weight (g) from pre-veraison to the third harvest

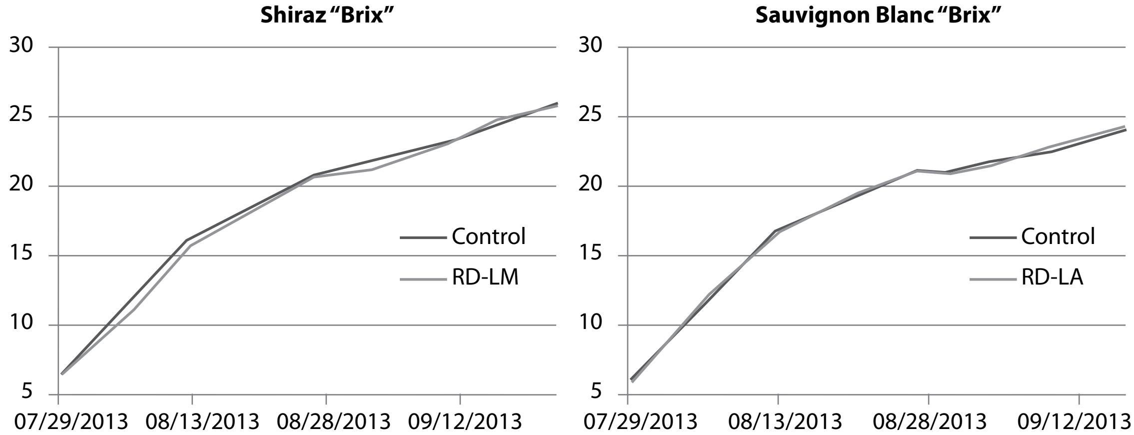



**Figure 2**. RD-LM treatment effect on Shiraz and RD-LA treatment effect on Sauvignon Blanc re °Brix from pre-veraison to the third harvest

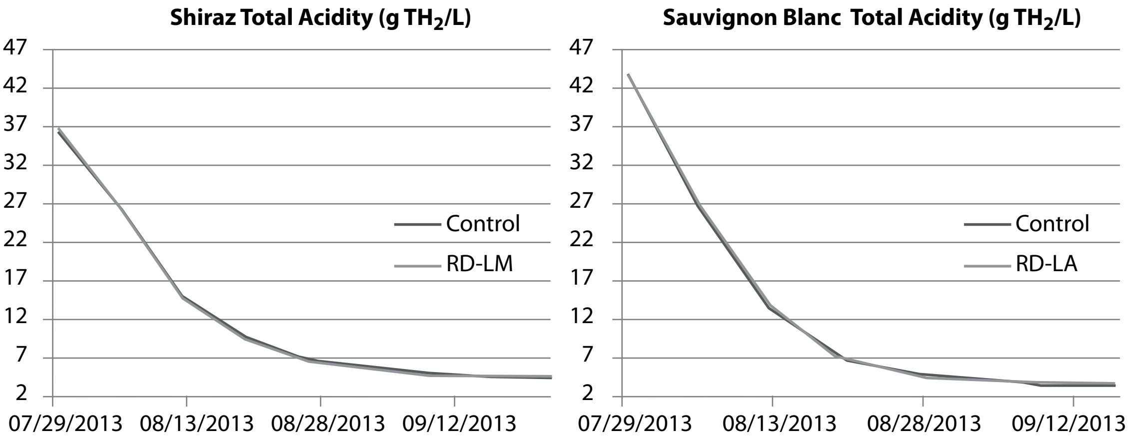



**Figure 3**. RD-LM treatment effect on Shiraz, and RD-LA and RD-LT treatment effect on Sauvignon Blanc re total ccidity (g $TH_2/L$) from pre-veraison to the third harvest

