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Ultraviolet Radiation as a Regulator of Grapevine Leaf Physiology and Berry and Wine Quality
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DEPARTAMENTO DE AGRICULTURA Y
ALIMENTACIÓN

Ultraviolet radiation as a regulator of grapevine leaf physiology and berry and wine quality

Memoria presentada por:

MARÍA ÁNGELES DEL CASTILLO ALONSO

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Fdo. MARÍA ÁNGELES DEL CASTILLO ALONSO

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LOS DOCTORES JAVIER MARTÍNEZ ABAIGAR Y ENCARNACIÓN NÚÑEZ OLIVERA, CATEDRÁTICOS DE BOTÁNICA Y DE FISIOLOGÍA VEGETAL DE LA UNIVERSIDAD DE LA RIOJA,

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Que la Memoria titulada "**Ultraviolet radiation as a regulator of grapevine leaf physiology and berry and wine quality**" ha sido realizada en las Áreas de Fisiología Vegetal y Botánica de la Universidad de La Rioja bajo nuestra dirección por MARÍA ÁNGELES DEL CASTILLO ALONSO, Licenciada en Ciencias Ambientales. Considerando que se encuentra concluida, autorizamos su presentación para ser juzgada por el tribunal correspondiente.

Y para que conste, expedimos el presente informe en Logroño, a 15 de Julio de 2019.

Fdo. Javier Martínez Abaigar

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Ultraviolet radiation as a regulator of grapevine leaf physiology and berry and wine quality

Mª Ángeles del Castillo Alonso

2019

Dedicado a Emma,

sin ti no soy nada, pero hubiera

acabado esta tesis un año antes.

Gracias por ocupar aún más mi tiempo

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Chapter 1. Introduction

INTRODUCTION

The grapevine plant and its economic impact

Grapevine (*Vitis vinifera* L.) is a sufruticose plant with a woody lower part and herbaceous annual branches, the vine shoots. Leaves are deciduous and have a palmate venation and mostly lobate and dentate margins. The shoots become woody as season progresses, being finally eliminated by pruning except some basal buds that will regenerate shoots in the following season. Typically, some shoots are transformed in tendrils, which allow the plant to climb up. The shoots produce bunches of small flowers that after pollination become berries with skin, flesh and usually 2-4 seeds per berry.

Grapevine cultivation probably began in Asia 7,000 years ago and was further extended firstly to the circum-Mediterranean areas and then to other Mediterranean-climate zones throughout the world. Grapevine has represented an important cultural symbol since ancient times, both in pagan and religious traditions. Other species of the genus *Vitis*, predominantly from the northern hemisphere, are mainly used as rootstocks because of their tolerance to phylloxere.

Grapevine is one of the most important crops throughout the world. This agricultural and economic sector includes table grapes, dried fruits, and the elaboration of, mainly, wine, but also juice, spirits and even non-alcoholic wines. The sector is economically crucial for many countries. Some data: the surface occupied by grapevine in the world is around 75 000 square kilometers. Half of this surface is located in Europe, mainly in Spain (13%), France (10%) and Italy (10%), and the other half in the rest of the world, mainly in China (8%), Turkey (6%) and the United States (5%). Worldwide wine production in good years may reach almost 30 billion liters, around 4 liters per person in the world, with rather the same countries as the main producers (Italy 16%, Spain 15%, France 15%, the United States 8%, Argentina 4%, Chile 4%, Australia 4%, China 4%, South Africa 4%).

The economic impact of the grape and wine sector on the world economy has been estimated at around 60 billion €. In the European Union, wine accounts for 3.5% of global trade in agricultural food products. And in the three biggest producers in the world (Italy, France and Spain), wine represents between 0.5 and 0.75% of their Gross Domestic Product (GDP). In La Rioja, this reaches 25%, with around 600 cellars for 350.000 inhabitants. Although there are differences from year to year, the biggest wine consumers in the world are the United States 12%, France 12%, Italy 9%, Germany 9%, China 7%, the United Kingdom 5%, Argentina 4%, Spain 4% and Australia 2% (Anderson *et al*., 2013).

Ultraviolet radiation and its effects on photosynthetic organisms

Ultraviolet (UV) radiation is a minority component (about 8-9%) of the total solar radiation reaching the Earth surface, and represents a natural environmental factor that has been involved in the appearance of diverse adaptive changes in organisms through the development of life (Cockell & Knowland, 1999). The CIE (Commission Internationale d'Eclairage) divided UV in three wavelength categories: UV-A (315-400 nm), the most abundant UV component and the less hazardous one; UV-B (280-315 nm), that provokes diverse biochemical and physiological responses, including some nocive effects; and UV-C (<280 nm) which is extremely harmful but absent at ground level due to stratospheric oxygen, ozone and other atmospheric gases absorption (Hollósy, 2002). The first two categories, UV-A and UV-B, play also a regulatory role on the physiology of plants (Hideg *et al*., 2013). Due to the anthropogenic reduction of stratospheric ozone, that causes an increase in UV-B in the biosphere, the study about UV effects on photosynthetic organisms has received particular attention in the last decades. Wavelength affects deeply these effects, so different biological weighting functions have been conceived to calculate the biologically effective UV (UV_{BE}). Although UV_{BE} includes UV-A and UV-B, UV-B dominates UV_{BE} because of the logarithmic increase in effectiveness with decreasing wavelength (Caldwell, 1971). Nevertheless, recent biological weighting functions pay especial attention to UV-A to develop biological weighting functions (Flint & Caldwell, 2003).

UV irradiance that reaches Earth surface is affected by several factors, such as latitude, season, hour of the day, altitude, presence of clouds or aerosols, surface reflectivity, and ozone levels (McKenzie *et al*., 2007). The anthropogenic emissions of halogenated carbon compounds have led to an ozone loss. This fact has been observed in Arctic and mid-latitudes but it is most dramatic in the Antarctic continent. An increase in solar UV-B has been estimated at 6-12% in mid-latitudes over the 1980 levels (McKenzie *et al*., 2003). The ozone layer is recovering in the last years, but due to other phenomena associated to climate change, as variations in cloud cover, models predict an increase of UV radiation at ground level, although with notable uncertainty (Bais *et al*., 2015).

Traditionally, UV radiation has been considered as a stressing factor for the photosynthetic organisms. An excess of UV may cause diverse damage in the photosynthetic apparatus, such as pigment degradation, photoinhibition, and decreases in quantum yield, photosynthetic rates, and the activity of the Calvin cycle enzymes (Jansen *et al*., 1998; Hollósy, 2002). Also, it can generate DNA alterations, oxidative damage, and changes in mineral absorption. At the ecosystem level, UV can affect litter decomposition, nutrient cycling, trophic interactions, and the competitive balance between species (Bornman *et al*., 2015). Nevertheless, in last years it has been suggested that UV radiation is also an environmental regulator controlling gene expression to activate protective and repair mechanisms, mediated by the UV-B specific photoreceptor ULTRAVIOLET RESISTANCE LOCUS 8 (UVR8) (Jenkins, 2009; Hideg *et al*., 2013; Morales *et al*., 2013). In that way, photosynthetic organisms have developed a number of mechanisms directed to minimize the penetration of UV, prevent oxidative stress and repair the damage caused (Jansen *et al*., 1998): accumulation of UV-absorbing compounds (UVACs: flavonoids, phenyl-propanoids, mycosporine-like aminoacids, etc.), antioxidant and photoprotective mechanisms, and repairing or turnover of damaged biomolecules such as DNA and proteins. The effects of UV on photosynthetic organisms have been studied mainly in

terrestrial plants, especially of agricultural interest, and in marine phytoplankton and macroalgae (Wargent & Jordan, 2013; Bornman *et al*., 2015; Häder *et al*., 2015).