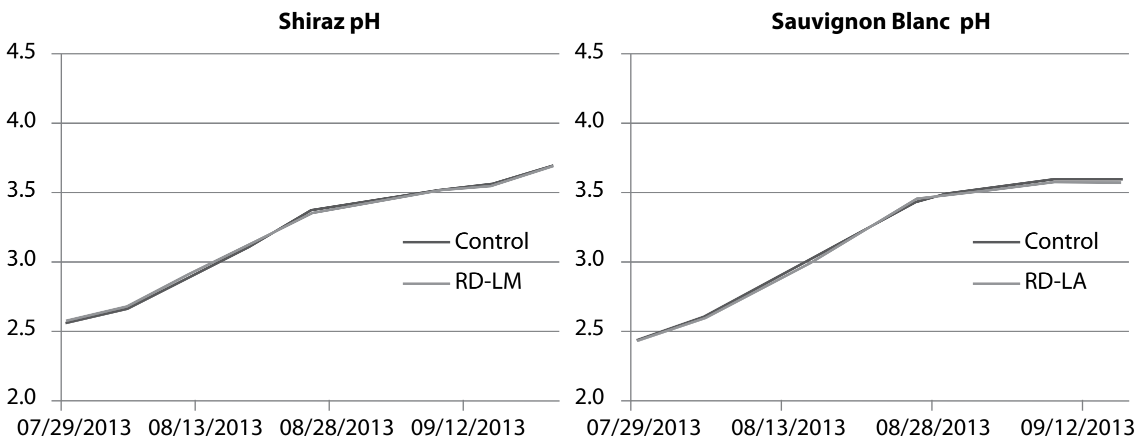



**Figure 4.** RD-LM treatment effect on Shiraz and RD-LA treatment effect on Sauvignon blanc re pH from pre-veraison to the third harvest

tent, expressed as °Brix, total acidity and pH. The control and yeast derivative treatments had similar values.

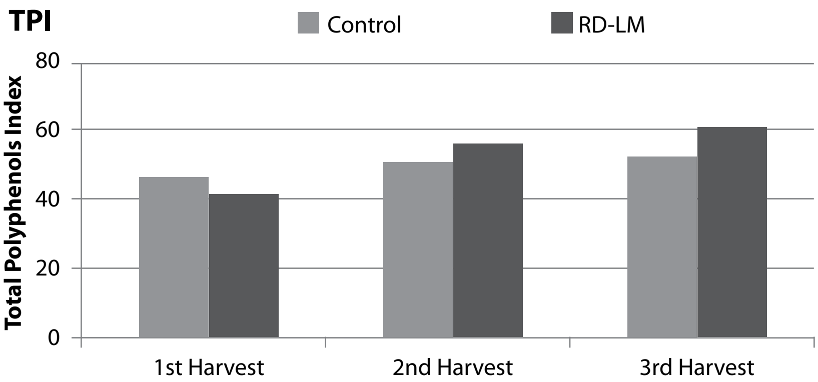
*e. Total Polyphenol Index*

The Index of Total Polyphenols theoretically represents the sum of the contribution of phenolic anthocyanins from the skins, the tannins from the skins and the tannins from the seeds. (See figure 5)

*f. Total Anthocyanins and Extractable Anthocyanins*

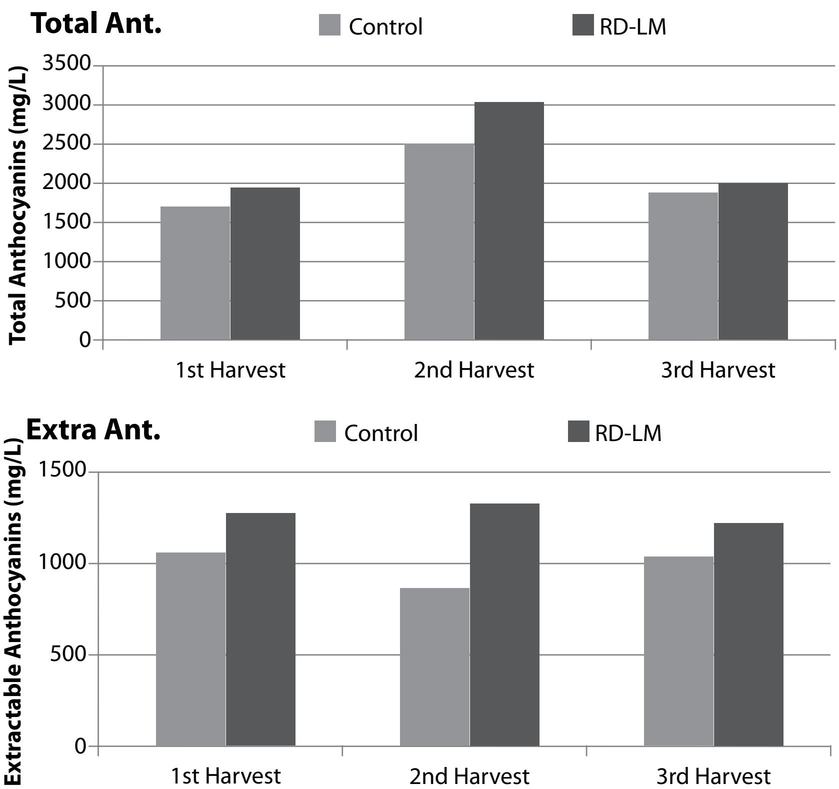
In figure 6, as expected, the anthocyanin content increases during ripening to a maximum at second harvest, then decreases due to "overripening" linked to a phenomenon of degradation and usually an aging phenomenon in the berry. As a consequence of this cellular aging, a substantial decrease in cohesion between the cells occurs, with degradation of cell walls and membranes. This increases the extractability of anthocyanins.

*g. Tannins*




| SHIRAZ | IPT |
|---|---|
| Harvest x Application | *** |
| Harvest | * |
| Application | ns |

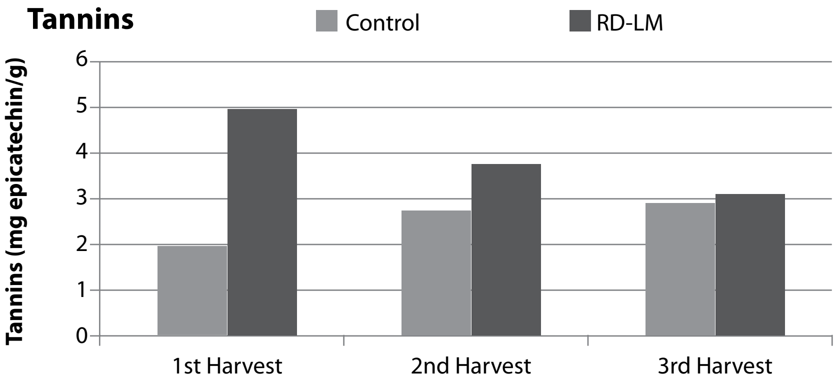
**Figure 5.** RD-LM treatment effect on Shiraz re Total Polyphenol Index (TPI) from pre-veraison to the third harvest. Statistical significance: *, **, ***, ns: significant differences for p≤0.05, 0.01, 0.001, or not significant, respectively.




| SHIRAZ | Ant TOTAL |
|---|---|
| Harvest x Application | *** |
| Harvest | * |
| Application | ns |

| SHIRAZ | Ant EXTRA |
|---|---|
| Harvest x Application | *** |
| Harvest | *** |
| Application | * |

**Figure 6.** RD-LM treatment effect on Shiraz re total anthocyanins and extractable anthocyanin concentration (mg malvidin/L) at the three harvests. Statistical significance: *, **, ***, ns: significant differences for p≤0.05, 0.01, 0.001, or not significant, respectively.




| SHIRAZ | Tannins |
|---|---|
| Harvest x Application | *** |
| Harvest | ns |
| Application | *** |

**Figure 7.** RD-LM treatment effect on Shiraz re tannin concentration (mg epicatechin/g) at the three harvests. Statistical significance: *, **, ***, ns: significant differences for $p \leq 0.05, 0.01, 0.001$, or not significant, respectively.

Tannins are synthesized during the first stage of berry growth or "herbaceous growth," and synthesis ends shortly after veraison.

When skin maturity was studied in Shiraz to ascertain the influence of yeast derivatives (LM) on phenolic maturity, it was found that the effect depended on harvest time and generally on interaction between harvest time and treatment with yeast derivatives. This was the case both for the total polyphenol index and for total and extractable anthocyanins. In the latter case, the treatment regardless of the harvest date was also shown to have a significant effect. Also noteworthy was that Shiraz tannin content increased significantly at all harvest times when yeast derivatives were applied.

We find this significant effect on Shiraz phenolic maturity when LM yeast derivatives are applied of particular note. Values were always higher on the last two harvest dates compared to the control, only lower on the first harvest date in the case of total polyphenols. The best and maximum values obtained with yeast derivatives products suggest that research needs to continue

### 2.6 Vinification

All repetitions were fermented at the three harvest times in both cultivars and all treatments studied, for a total of

**Table 7.** Actions and timing of winemaking process on Shiraz and Sauvignon Blanc at the three harvest times for each treatment. In both cases the yeast used for fermentation was EC-1118 (Lallemand Inc.). The enzyme added in Sauvignon Blanc musts was lalzyme Cmax (Lallemand Inc).

| Variety | Harvest | Action | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Harvest + Vatting + Inoculation | Nutrient Vit™ Addition | Reduless™ Addition | End of Fermentation | End of Maceration Devatting | End of Stabilization Homogenized + Bottled | Tasting |
| Shiraz | 1st Harvest | 03 Sep | 04 Sep | 09 Sep | 15 Sep | 17 Sep | 27 Sep | 10 Feb<br>09 May |
| | 2nd Harvest | 18 Sep | 19 Sep | 25 Sep | 03 Oct | 03 Oct | 14 Oct | 10 Feb<br>09 May |
| | 3rd Harvest | 24 Sep | 25 Sep | 03 Oct | 16 Oct | 18 Oct | 05 Nov | 10 Feb<br>09 May |

| Variety | Harvest | Action | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | Harvest + Vatting + Enzyme + Refrigeration | Clarification + Inoculation | Nutrient Vit™ Addition | Reduless™ Addition | End of Fermentation Devatting | End of Stabilization Homogenized + Bottled | Tasting |
| Sauvignon Blanc | 1st Harvest | 29 Aug | 01 Sep | 05 Sep | 09 Sep | 12 Sep | 23 Sep | 20 Jan<br>09 May |
| | 2nd Harvest | 10 Sep | 12 Sep | 19 Sep | 22 Sep | 24 Sep | 04 Oct | 20 Jan<br>09 May |
| | 3rd Harvest | 18 Sep | 20 Sep | 25 Sep | 03 Oct | 08 Oct | 18 Oct | 20 Jan<br>09 May |