Grapevine and UV radiation

Manipulation of UV radiation

To our knowledge, around 143 papers containing original data have been published on this topic (Table 1.1). Until recent times, both the quantity and quality of grapes, and consequently of wines, have mainly been driven by the meteorological conditions of temperature, solar radiation and water availability experienced by the plants in the field (Nicholas *et al*., 2011; Cuneo *et al*., 2013). In fact, climate is one of the components of the so-called "terroir", together with soil, geography and variety (Van Leeuwen *et al*., 2004). Seasonal variability in the prevailing climate is also important in determining year-on-year variation in the yield, quality and value of berries, reflected in the concept of wine vintages of varying quality (Kemp *et al*., 2011; Verzera *et al*., 2016). Nowadays, new technological tools allow to modulate this meteorological dependence, and manipulation of UV radiation is one of these tools (Jordan, 2017).

Potentially, UV-C, UV-B and UV-A wavelengths can be manipulated. UV-C has extensively been used in postharvest table grapes to enhance the production of stilbenes, a type of phenolic compounds (Wang *et al*., 2010; Kiselev *et al*., 2017; Ma *et al*., 2019). The most famous stilbene is resveratrol, which is considered a nutraceutical because of its apparent multiple healthy properties as antioxidant, anticarcinogenic, antiinflammatory, cardioprotective, and neuroprotective, although there are not solid evidences about it in humans (González-Barrio *et al*., 2009). This use of UV-C has also been demonstrated in juice and wine grape varieties, which could serve to produce stilbene-enriched grape juices and wines. The increase in stilbenes could also improve plant immunity and defense mechanisms against, mainly, fungal diseases, consequently reducing the use of biocides (Hasan and Bae, 2017).

UV-A manipulation has been much less studied, despite that some pests need UV-A for a correct behaviour, and some parasitic fungi need it for spore production. Thus, UV-A could be eliminated to prevent the normal development of these pests and diseases. However, some pollinator insects, and probably the plant itself, need UV-A for a normal function (Verdaguer *et al*., 2017). For example, some phenolics are induced by UV-A (Kolb *et al*., 2003; Fernandes de Oliveira *et al*., 2015). Hence, total elimination of UV-A may not be positive. On the other hand, many studies do not discriminate the effects of UV-A and UV-B, and some UV-B lamps emit a certain amount of UV-A. Thus, we need more studies on the specific effects of each wavelength. Given that UV-B is the UV fraction that has been mostly studied, it Will be the focus of the remaining introductory text.

Organs and metabolites studied

Regarding the organs studied, UV-B influences leaf physiology at very different scales, from the molecules to the field (Table 1.1). The clearest responses are the accumulation of certain phenolic compounds, mainly flavonols, and the enhancement of antioxidant and photoprotection systems (Kolb *et al*., 2001; Csepregi and Hideg, 2017). In addition, genes involved in the polyphenol formation pathways are upregulated by UV-B (Carbonell-Bejerano *et al*., 2014). Some negative responses have been also described, such as oxidative damage, chlorophyll degradation, and a decrease in photosynthesis rates and growth (Choudhary *et al*., 2014; Zhu *et al*., 2015). UV-B can also affect morphology, with a reduction in leaf area and an increase in leaf thickness (Robson *et al*., 2015). In general, negative effects are modest and lie in the concept of eustress (Wargent *et al*., 2013), so that plants are aware of being exposed to UV-B through a specific photoreceptor (UVR8) and respond adjusting their metabolism in diverse ways, being well adapted to UV-B (Núñez-Olivera *et al*., 2006).

Studies on berries are more frequent than those on leaves (85 against 55, approximately, Table 1.1), and they are inevitably necessary to have a commercial approach to the subject, because

UV-B effects on berries are more critical for wine quality (Blancquaert *et al*., 2018). The berry is not a homogeneous organ, and it can be divided in at least three parts: the skin, the flesh and the seeds. Most studies have been carried out on skins, but the responses of flesh and seeds could be different. Not only the different phenolic compounds can be located in different parts of the berry, but also in different cell compartments: cell walls, nuclei, vacuoles, etc. (Teixeira *et al*., 2013). This can be related to the diverse functions of phenolic compounds in the plant: antioxidants, UV screens, signaling molecules, herbivore deterrents, etc., and can be relevant for wine characteristics, because the different location of phenolic compounds may influence their extractability in the enological process (Blancquaert *et al*., 2018). Thus, it would be desirable to analytically differentiate the cellular location of phenolic compounds.

As occurs in leaves, UV-B radiation determines the accumulation of different secondary metabolites in berries. The most studied metabolites have been phenolic compounds and volatile organic compounds (VOCS), that are crucial for wine quality because they determine aroma, astringency, colour and stability (Gil *et al*., 2013; Feng *et al*., 2015; Liu *et al*., 2018). Thus, a controlled UV-B supplement can noticeably modify the quality of berries and wine.

Regarding phenolic compounds, the first thing to remark is that their synthesis pathway is very complex, in line with their high diversity (Jug and Rusjan, 2012). The effect of UV-B depends on the type of compound. Phenolic acids (that is hydroxycinnamic and hydroxybenzoic acids and their derivatives) are simple phenols rarely affected by UV-B (Berli *et al*., 2011; Del-Castillo-Alonso *et al*., 2015). Stilbenes, the most famous of which is resveratrol, are physiologically phytoalexins typically responding to wounding or pathogens. The remaining phenolic compounds are flavonoids and all of them have the flavan nucleus. Flavanols are little responsive to changes in environmental factors, including radiation (Cortell and Kennedy, 2006; Koyama *et al*., 2012). Flavonols are probably the most interesting phenolic compounds regarding UV-B since flavonol accumulation may be the most reliable response of grape skins to increasing UV-B radiation (Jordan, 2017). This occurs with the diverse types of flavonols, which differ in the

hydroxylation level: myricetins are trihydroxylated, quercetins are dihydroxylated, and kaempferols, isorhamnetins and syringetins are monohydroxylated. In addition, flavonols can be glycosylated in different ways. The last phenolic family, anthocyanidins, can also be diversely substituted, and also glycosylated to form anthocyanins. Anthocyanins usually increase under high photosynthetic radiation levels in combination with low temperatures, but the specific effect of UV-B is quite variable, because they may increase, decrease or remain unaltered (Mori *et al*., 2007; Carbonell-Bejerano *et al*., 2014).

As mentioned, the phenolic synthesis pathway is complex, with many genes, transcription factors and enzymes involved. UV-B upregulates a number of components of this molecular machinery (Pastore *et al*., 2013; Carbonell-Bejerano *et al*., 2014; Liu *et al*., 2015). In addition, the accumulation of different phenolic compounds is mediated, at least to a certain extent, by the specific UV-B receptor UVR8 (Jordan, 2017). These findings diversify the possibilities for influencing the metabolite profile of berries through molecular responses, and thus for implementing adequate management practices.

High UV-B seems to increase VOCs content, but data are much more scarce than those available for phenolic compounds (Matus, 2016).

Effects of UV-B on sugar content of berries (and thus alcoholic degree of wines) and volume and weight of berries are not conclusive (Berli *et al*., 2011; Carbonell-Bejerano *et al*., 2014; Song *et al*., 2015). Clearly, more studies are needed on all these variables. Studies on the influence of UV-B on other grapevine organs, such as flowers, flesh and seeds, are merely testimonial.