**Table 8.** RD-LA treatment effect on Sauvignon Blanc and RD-LM treatment effect on Shiraz re triangle taste test. Level of significance 5%, 1% and 0.1% for a panel of 11 tasters (according to Roessler et al. 1948).

| Variety/ Product | Harvest | Level of signifiance | Harvest | Level of signifiance | Harvest | Level of signifiance |
|---|---|---|---|---|---|---|
| Sauvignon Blanc RD-LA | 1st Harvest | ns | 2nd Harvest | 5% | 3rd Harvest | 1% |
| | 1st Harvest | 1% | 2nd Harvest | 5% | 3rd Harvest | ns |
| | 1st Harvest | 5% | 2nd Harvest | 5% | 3rd Harvest | 5% |
| Shiraz RD-LM | 1st Harvest | 5% | 2nd Harvest | 1% | 3rd Harvest | 1% |
| | 1st Harvest | 5% | 2nd Harvest | 1% | 3rd Harvest | 1% |
| | 1st Harvest | 1% | 2nd Harvest | 1% | 3rd Harvest | 1% |

48 microvinifications. The table on page 74 shows the actions and dates relative to the winemaking process for the experimental vinifications in both cultivars.

### 2.7 Sensory Evaluations

**Triangle Test**

The Triangle Taste Test makes it possible to determine differences between two products with similar qualities. Tasters are offered three samples at random, two of which are from the same wine. Three repetitions of each treatment were tested.

For a panel of 11 tasters, the level of significance according to Roessler et al. (1948) for the triangle taste test is 5% if 7 out of 11 answers are correct, 1% if 8 out of 11 answers are correct and 0.1% if 10 out of 11 answers are correct. Below are the results:

Except in one of the comparisons of the first harvest and in another of the third harvest, the difference was significant between Sauvignon Blanc wines from vines treated with RD-LA versus those from untreated vines.

In the case of Shiraz, the wines from vines treated with RD-LM showed high levels of significance when compared to wines from untreated vines.

It is worth noting that the differences in many cases exceeded 70%, which adds substantial weight to the sensory analysis that revealed a general preference for wines from vines treated with yeast derivatives.

**Descriptive Test**

A sensory descriptive analysis was performed after it was determined that the wines from vines treated with RD-LM and RD-LA were significantly different from the wines from untreated vines.

Figures 9 and 10 (next page) present the results of the descriptive analysis.

SAUVIGNON BLANC

The particularly interesting sensory analysis of Sauvignon Blanc reveals that depending on the time of harvest, the wines are more aromatic and feature a more enjoyable mouthfeel when treated with yeast derivatives. In the case of Shiraz, significantly favourable mouthfeel and color with LM treatment always depended on the time of harvest.

## Conclusions

Applying LA in Sauvignon Blanc and LM in Shiraz improved the aromatic expression of Sauvignon Blanc wines and the phenolic maturity of Shiraz, with higher anthocyanin and tannin content and enhanced sensory impressions in mouthfeel.

Applying yeast derivatives between veraison and the start of ripening (LA and LT in Sauvignon Blanc and LM in Shiraz) did not affect the functionality of the vine, leaf activity, yield components, vegetative growth or technological maturity of the grapes.

These conclusions should be consolidated with results of future trials

## Acknowledgments

We would like to thank Lallemand Inc for its experimental and economical support.

We would also like to make special mention of CEIGRAM (Centro de Estudios e Investigación para la Gestión de Riesgos Agrarios y Medioambientales) for its help in project management.

Special thanks, too, to Grupo de Investigación en Viticultura de la UPM for its major contribution to this research.

We would also like to thank Finca Contancia for its full cooperation in the use of its facilities.

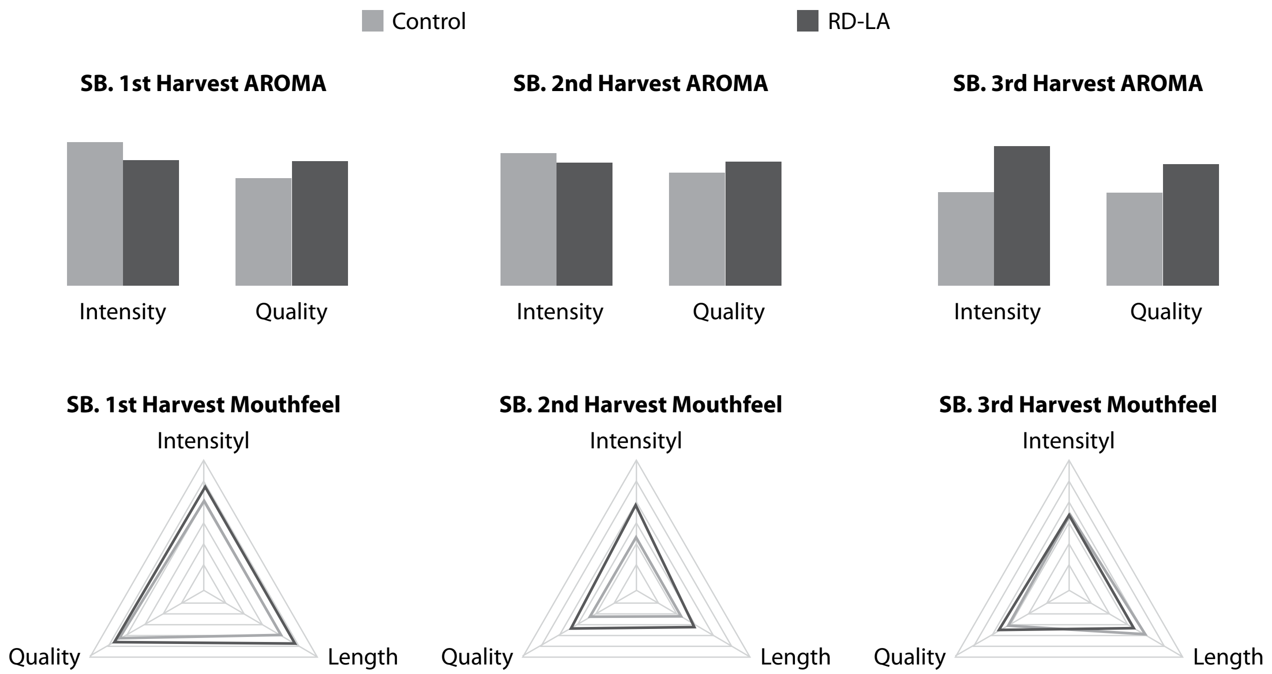



**Figure 8**. Tasting analysis examining the impact of RD-LA treatment on Sauvignon Blanc wines at the three different harvest times. Statistical significance: *, **, ***, ns: significant differences for p≤0.05, 0.01, 0.001, or not significant, respectively.

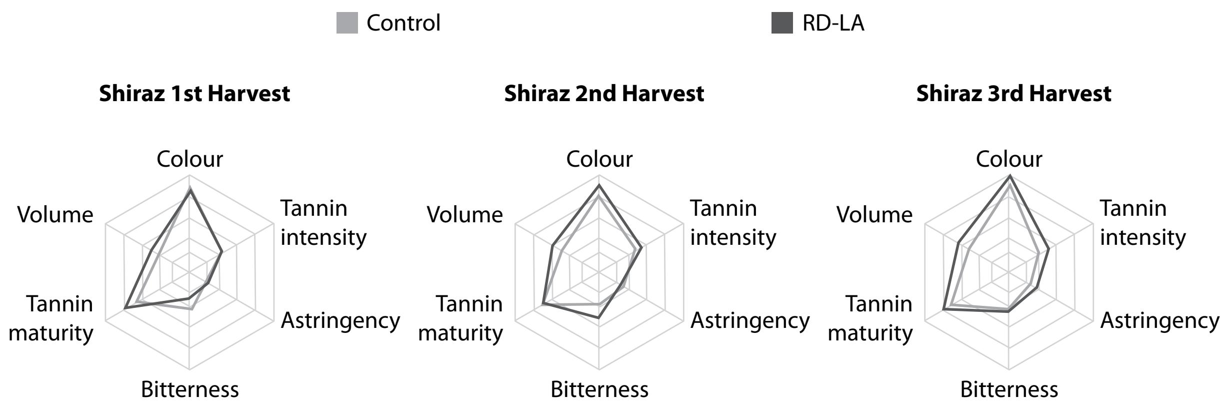



**Figure 9**. Palette evaluation examining the impact of RD-LM treatment on Shiraz wines at three different harvest times. Statistical significance: *, **, ***, ns: significant differences for p≤0.05, 0.01, 0.001, or not significant, respectively.