The effect of variety

It is also important to note that the variety used determines the responses to UV-B. Many different varieties have been used among the 1,271 cataloged in the world, specifically half of the 30 major varieties: Cabernet Sauvignon (17), Merlot (2), Tempranillo (12), Chardonnay (5), Syrah (4), Grenache (7), Sauvignon Blanc (8), Pinot Noir (14), Sangiovese (13), Monastrell (14),

Riesling (18), Macabeo or Viura (20), Cot or Malbec (21), Gamay (30). Both red and white varieties have been studied (Table 1.1).

Interactions of UV-B with other environmental factors

Under field conditions, UV-B interacts with other environmental factors that modulate UV-B effects: other wavelengths, such as PAR, UV-A, blue or red, that may have other photoreceptors; temperature, that influences the levels of certain secondary metabolites and, particularly, water availability (Downey *et al*., 2004; Azuma *et al.,* 2012; Verdaguer *et al.,* 2017). Drought is frequently associated with high UV-B levels, high PAR and high temperature. There are some common physiological responses to these factors and, for example, UV-B may enhance resistance to drought and vice versa (Niculcea *et al*., 2015). Hydrogen peroxide, nitric oxide, abscisic acid, jasmonic acid, ethylene, and salicylic acid participate in the activation of defense mechanisms against both factors: enzymatic and non-enzymatic antioxidant systems, flavonoid synthesis, accumulation of osmolytes and regulation of stomatal closure (Agati and Tattini *et al.,* 2010; Alonso *et al*., 2016). This kind of interactions should be taken into account, particularly in the context of climate change, because there are many uncertainties about how the areas suitable for growing wine grapes will change in the future, both in latitude and altitude.

Technical approaches for the manipulation and management of UV-B

Different technical approaches can be applied for the manipulation and management of UV-B (Table 1.1):

1) Using filters to eliminate ambient UV-B. This method is useful to understand the specific effects of ambient UV-B levels on leaves or berries and can easily be implemented under field conditions. Filters may affect the microclimatic environment of the plants, and thus it is convenient to establish some kind of control plants out of filters. This method would be useful if natural UV-B is in excess,

and is indispensable to understand the effects of ambient UV-B and, consequently, to derive potential applications.

2) Using lamps to artificially supplement UV-B. This method is more complex technically and more expensive, but allows to explore both basic aspects (such as the UV-B tolerance of the plants, the UV-B-induced physiological and molecular responses, the functionality of UV-B photoreceptors) and applied aspects, particularly the improvement of berry and wine quality through the induction of important metabolites. This method can also mimic the conditions of stratospheric ozone degradation.

3) Using a combination of filters and lamps, to achieve the objectives of the first two approaches.

4) Taking advantage of natural altitudinal or latitudinal UV-B gradients. This is a non-manipulative and technically easy approach, but the effects of ambient UV-B levels are mixed with those of other environmental factors, such as photosynthetic radiation, UV-A, temperature or water availability. Consequently, the responses of the plants cannot unequivocally be attributed to UV-B, as occurs in other studies that analyze the effects of the full solar radiation spectrum through leaf removal or imposing shading or total darkness to the plants. Nevertheless, results can be reasonably reliable if the plant responses are thoroughly correlated with the different environmental factors involved. For applied purposes, this method would require to cultivate plants at different altitudes or latitudes, which limits its efficiency out of basic research.

5) Using postharvest UV-B applications, as it has been successfully done with UV-C. This method has rarely been applied regarding UV-B because, for example, UV-B is less efficient than UV-C for resveratrol induction, but could be useful to enrich grapes in other metabolites specifically induced by UV-B.

It should be mentioned that the research on grapevine and UV-B has frequently been carried out on high-altitude vineyards (Alonso *et al.,* 2016; Marfil *et al.,* 2019) or more or less controlled conditions, for example growing plants in pots and/or in greenhouses (Schoedl *et al*., 2013; Grifoni *et al*., 2016), or using special cuttings (Martínez-Lüscher *et al.,* 2014a; 2014b; 2016). These studies, although valuable, may not totally replicate the typical commercial conditions, which is the type of knowledge preferentially needed to derive applications.

Table 1.1. Original papers and reviews about the effects of UV radiation on grapevines. Key for "Variety": Mr, *Muscadinia rotundifolia*, Vv, *Vitis vinifera*; Vl, *Vitis lambrusca*; Vr, *Vitis rupestris*; Vvs, *Vitis vinifera* ssp. *sylvestris*, Vs, *Vitis sylvestris*; Vq, *Vitis quinquangularis*; Va, *Vitis amurensis*, Vri, *Vitis riparia*; Vb, *Vitis berlandieri*. Key for "Organ studied": L, leaves; B, berries; F, flowers; ?, undetermined; W, wine; J: juice; C, cells. Key for "Type of experiment": F, field; Gh, greenhouse; L, laboratory. Key for "Manipulation of UV": E, exclusion; S, supplement (UV-A, UV-B or UV-C); N, natural. Key for "Duration of experiment": S, short duration (< 15 days); M, medium duration (16-60 days); L, long duration (> 60 days). Key for "Variables used": A, alterations in DNA; C, cover; Scl, sclerophylly; Pho, photosynthesis; Fl, chlorophyll fluorescence; Gn, genetic responses; H, hydric relations; Hr, hormones; M, morphology; Mt1, primary metabolites (glucids, proteins, lipids…); Mt2, secondary metabolites, including UV-absorbing compounds; N, mineral nutrients; Ox, variables of oxidative stress (peroxide content, lipid peroxidation, ascorbate, superoxide dismutase, peroxidase, catalase); AxC, antioxidant capacity; P, photosynthetic pigments; R, respiration; Rf, reflectance indices; Pr: proteins; U, ultrastructure; Z, other variables.

Reference	Variety	Organ studied	Type of experiment	Manipulation of UV	Duration of experiment	Variables used
Abyari *et al.* (2006)	Siahe Sardasht (Vv)	B	L	S (UV)	S	Mt2
Adrian *et al.* (2000)	Pinot noir (Vv), Gamay (Vv)	B	L	S (UV-C)	S	Mt2
Alonso *et al.* (2015)	Malbec (Vv)	L	F	E (UV-B)	L	Pho; Mt2
Alonso *et al.* (2016)	Malbec (Vv)	B	F	E (UV-B)	L	Mt2; Ox
Azuma *et al.* (2012)	Pione (Vv x Vl)	B	L	S (UV)	S	Mt2
Bai *et al.* (2019)	Concord (Vl), Cabernet sauvignon (Vv)	L	L	S (UV-C)	S	Sti; Gn
Becker *et al.* (2017)	(Vv x Mr)	L	L	S (UV-C)	S	Sti; U
Berli *et al.* (2008)	Malbec (Vv)	B	F	E (UV-B)	L	Mt2; Ox
Berli *et al.* (2010)	Malbec (Vv)	L	F	E (UV-B); S (UV-B)	L	Mt2; Ox; P