## References

Baumes, R., J. Wirth, S. Bureau, Y. Gunata, and A. Razungles. 2002. Biogeneration of C< sub> 13</sub>-norisoprenoid compounds: experiments supportive of an apo-carotenoid pathway in grapevines. *Analytica Chimica Acta*. 458(1):3-14.

Bell, S. J., and P. A. Henschke. 2005. Implications of nitrogen nutrition for grapes, fermentation and wine. *Australian Journal of Grape and Wine Research*. II: 242-295.

Bindon, K. A. 2004. *Influence of partial rootzone drying on aspects of grape and wine quality*. Dissertation. University of Adelaide, School of Wine and Agriculture.

Bindon, K. A., P. R. Dry, and B. R. Loveys. 2007. Influence of plant water status on the production of C13-norisoprenoid precursors in *Vitis vinifera* L. cv. Cabernet Sauvignon grape berries. *Journal of Agricultural and Food Chemistry*. 55(11):4493-4500.

Blancher, G., S. Chollet, R. Kesteloot, D. N. Hoang, G. Cuvelier, and J.-M. Sieffermann. 2007. French and Vietnamese: How do they describe texture characteristics of the same food? A case study with jellies. *Food Qual. Pref.*. 18:560-575.

Bogs, J., M. O. Downey, J. S. Harvey, A. R. Ashton, G. J. Tanner, and S. P. Robinson. 2005. Proanthocyanidin synthesis and expression of genes encoding leucoanthocyanidin reductase and anthocyanidin reductase in developing grape berries and grapevine leaves. *Plant Physiology*. 139(2):652-663.

Bonnisseau, M., and T. Dufourcq. 2004. Adaptation de la conduite du vignoble : la gestion du rapport feuilles/fruits. MONDIAVITI-Bordeaux 1º-2º Dic.: 139-149.

Boss, P. K.; J. Dunlevy, A. Cox, A. Tomas, E. Nicholson, L. Krake, and C. Davies. 2008. The pathways to a greater understanding of grape flavour development. *Proceedings 13th Australian Wine Industry Technical Conference*. R. Blair, P. Williams and S. Pretorius, eds. 47-51.

Bravdo, B., Y. Hepner, C. Loinger, and H. Tabacman. 1985. Effect of irrigation and crop level on growth, yield and wine quality of Cabernet Sauvignon. *Am. J. Enol. Vitic.*36(2):132-139.

Carbonneau, A., and P. Casteran. 1986. Optimization of vine performance by the lyre training sytems. *Proceedings of the 6th Australian Wine Industry Technical Conference*. 194-204.

Carbonneau, A. 1987. Stress modérés sur feuillages induits par le système de conduite et régulation photosynthétique de la vigne. *Proceedings of the III Symposium International de Physiologie de la Vigne*. 376-385.

Carbonneau, A., and P. Costanza. 2004. Response of vine leaf water potential to quick variation in canopy exposure. Example of canopy opening manipulation of Merlot (*Vitis vinifera* L.). *J. Int. Sci. Vigne Vin*. 38(1):27-33.

Cartier, R., A. Rytz, A. Lecomte, F. Poblete, J. Krystlik, E. Belin, and N. Martin. 2006. Sorting procedure as an alternative to quantitative descriptive analysis to obtain a product sensory map. *Food Qual. Pref*. 17:562-571.

de la Fuente, M., R. Linares, P. Baeza, and J. R. Lissarrague. 2007. Efecto del sistema de conducción en climas semiáridos sobre la maduración, composición de la baya y la exposición de los racimos en *Vitis vinifera* L. Cv. Syrah.*Acta del CONCLIVIT*. 1:132-138.

Downey, M. O., J. S. Harvey, and S. P. Robinson. 2003. Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. *Australian Journal of Grape and Wine Research*. 9(1):15-27.

Dühring, H. 1988. CO2 assimilation and photorespiration of grapevine leaves: responses to light and drought. *Vitis*. 27:199-208.

Esteban, M. A., M. J. Villanueva, and J. R. Lissarrague. 1999. Effect of irrigation on changes in berry composition of Tempranillo during maturation. Sugars, organic acids and mineral elements. *Am. J. Enol. Vitic.* 50(4):418-434.

Fernandez, R. T., R. L. Perry, and J. A. Flore. 1997. Drought response of young apple trees on three rootstocks. II. Gas exchange, chlorophyll fluorescence, water relations, and leaf abscisic acid. *Journal of the American Society for Horticultural Science*. 122(6):841-848.

Flexas, J., M. Badger, W. Soon Chow, H. Medrano, and C. B. Osmond. 1999. Analysis of the relative increase in photosynthesis O2 uptake when photosynthesis in grapevine leaves is inhibited following low night temperatures and/or water stress. *Plant Physiology*. 121(2):675-684.

Flexas, J., J. Bota, J. M. Escalona, B. Sampol, and H. Medrano. 2002. Effects of drought on photosynthesis in grapevines under field conditions: an evaluation of stomatal and mesophyll limitations. *Functional Plant Biology*. 29(4):461-471.

Fregoni, M. 1980. Nutrizione e fertilizzazione della vite. Edagricole. Bologna, Italy.

Garcia-Moruno, E., M. Ribaldone, and R. Stefano. 2000. Hydrolysis of terpene glycosides of grape skins in the preparation of base musts from aromatic red-berried grapes, *Rivista di Viticoltura e di Enologia*. 53(1):27-36.

Glories, Y., and M. Augustin. 1993. Maturité phénolique du raisin, conséquences technologiques : application aux millésimes 1991 et 1992. *Actas du Colloque Journée Techn. Bordeaux : CIVB*. 56-61).

Harbertson, J. F., J. A. Kennedy, and D. O. Adams. 2002. Tannin in skins and seeds of Cabernet Sauvignon, Syrah, and Pinot noir berries during ripening. *Am. J. Enol. Vitic.* 53(1):54-59.

Harborne, J. B. 1984. *Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis*. Chapman and Hall. London.

Holt, H. E., W. Birchmore, M. J. Herderich, and P. G. Iland. 2010. Berry phenolics in Cabernet Sauvignon (*Vitis vinifera* L.) during late-stage ripening. *Am. J. Enol. Vitic.* 61(3):285-299.

Hrazdina, G., G. F. Parsons, and L. R. Mattick. 1984. Physiological and biochemical events during development and maturation of grape berries. *Am. J. Enol. Vitic.* 35(4):220-227.

Huglin, P. 1986. *Biologie et écologie de la vigne*. Ed. Payot Lausanne. Paris. 371.

Iland, P., N. Bruer, G. Edwards, S. Weeks, and E. Wilkes. 2004. *Chemical Analysis of Grapes and Wine: Techniques and Concepts*. Patrick Iland Wine Promotions. Campbelltown, Australia. 32-58.

Iland, P., P. Dry, T. Proffitt, and S. Tyerman. 2004. *The Grapevine from the Science to the Practice of Growing Vines for Wine*. Patrick Iland Wine Promotions. Australia.

Jackson, D. I., and P. B. Lombard. 1993. Environmental and management practices affecting grape composition and wine quality - A review. *Am. J. Enol. Vitic.* 44(4):409-430.

Jackson, R. S. 2008. *Wine Science: Principles and Applications*. Academic Press.

Keller, M., and G. Hrazdina. 1998. Interaction of nitrogen availability during bloom and light intensity during veraison. II. Effects on anthocyanin and phenolic development during grape ripening. *Am. J. Enol. Vitic.* 49(3):341-349.

Kriedemann, P. E., and R. E. Smart. 1971. Effects of irradiance, temperature and leaf water potential on photosynthesis of vine leaves. *Photosynthetica*. 5(1):6-15.

Marais, J., C. van Wyk, and A. Rapp. 1991. Carotenoid levels in maturing grapes as affected by climatic regions, sunlight and shade. *S. Afr. J. Enol. Vitic*. 12(2):64-69.

Maroco, J. P., M. L. Rodrigues, C. Lopes, and M. M. Chaves. (2002). "Limitations to leaf photosynthesis in field-grown grapevine under drought – metabolic and modelling approaches. *Functional Plant Biology*. 29(4):451-459.

Medrano, H., J. Bota, A. Abadía, B. Sampol, J. M. Escalona, and J. Flexas. 2002. Effects of drought on light-energy dissipation mechanisms in high-light-acclimated, field-grown grapevines. *Functional Plant Biology*. 29:1197-1207.

Mercurio, M. D., and P. A. Smith. 2008. Tannin quantification in red grapes and wine: comparison of polysaccharide- and protein-based tannin precipitation techniques and their ability to model wine astringency. *Journal of Agricultural and Food Chemistry*. 56(14):5528-5537.

Naor, A., B. Bravdo, and J. Gelobter. 1994. Gas Exchange and Water Relations in Field-Grown Sauvignon Blanc Grapevines. *Am. J. Enol. Vitic*. 45(4):423-428.

Razungles, A., Z. Gunata, S. Pinatel, R. Baumes, and C. Bayonove. 1993. Étude quantitative de composés terpéniques, norisoprénoïdes et de leurs précurseurs dans diverses variétés de raisins. *Sciences des aliments*. 13(1):59-72.