Reference	Variety	Organ studied	Type of experiment	Manipulation of UV	Duration of experiment	Variables used
Berli *et al.* (2011)	Malbec (Vv)	B	F	E (UV-B)	L	C; Mt1; Mt2
Berli *et al.* (2013)	Malbec (Vv)	L	F	E (UV-B)	L	C; Pho; F;, Mt2; Ox; P
Berli *et al.* (2015)	Malbec (Vv)	B	F	E (UV-B)	L	Mt2
Blancquaert *et al.* (2018)	(Vv)	B; W	F	UV	L	Mt2
Bonomelli *et al.* (2004)	Chardonnay (Vv), RupStiris du lot (Vr)	L	L	S (UV-C)	S	Mt1; Mt2; Gn
Cantos *et al.* (2000)	Napoleon (Vv)	B	L	S (UV-C, UV-B)	S	Mt2
Cantos *et al.* (2001)	Napoleon (Vv)	W	L	S (UV-C)	S	Mt2
Cantos *et al.* (2002)	Flame (Vv), Red globe (Vv), Crimson (Vv), Napoleon (Vv), Superior (Vv), Dominga (Vv), Moscatel italica (Vv)	W	L	S (UV-C)	S	Mt2
Cantos *et al.* (2003a)	Monastrell (Vv)	W	L	S (UV-C)	S	Mt2
Cantos *et al.* (2003b)	Tempranillo (Vv), Cabernet sauvignon (Vv), Merlot (Vv), Syrah (Vv), Monastrell (Vv), Garnacha (Vv), Cariñena (Vv)	W	L	S (UV-C)	S	Mt2
Carbonell-Bejerano *et al.* (2014)	Tempranillo (Vv)	B	F	E (UV)	L	Mt2; Gn
Carini *et al.* (1998)	(Vv)	B	L	S (UV-B)	S	Mt2
Castagna *et al.* (2017)	Pinot noir (Vv)	L	F	N	L	Mt2; AxC; P
Cavallini *et al.* (2015)	Cabernet sauvignon (Vv)	B	F	E (UV-B); S(UV-C)	L	Mt2; Gn

Reference	Variety	Organ studied	Type of experiment	Manipulation of UV	Duration of experiment	Variables used
Cetin *et al.* (2014)	Öküzgözü (Vv)	L	L	S (UV-C)	S	Mt2
Colas *et al.* (2012)	Pinot noir (Vv)	B	L	S (UV-C)	S	Mt1; Gn
Cortell and Kennedy (2006)	Pinot noir (Vv)	B	F	E (N)	L	Mt2
Crupi *et al.* (2013)	Red globe (Vv)	B	L	S (UV-C)	S	Mt2
Csepregi and Hideg (2017)	*V. vinifera*	L	L	UV-A , UV-B	S	Mt2
Csepregi *et al.* (2019a)	Emperor (Vv)	B	L	S (UV-A , B)	S	Mt2; AxC
Csepregi *et al.* (2019b)	Pinot noir (Vv)	L	F	N	S	Mt2; Ox; F
Cuneo *et al.* (2013)	Pinot noir (Vv)	W	F	N	L	Mt2
Del-Castillo-Alonso *et al.* (2015)	Graciano (Vv)	L; B	F	E (UV)	L	Mt2; Scl; Pho; Fl; P
Del-Castillo-Alonso *et al.* (2016a)	Pinot noir (Vv)	B	F	N	L	Mt2; AxC
Del-Castillo-Alonso *et al.* (2016b)	Tempranillo (Vv)	L; B	F	E (UV)	L	Pho; Fl; Mt2
Diago *et al.* (2012)	Tempranillo (Vv)	W	F	N	L	Mt2
Dokoozlian *et al.* (1996)	Cabernet sauvignon (Vv), Pinot noir (Vv)	B	L	N	L	Mt1; Mt2; C
Doupis *et al.* (2011)	Soultanina (Vv)	L	F	S (UV-B)	M	C; H; Hr; Mt2; Ox
Doupis *et al.* (2012)	Romeiko (Vv)	L	L	N; S (UV-B)	S	Ox
Doupis *et al.* (2016)	Romeiko (Vv), Sultanina (Vv)	L	F	S (UV-B)	S	Pho; Fl
Downey *et al.* (2004)	Syrah (Vv)	B	F	E (N)	L	Mt2
Downey *et al.* (2006)	(Vv)	?	?	?	?	Mt2

Reference	Variety	Organ studied	Type of experiment	Manipulation of UV	Duration of experiment	Variables used
Duan *et al*. (2015)	Augster weiss (Vv), Pinot blanc (Vv), Pinot noir (Vv), Müller-thurgau (Vv), Chardonnay (Vv),Cabernet sauvignon (Vv), (Vvs), (Vs), (Vq)	B	L	S (UV-C)	S	Mt2; Gn
Duan *et al*. (2016)	Augster weiss (Vv), (Vvs)	L; C	L	S (UV-C)	S	Mt2
Feng *et al*. (2015)	Pinot noir (Vv)	B	F	E (N)	L	Mt2
Fernandes de Oliveira and Nieddu (2013)	Cannonau (Vv)	B	F	N	L	Mt2
Fernandes de Oliveira *et al*. (2015)	Cariñena (Vv), Garnacha (Vv)	B	F	E (UV-A)	L	Mt2; Mt1
Fernandes de Oliveira and Nieddu (2016a)	Cariñena (Vv), Garnacha (Vv)	B	F	E (UV)	L	Mt2
Fernandes de Oliveira and Nieddu (2016b)	Cariñena (Vv), Garnacha (Vv)	L; B	F	E (UV)	L	Pho; Mt2
Ferrandino and Lovisolo (2014)	(Vv)	?	?	N	?	Mt2
Fracassetti *et al*. (2019)	Chardonnay (Vv), Crimson (Vv)	J	L	S (UV-C)	S	Z
Fujimori *et al*. (2014)	Merlot (Vv)	B	F	S (UV-B)	L	Gn
Fujita *et al*. (2018)	Koshu (Vv)	C	L	S (UV-C)	S	Gn
Gil *et al*. (2012)	Malbec (Vv)	L	Gh	E (UV-B), S (UV-B)	S	Mt2
Gil *et al*. (2013)	Malbec (Vv)	B	L; F	E (UV-B), S (UV-A)	L	Mt2
Gil *et al*. (2014)	Malbec (Vv)	F	F	E (UV-B)	L	Mt2
González *et al*. (2015)	Malbec (Vv)	B	F	E (N)	L	Mt2

Reference	Variety	Organ studied	Type of experiment	Manipulation of UV	Duration of experiment	Variables used
González-Barrio *et al*. (2006)	Superior (Vv)	B	L	S (UV-C)	S	Mt2
González-Barrio *et al*. (2009)	*V. vinifera*	B	L	S (UV-C)	M	Mt2
Gregan *et al*. (2012)	Sauvignon blanc (Vv)	B	F	E (UV-B)	L	Mt2
Grifoni *et al*. (2008)	*V. vinifera*	L	F	N	S	Z
Grifoni *et al*. (2016)	Sangiovese (Vv)	L	Gh	E (UV)	L	Mt2; F; Fl
Guan *et al*. (2016)	Bordeaux (Vv)	B	F	N	L	Mt2
Guerrero *et al*. (2010)	Syrah (Vv), Merlot (Vv), Graciano (Vv), Tempranillo (Vv), Palomino fino (Vv), Palomino negro (Vv),Tintilla roja (Vv), Orion (Vv), Regent (Vv)	B	L	S (UV-C)	S	Mt2
Guerrero *et al*. (2016)	*V. vinifera*	B	L	S (UV-C)	S	Mt2
Hasan and Bae (2017)	*V. vinifera*	B; W	?	(UV-B; UV-C)	?	Mt2
Henrique *et al*. (2016)	*V. labrusca*	Z	L	S (UV-C)	S	Mt2
Jordan (2017)	*V. vinifera*	B	F	UV-B	L	Mt2; Gn
Joubert *et al*. (2016)	Sauvignon blanc (Vv)	B	F	E (UV)	L	Pho; P; Mt2
Jug and Rusjan (2012)	*V. vinifera*	?	?	N	?	Mt2; C; F
Kataoka *et al*. (2003)	Gros colman (Vv)	B	L	E (UV); S (UV)	S	Mt2