Razungles, A. J., I. Babic, J. C. Sapis, and C. L. Bayonove. 1996. Particular behavior of epoxy xanthophylls during veraison and maturation of grape. *Journal of Agricultural and Food Chemistry*. 44(12):3821-3825.

Reyero, R., J. Garijo, E. Díaz Plaza, H. Cuartero, M. R. Salinas, and F. Pard. 2000. Comparison of aroma composition of six monovarietal red wines. *Alimentación, Equipos y Tecnología*. 19:101-110.

Reynolds, A., ed. 2010. *Managing Wine Quality: Viticulture and Wine Quality* (Vol. 1). Elsevier.

Reynolds, A. G., and D. A. Wardle. 1997. Flavour development in the vineyard: impact of viticultural practices on grape monoterpenes and their relationship to wine sensory response. *S. Afr. J. Enol. Vitic*. 18:3-18.

Roessler, E. B., J. Warren, and J. F. Guymon. 1948. Significance in triangular test. *Food Res*. 13:503.

Roubelakis-Angelakis, K. A., ed. 2009. *Grapevine molecular physiology & biotechnology*. Springer.

Ryona, I., B. S. Pan, D. S. Intrigliolo, A. N. Lakso, and G. L. Sacks. 2008. Effects of cluster light exposure on 3-isobutyl-2-methoxypyrazine accumulation and degradation patterns in red wine grapes (*Vitis vinifera* L. Cv. Cabernet Franc). *Journal of Agricultural and Food Chemistry*. 56(22):10838-10846.

Sampaio, T. L., J. A. Kennedy, and M. C. Vasconcelos. 2007. Use of microscale fermentations in grape and wine research. *Am. J. Enol. Vitic.* 58(4):534-539.

Sánchez-de-Miguel, P. 2007. *Producción y distribución de fotoasimilados en la vid (*Vitis vinifera *L.) durante el período de maduración. Cambios en la respuesta fotosintética a la luz de las hojas por factores biológicos, ambientales y culturales*. Dissertation. Departamento de Producción Vegetal: Fitotecnia. ETSI. Agrónomos.Universidad Politécnica de Madrid.

Sarneckis, C. J., R. G. Dambergs, P. Jones, M. Mercurio, M. J. Herderichy, and P. A. Smith. 2006. Quantification of condensed tannins by precipitation with methyl cellulose: development and validation of an optimised tool for grape and wine analysis. *Aust. J. Grape Wine Res*.12:39-49

Seguin, G. 1975. Alimentation en eau de la vigne et composition chimique des moûts dans les Grands Crus du Médoc. Phénomènes de régulation. *Connaissance Vigne Vin*. 9:23-34.

Smart, R. E., and B. G. Coombe. 1983. Water relations of grapevines. In Water deficits and plant growth. Vol. II.

Additional Woody Crop Plants. T. T. Kozlowski, ed. Academic Press. New York. 137-196.

Somers, T. C. 1976. Pigment development during ripening of the grape. *Vitis*. 14:269-277.

Vallone, R. J. Pérez Peña, L. Nijensohn, and J. B. Cavagnaro. 1997. Efectos del estrés hídrico sobre el crecimiento vegetativo, reproductivo y calidad enológica en vid cv. Sangiovesse. *Proceedings of the XXII Congresso Mundial de la Vid y Vino*. Buenos Aires.

Vitalini, S., C. Gardana, A. Zanzotto, G. Fico, P. Simonetti, and M. Iriti. 2011. From vineyard to glass: agrochemicals enhance the melatonin and total polyphenol contents and antiradical activity of red wines. *J. Pineal Res*. 51:278-285.

Williams, L. E. 1996. *Grape*. In Photoassimilate Distribution in Plants and Crops: Source-Sink Relationships. Marcel Dekker.

Williams, L. E., and D. W. Grimes. 1987. Modelling vine growth development of a data set for a water balance subroutine. *Proceedings of the VI Australian Wine Industry Technical Conference*. Adelaide, South Australia. 169-174

Williams, L. E., and M. A. Matthews. 1990. *Grapevine*. Irrigation of Agricultural Crops. Agronomy monograph. 30:1019-1055.

Williams, L. E., and P. J. Biscay. 1991. Partitioning of dry weight, nitrogen, and potassium in Cabernet Sauvignon grapevines from anthesis until harvest. *Am. J. Enol. Vitic.* 42(2):113-117.

Yuste, J. 1995. *Comportamiento fisiológico y agronómico de la vid (*Vitis vinifera *L.) en diferentes sistemas de conducción en secano y regadío.* Dissertation. Departamento de Producción Vegetal: Fitotecnia. ETSI. Agrónomos. Universidad Politécnica de Madrid. 280.

Original Cover Design: Bruno Loste – Layout and Printing: MODULI INC.

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