Reference	Variety	Organ studied	Type of experiment	Manipulation of UV	Duration of experiment	Variables used
Keller *et al*. (2003)	Cabernet sauvignon (Vv), Chardonnay (Vv)	L	F	E (UV)	M	Pho; Mt2; P; R; Z
Kemp *et al*. (2011)	Pinot noir (Vv)	B; W	F	N	L	Mt2
Kiselev *et al*. (2017)	*V. amurensis*	L; B	L	S (UV-C)	S	Mt2; Gn
Kiselev *et al*. (2019)	*V. amurensis*	L	L	S (UV-C)	S	Mt2; Gn
Kobayashi *et al*. (2011)	Sauvignon blanc (Vv), Chardonnay (Vv), Koshu (Vv), Merlot (Vv)	L;B	F	S (UV-C)	S	Mt2; Gn
Kolb *et al*. (2001)	Silvaner (Vv)	L	F; Gh	E (UV)	S	Pho; Fl; Mt2; U
Kolb *et al*. (2003)	Bacchus (Vv)	B	Gh	N; E (UV-B , UV-A)	L	Mt2
Kolb *et al*. (2005)	Bacchus (Vv)	L	Gh	E (UV)	S	Fl
Kolb and Pfündel (2005)	Silvaner (Vv)	L	Gh	E (UV)	S	Fl; Mt2
Koyama *et al*. (2012)	Cabernet sauvignon (Vv)	B	F	E (N)	M	Gn; Mt2; AxC
Kuhn *et al*. (2014)	*Vitis vinifera*	B	?	N	?	Mt2
Lang *et al*. (2000)	Concord (Vv)	L	F; Gh	S (UV)	L	Rf
Guan *et al*. (2016)	Gamay (Vv)	B	F	E (N)	L	Mt2; Gn
Lee and Skinkis (2013)	Pinot noir (Vv)	B	F	N	L	Mt2
Lesniewska *et al*. (2004)	Gamay (Vv)	Cel	L	S (UV-B)	S	U
Liakopoulos *et al*. (2006)	Soultanina (Vv), Siriki (Vv), Athiri (Vv)	L	F	N	S	Pho; Fl; Mt2; P
Liu *et al*. (2015)	Sauvignon blanc (Vv)	B	L	S (UV-B)	L	Mt2; Gn

Reference	Variety	Organ studied	Type of experiment	Manipulation of UV	Duration of experiment	Variables used
Liu *et al*. (2018)	Sauvignon blanc (Vv)	B	F; L	E (UV-B); S (UV-B)	L	Mt2; Gn
Loyola *et al*. (2016)	*Vitis vinifera*	L	L	S (UV-B)	S	Mt2; Gn
Luo *et al*. (2019)	*Vitis vinifera*	L	L	S (UV-C)	S	Mt2; Gn; P
Ma *et al*. (2019)	Thompson seedless (Vv)	L	L	S (UV-C)	S	Mt2; Gn
Majer and Hideg (2012a)	Chardonnay (Vv)	L	F	UV	L	Ox; AxC
Majer and Hideg (2012b)	Chardonnay (Vv)	L	Gh	S (UV)	S	Pho; Fl; Mt2; Ox; P
Marfil *et al*. (2019)	Malbec (Vv)	B	F	E (UV-B)	L	Mt2; Gn
Martínez-Lüscher *et al.* (2013)	Tempranillo (Vv)	L	Gh	S (UV-B)	L	Pho; Ox
Martínez-Lüscher *et al*. (2014a)	Tempranillo (Vv)	B	Gh	S (UV-B)	S	Mt2; Gn
Martínez-Lüscher *et al*. (2014b)	Tempranillo (Vv)	B	Gh	S (UV-B)	S	Mt1; Mt2
Martínez-Lüscher *et al*. (2015a)	Tempranillo (Vv)	L	Gh	S (UV-B)	L	Pho; Mt2; C; H; Ox
Martínez-Luscher *et al.* (2015b)	Tempranillo (Vv)	L	Gh	S (UV-B)	L	Pho; Ox
Martínez-Lüscher *et al*. (2016)	Tempranillo (Vv)	B	Gh	S (UV-B)	L	Mt2; Gn
Martínez-Lüscher *et al*. (2019)	Cabernet sauvignon (Vv)	B	F	N	L	Mt2
Matus *et al*. (2009)	Cabernet Sauvignon (Vv)	B	F	N	L	Mt2; Gn
Matus *et al*. (2017)	Cabernet sauvignon (Vv), Corniva veronese (Vv), Viura (Vv), Chardonnay blanc (Vv), Sauvignon blanc (Vv)	B; L; Z	F	S (UV-B)	L	Mt2; Gn

Reference	Variety	Organ studied	Type of experiment	Manipulation of UV	Duration of experiment	Variables used
Maurer *et al*. (2017)	*V. labrusca* × *V. vinifera*	B	L	S (UV-C)	S	Mt2; AxC
Núñez-Olivera *et al*. (2006)	Tempranillo (Vv), Viura (Vv)	L	F	E (UV-B)	M	Scl; Pho; Fl; Mt2
Ozden *et al*. (2014)	Syrah (Vv)	B	F	N	L	Mt2; AxC
Pan *et al*. (2009)	Cabernet sauvignon (Vv)	B	L	S (UV-C)	L	Gn; Mt1; Mt2: Est; U
Parish-Virtue *et al*. (2019)	Sauvignon blanc (Vv)	W	L	S (UV)	S	Mt2
Petit *et al*. (2009)	Pinot meunier (Vv)	B; F	L	S (UV-C)	L	Mt2; Gn
Pfündel *et al*. (2003)	Silvaner (Vv), Bacchus (Vv)	L	Gh	E (UV); S (UV)	S	Fl
Pfündel *et al*. (2007)	Silvaner (Vv)	L	Gh	N	M	Fl
Pinto *et al*. (2016)	Concord (Vl)	B	L	S (UV-C)	L	Mt2; Gn; AxC
Pollastrini *et al*. (2011)	Sangiovese (Vv)	L	F	E (UV)	L	Pho; Fl; M; Mt2; R
Pontin *et al*. (2010)	Malbec (Vv)	L	Gh	S (UV-B)	S	Gn
Price *et al*. (1995)	Pinot noir (Vv)	B; W	F	N	L	Mt2; Gn
Reshef *et al*. (2018)	Cabernet sauvignon (Vv)	B; W	F	E (N)	L	Mt2
Sasaki *et al*. (2016)	Sauvignon blanc (Vv)	B	F	E (UV-B); S (UV-B)	L	Mt2; Gn
Schoedl *et al*. (2013)	Pinot noir (Vv), Riesling (Vv)	L	Gh	S (UV-B)	L	Pho; Mt2
Schultz *et al*. (1998)	Riesling (Vv)	B	F	E (UV-B)	L	Mt1; P
Schultz et *al*. (2000)	*Vitis vinifera*	?	?	N	?	Mt2
Schultz et *al*. (2004)	*Vitis vinifera*	?	?	N	?	Mt2

Reference	Variety	Organ studied	Type of experiment	Manipulation of UV	Duration of experiment	Variables used
Song *et al*. (2015)	Pinot noir (Vv)	W	F	N	L	Mt2
Spayd *et al*. (2002)	Merlot (Vv)	B	F	N	L	Mt2
Steel *et al*. (2000)	Cabernet sauvignon (Vv)	L; B	F	E (UV)	L	P
Steel and Greer (2005)	Cabernet sauvignon (Vv)	L	L	S (UV-B)	S	Mt1
Sternad Lemut *et al*. (2013)	Pinot noir (Vv)	B	F	N	L	Mt2
Suklje *et al*. (2014)	Sauvignon blanc (Vv)	W	F	E (UV)	L	Mt2
Sunitha *et al*. (2019)	Cabernet sauvignon (Vv)	L; B	L; F	E (UV-B); S (UV-B)	L	Gn
Suzuki *et al*. (2015)	*Vitis vinifera*	B	L	S (UV-C)	S	Mt2; Gn
Takayanagi *et al*. (2004)	Chardonnay (Vv), Koshu (Vv), Bailey x Muscat Hamburg	B	F	S (UV-C)	S	Mt2
Teixeira *et al*. (2013)	*Vitis vinifera*	?	?	N	?	Mt2
Versari *et al*. (2001)	Corvina (Vv)	B	F	S (UV)	S	Mt2
Verzera *et al*. (2016)	Nero d'Avola (Vv)	W	F	N	L	Mt2
Vilanova *et al*. (2011)	Tempranillo (Vv)	W	F	N	L	Mt2
Wang *et al*. (2010)	Cabernet sauvignon (Vv)	B	Gh	S (UV-C)	S	Mt2; Gn; U
Wang *et al*. (2013)	*V. vinifera, V. vinifera* x *V. amurensis*	L; B	L	S (UV-C)	S	Mt2; Gn
Wang *et al*. (2015)	*V. vinifera x V. amurensis*	B	L	S (UV-C)	S	Mt2

Reference	Variety	Organ studied	Type of experiment	Manipulation of UV	Duration of experiment	Variables used
Wen *et al*. (2015)	Cabernet sauvignon (Vv)	B	L	S (UV-C)	S	Mt2; Gn
Xi *et al*. (2014)	Hongbaladuo (Vv)	L	L	S (UV-C)	S	Mt2; Gn
Xu *et al*. (2012)	*V. pseudoreticulata*	L	L	S (UV-C)	S	Mt2; Gn
Yin *et al*. (2016)	*V. amurensis*	B	L	S (UV-B; UV-C)	S	Mt2; Gn
Zhang *et al*. (2012)	Cabernet sauvignon (Vv)	B	F	S (UV)	L	Mt2; Gn
Zhang *et al*. (2013)	Cabernet sauvignon (Vv)	B	F	S (UV)	S	Mt2; Gn

Chapter 2. Objectives

OBJECTIVES

The general objective of the present Doctoral Thesis was to study the effects of UV radiation on grapevine from a global and integrative perspective, and from both basic and applied viewpoints. In this sense, this study was conceived to be developed under typical Mediterranean field conditions and at different scales, comprising:

- Different UV wavelengths, distinguishing the effects of the UV bands reaching the Biosphere (UV-A and UV-B).
- Different experimental approaches, including the use of filters for the exclusion of solar UV (thus assessing the effects of ambient solar levels of UV radiation), lamps to enhance UV (hence evaluating the potential ecological effects of climate change and ozone depletion, but also exploring the options of manipulating UV for a better production), and UV natural gradients (whose use could inform management of UV and other environmental parameters affecting berry composition).
- Different grapevine varieties, including minor (Graciano) and major ones, both typically Spanish (Tempranillo) to international (Pinot noir).
- Different plant organs, from leaves to grapes, to understand the whole functioning of the plant.
- Different berry components (skin, flesh and seeds) representing different sources of metabolites for wine.
- Different phenological stages, from bud break to harvest, but paying particular attention to the stages of berry development.
- Different variables including morphology, physiology and agricultural exploitation. Hence, we measured from variables exploring the molecular mechanisms (gene expression) underlying the metabolic responses of grapes to UV, to those assessing the physiology of the plant (such as photosynthesis, photosynthetic pigment composition,

etc.), and those defining the commercial quality of grapes and wine, including a wide diversity of phenolic and volatile compounds determining key sensorial characteristics.

- And different cell locations of the potentially protecting phenolic UV-absorbing compounds that, also, could represent different ways of incorporating these compounds to the wine.

In addition, we aimed to study the influence of UV on grapevine walking along a rarely circulated track: the whole way of grapevine commercial exploitation from the grapes to the wine. As a consequence of this experience, and having tried to establish to what extent the effects of UV radiation on grapes were conserved in the resulting wines, our further aim was to offer grapevine growers and winemakers new, realistic and easy-to-apply methods to improve the quality of grapes and wine through the manipulation of UV radiation at a crop scale.

These general objectives were more thoroughly and specifically developed in the more specific aims of the different chapters of the Thesis, which are not mentioned here for the sake of efficiency.

Chapter 3. Effects of UV exclusion on the physiology and phenolic composition of leaves and berries of Vitis vinifera *cv. Graciano*

Effects of UV exclusion on the physiology and phenolic composition of leaves and berries of *Vitis vinifera* cv. Graciano

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ABSTRACT

BACKGROUND: UV radiation induces adaptive responses which can be used for plant production improvement. Our aim was to assess the effect of solar UV exclusion on the physiology and phenolic compounds of leaves and berry skins of *Vitis vinifera* L. cv. Graciano under field conditions. Phenolic compounds were analyzed globally and individually in both the vacuolar fraction and, for the first time in grapevine, the cell wall-bound fraction. These different locations may represent diverse modalities of phenolic response to and protection against UV.

RESULTS: UV exclusion led to a decrease in Fv/Fm in leaves, revealing that solar UV is needed for an adequate photoprotection. Only p-caffeoyl-tartaric acid from the soluble fraction of leaves and myricetin-3-O-glucoside from the soluble fraction of berry skins were significantly higher in the presence of UV radiation, and thus they could play a role in UV protection. Other hydroxycinnamic acids, flavonols, flavanols and stilbenes did not respond to UV exclusion.

CONCLUSION: UV exclusion led to subtle changes in leaves and berry skins of Graciano cultivar, which would be well adapted to current UV levels. This may help support decision-making on viticultural practices modifying UV exposure of leaves and berries, which could improve grape and wine quality.

INTRODUCTION

Ultraviolet (UV) radiation is a minor component (8-9%) of the total solar radiation reaching the Earth surface, representing an environmental factor which has induced diverse adaptive changes during life evolution (Cockell and Knowland, 1999). UV-A (315-400 nm) is the most abundant UV component but the less harmful one, UV-B (280-315 nm) causes several physiological damage despite its relatively low proportion, and finally UV-C (<280 nm) is extremely hazardous but absent at ground level due to stratospheric oxygen and ozone absorption. In the last decades, the anthropogenic reduction of stratospheric ozone has led to an increase in UV-B in the biosphere (6-12% since 1980 in mid-latitudes) (McKenzie *et al.,* 2007), and, as a consequence, the study of UV effects on organisms has deserved increased interest.

In photosynthetic organisms, increased UV radiation may cause critical impairments in the photosynthetic apparatus leading to pigment degradation, photoinhibition, and reductions in quantum yield, net photosynthesis and the Calvin cycle enzyme activity, together with DNA and oxidative damage (Jansen *et al.,* 1998). Plants are unavoidably exposed to UV radiation, as they require sunlight to carry out photosynthesis, and therefore they have evolved protection and repairing mechanisms resulting in UV acclimation. Nowadays, UV radiation is rather considered a regulation factor triggering a global response in the plant than a simple stressing factor (Hideg *et al*., 2013).

In grapevine (*Vitis vinifera* L.), the responses to UV radiation depend on the cultivar, the incident UV dose, the ratio between UV and PAR (photosynthetically active radiation) and other environmental conditions (Majer and Hideg, 2012a,b; Berli *et al*., 2013). Furthermore, differential consequences seem to occur depending on the plant organ (leaf, stem or berry) and its developmental stage (Berli *et al.,* 2013). Phenolic compounds are key secondary metabolites present in grapevine leaves and, especially, in the skins and seeds of berries. They are responsible for most of the sensory quality traits of grape and wine composition, such as color, taste and mouthfeel (Kennedy *et al.,* 2006). Phenolic acids (hydroxybenzoic and

hydroxycinnamic acids), which are mainly located in cell walls, together with stilbenes and flavonoids (anthocyanins, flavanols and flavonols) accumulated in the vacuoles, comprise the pool of phenolic compounds found in grapevine berries (Braidot *et al.,* 2008). Phenolic compounds may act as both UV-absorbers and antioxidants, and their accumulation in leaves and berries is probably the most important adaptive mechanism of grapevine to UV (Kolb *et al*., 2001; Berli *et al*., 2008, 2010, 2011; Pontin *et al*., 2010; Koyama *et al*., 2012; Zhang *et al*., 2012, 2013). However, the vacuolar and cell wall-bound fractions of phenolic compounds have never been differentially analyzed in grapevine, in contrast to other species (Schnitzler *et al*., 1996).

In recent years, the effects of UV radiation on grapevine physiology, berry metabolism and gene expression have been deeply studied (Berli *et al*., 2008, 2010, 2011, 2013; Pontin *et al*., 2010; Núñez-Olivera *et al*., 2006; Pollastrini *et al*., 2011). However, most of these studies have focused on high-altitude vineyards (1000-1500 m). Since UV irradiance increases with elevation, notoriously inducing the synthesis of phenolic compounds, further experimentation is needed in vineyards located at lower altitudes, where most of worldwide viticulture is carried out.

The goal of this study was to assess, under mid-altitude field conditions, the effect of UV exclusion (from veraison to harvest) on grapevine physiology and non-anthocyanin phenolic compounds in leaves and berry skins of *Vitis vinifera* L. cv. Graciano. To our knowledge, this is the first study in grapevine where the accumulation of phenolic compounds is separately analyzed in the methanol-soluble (vacuoles) and -insoluble (cell walls) fractions in both leaves and berry skins.

MATERIALS AND METHODS

Plant material and experimental design

The experiment was conducted in the 2011 season in a commercial, dry-farmed vineyard (*Vitis vinifera* L. cv. Graciano) located in Ollauri (La Rioja, northern Spain: 42º31'N, 2º49'W, 527 m). Graciano vines were grafted onto 110R rootstock and planted in 1995 in a clay-loam soil. Vines had a between-row and within row spacing of 2.70 m x 1.15 m, respectively, and row orientation was east-west. The vines were spur-pruned (12 buds per vine) in a bilateral cordon and trained to a vertically shoot-positioned (VSP) trellis system. The trellis featured a supporting wire at 0.70 m, two wires at 1.00 m aboveground for protection against wind damage, and a pair of movable shoot-positioned wires at 1.45 m. Vines were not irrigated during the growing season. During the duration of the study (from 2 August to 14 October 2011), total rainfall was 21.8 mm, average mean temperature (± SD) was 17.8 ± 1.6 ºC, and absolute maximum and minimum temperatures were 36.2 ºC and 3.3 ºC, respectively (weather station of El Naval, 510 m altitude, Casalarreina, La Rioja, Spain).

At pre-bloom (3 June 2011, seven days before flowering), all vines were defoliated by removing the first eight main basal leaves of each shoot, but no laterals were detached, to increase and homogenize the exposure of fruits to solar radiation. Shoots were trimmed once at the end of July, before veraison.

The experimental layout included a completely randomized block design. Three blocks of nine vines each were divided into three experimental conditions: no filter (Ambient), UV-transmitting filter (FUV+), and UV-blocking filter (FUV-), allocating three vines per replicate. The two filtering treatments involved placing transparent polymetacrylate filters (PMMA XT Vitroflex 295 and XT Vitroflex 395 Solarium Incoloro, Polimertecnic, Girona, Spain), which allowed and blocked, respectively, the transmission of UV radiation. Filters (1.30 x 0.75 m) were placed at 45º from the vertical axis of the plant, on both sides of the canopy, covering the fruiting zone and the first

0.7 m of the canopy of each grapevine. Filters were installed at veraison (2 August 2011) and were kept until harvest (14 October 2011). Spectral irradiances under the filters were measured regularly from the beginning of the experiment using a spectroradiometer (Macam SR9910, Macam Photometrics Ltd, Livingstone, Scotland) to confirm the stability of their filtering characteristics (Figure 3.1). Ambient photosynthetic (PAR), UV-A, and UV-B irradiances were continuously recorded by broad band radiometers (Skye Quantum SKP 215, SKU 420 and SKU 430, respectively, Skye Instruments Ltd, Powys, UK) installed at Universidad de La Rioja (Logroño). An action spectrum was applied to calculate the biologically effective UV radiation (UV_{BE}) (Flint and Caldwell, 2003).

Collection of leaves and berries

For all treatments, leaf and berry samples were collected around noon on a sunny day (14 October 2011), prior to harvesting. Leaf discs of 3.98 cm^2 from secondary leaves of the mid-upper part of the shoot, and berries from the basal position of a south-oriented shoot were collected for each replicate. Berry densities were determined by floatability in a NaCl solution series as a non-invasive indication of the internal sugar concentration (Rolle *et al*., 2011). Berries with density between 150-170 g NaCl L^{-1} (corresponding to approximately 24 ^{o}Brix) were rinsed in distilled H_2O, and both berries and leaf discs were frozen *in situ* in liquid nitrogen and then kept at -80ºC until further analyses.

Physiological measurements

The *in vivo* chlorophyll fluorescence of Photosystem II (PSII) was measured *in situ* on the same leaves described above, using a portable fluorimeter (MINI-PAM, Walz, Effeltrich, Germany) following the saturating pulse methodology (Schreiber *et al*., 1995). Maximum (F_m) and mínimum (F_0) fluorescence values were determined in intact leaves, previously subjected to dark adaptation, and the maximum quantum yield of PSII (F_v/F_m) was determined, where $F_v = F_m – F_o$.

For the measurement of Sclerophylly Index (SI), leaf discs were weighed (fresh weight, FW) and oven-dried at 60ºC during 24 h. Dry weight (DW) was recorded afterwards and SI was computed as the quotient between DW and leaf surface (mg DW cm^{-2}).

Chlorophylls and carotenoids were analysed in leaf discs by HPLC (García-Plazaola and Becerril, 2001; Otero *et al*., 2006). Frozen leaf discs were twice ground for 45 s at 30 Hz using a Tyssue-Lyser (Qiagen, Hilden, Germany). Then, pigments were extracted using 100% acetone (with 0.5 g L^{-1} of $CaCO_3$) in darkness for 24 h at 4 ºC. Identification and quantification were carried out on a HP1100 HPLC (Agilent Technologies, Palo Alto, CA, USA). Commercial standards of chlorophyll *a* and *b* (Fluka, Buchs, Germany), and carotenoids (CaroteNature, Ostermundigen, Switzerland) were used to build calibration curves for quantification. Total chlorophylls (Chl *a + b*), Chl *a / b* ratio, total carotenoids, β-carotene, and xanthophylls such as lutein (L), neoxanthin (N), violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z), were determined. The xanthophyll index was computed as the (A+Z)/(A+Z+V) ratio.

The chlorophyll content of leaves was also determined *in situ* on the sampling day using the non-invasive leaf clip sensor Dualex® 4 SCIENTIFIC (FORCE-A, Orsay, France). This sensor can perform measurements of chlorophyll content from leaf transmittance and has shown a linear response to leaf chlorophyll contents for both monocotyledons and dicotyledons with coefficients of determination of 0.96 (Cerovic *et al*., 2012).

Phenolic compounds were analysed in both leaves and berry skins, differentiating methanol-soluble and –insoluble compounds, that are mainly located, respectively, in the vacuoles or bound to the cell walls (Schnitzler *et al.,* 2006). The bulk level of phenolic compounds was measured by spectrophotometry and the concentrations of individual compounds by HPLC (leaves) or UPLC (berry skins). For these analyses, berry skins were removed from the flesh and seeds using a scalpel and then immediately submerged in liquid nitrogen and ground for 20 s with an analytical mill (A11 basic, IKA, Staufen, Germany) until a fine paste was obtained. Samples of this paste (100 mg each) and leaf discs were frozen in liquid nitrogen and ground in

a TissueLyser. Then, five ml of methanol: water: 7M HCl (70:29:1 v:v:v) was added for extraction (24 h at 4ºC in the dark) for each analytical sample. The extract was centrifuged at 6000 *g* for 15 min and the supernatant and pellet were considered the source of, respectively, methanol-soluble and methanol-insoluble phenolic compounds (MSPC and MIPC, respectively). The pellet was then hydrolyzed with 1 mL of 1M NaOH for 3 hours in a water bath at 80ºC. Afterwards, 1 ml of HCl (5.6 N) was added and the sample was rinsed three times with ethyl acetate. The supernatant obtained from the rinsing process was then allowed to evaporate (Büchi R-200) at 40ºC, and the remaining material was resuspended in methanol 100% up to a final volume of 2 mL (for leaf samples) or 1 mL (for berry skin samples).

In both soluble and insoluble fractions, bulk levels of phenolic compounds were measured as the area under the absorbance curve in the interval 280–400 nm ($AUC_{280–400}$) per unit of FW (Fabón *et al*., 2010), using a Perkin-Elmer *λ*35 spectrophotometer (Perkin-Elmer, Wilton, CT, USA). Individual phenolic compounds were analysed in both fractions by HPLC (leaves) or UPLC (berry skins). HPLC determinations were performed using an Agilent HP1100 HPLC system (Arróniz-Crespo *et al*., 2006). UPLC analyses were performed using a Waters Acquity Ultra Performance LC system (Waters Corporation, Milford, USA) (Sáenz-Navajas *et al*., 2010). The UPLC system was coupled to a micrOTOF II high-resolution mass spectrometer (Bruker Daltonik, Germany) equipped with an Apollo II ESI/APCI multimode source and controlled by the Bruker Daltonics DataAnalysis software. The electrospray source was operated in negative mode. The capillary potential was set to 4 kV; the drying gas temperature was 200 ºC and its flow 9 $L·min^{-1}$; the nebulizer gas was set to 3.5 bar and 25 ºC. Spectra were acquired between *m/z* 120 and 1505 in negative mode.

The different phenolic compounds analysed were identified according to the order of elution and retention times of pure compounds: catechin, epicatechin, catechin gallate, epicatechin gallate, myricetin, quercetin, caffeic acid, coumaric acid, ferulic acid and *t*-resveratrol (Sigma, St. Louis, USA); procyanidin B1, quercetin, kaempferol, isorhamnetin glucoside, and kaempferol-3-

rutinoside (Extrasynthese, Genay, France); quercetin-3-rutinoside, isorhamnetin and quercetin-3-galactoside (Fluka, Buchs, Germany). Quantification of non-commercial compounds was made using the calibration curves belonging to the most similar compound: caffeic acid for *p*-caffeoyl-tartaric acid; *p*-coumaric acid for *p*-coumaroyl-tartaric acid; and *t*-resveratrol for its glucoside. Amounts of flavonols, hydroxycinnamic acid derivatives, stilbenes and flavanols were expressed in µg g^{-1} FW in the case of berry skins, and in µg cm^{-2} DM for leaves.

The flavonoid content of leaves was also determined *in situ* on the sampling day using the Dualex® 4 SCIENTIFIC. Basically, the method compares the chlorophyll fluorescence signal measured at two excitation wavelengths. Due to the sub-epidermal localization of chlorophyll, the excitation-light reaching the chloroplasts is attenuated by compounds located in the epidermal cell compartments. As a result, an inverse relationship between the concentration of UV-absorbing compounds and the intensity of chlorophyll fluorescence is observed (Bilger *et al*., 2001). Dualex® 4 SCIENTIFIC is capable of measuring leaf epidermal flavonoids at 375 nm using the chlorophyll screening method (Cerovic *et al*., 2002), and has shown a linear response to leaf extracts at 375 nm (Goulas *et al*., 1996).

Statistical analysis

Pearson correlation coefficient (r) was used to examine the relationships between variables. The effect of treatment (radiation regime) on the variables measured was tested using analysis of variance (ANOVA), once proved that the data met the assumptions of normality (Shapiro–Wilks's test) and homoscedasticity (Levene's test). In the case of significant differences, means were compared by Tukey's test. Non-parametric tests (Kruskal-Wallis) were used if the data did not meet those assumptions. In this case, and when significant differences occurred, means were compared by Mann-Whitney's test. All the statistical procedures were performed with SPSS 19.0 for Windows (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

Characterization of radiation experimental conditions

UV radiation was almost absent under FUV-, while FUV+ treatment only led to a slight irradiance reduction compared to the Ambient condition (Figure 3.1). Similar doses of PAR were received by the plants under each radiation regime from the onset of treatments until harvest time (Table 3.1). However, UV_{BE} and unweighted UV-A and UV-B doses were significantly lower for FUV- than for the rest of treatments (Table 3.1).

Phenolic compounds in Graciano leaves and berry skins

We analyzed phenolic compounds both in the methanol-soluble vacuolar fraction (MSPC), which is the usually analyzed fraction in grapevine,(Berli *et al*., 2013, 2010; Núñez-Olivera *et al*., 2006; Keller *et al*., 2003) and, for the first time in grapevine, the methanol-insoluble cell wall-bound fraction (MIPC). This differentiation can be important, given that the cell wall-bound fraction would represent a more efficient UV screen than the vacuolar fraction, whereas the latter fraction could be more related to the antioxidant role of phenolic compounds. The MSPC fraction was the main reservoir of phenolics in Graciano leaves and berry skins (Tables 3.2, 3.3); in berry skins, flavonols were the most abundant group, followed by the hydroxycinnamic acids, whereas the contrary occurred in leaves. This also occurs in other grapevine varieties (Kolb *et al.*, 2001; Berli *et al*., 2010). In the MIPC fraction, only hydroxycinnamic acids were detected. They were not bound to an organic acid, whereas those extracted from the vacuoles were all bound to tartaric acid, probably to increase their solubility. Among the flavonols, quercetins were much more abundant than kaempferols, which would confer a stronger antioxidant capacity to Graciano leaves and berry skins because of the higher number of hydroxyl groups present in quercetins as compared to kaempferols. Similar results were obtained in Malbec leaves and berry skins (Berli *et al*., 2010, 2011).

Effects of radiation treatments on leaves

Among leaf traits, only F_v/F_m was affected by the radiation treatment and significantly decreased when UV radiation was excluded (FUV-) (Table 3.2), revealing a lower physiological vitality (Maxwell and Johnson, 2000), and suggesting that natural UV levels are necessary for Graciano leaves to develop an adequate photoprotection preventing photoinhibition or PSII damage. In other varieties, this occurs through the stimulation of both the synthesis of screening UV-absorbing compounds and efficient mechanisms for energy dissipation in the antenna complexes (Berli *et al.,* 2013; Pollastrini *et al*., 2011). Hence, natural UV levels would not affect photosynthesis through damage to the photochemical apparatus but rather through alterations in gas exchange and stomatal conductance (Kolb *et al*., 2001). This brings into question the suitability of F_v/F_m to measure the impact of natural levels of UV radiation on PSII functionality in grapevine, and it has been proposed that the specific study of F_0 and F_m separately would be more illustrative (Pfündel, 2003).

None of the variables related to chlorophylls or carotenoids revealed significant differences due to UV exclusion, as occurred in other grapevine varieties (Cabernet Sauvignon, Chardonnay, Viura and Malbec) grown at altitudes lower than 900 m (Berli *et al*., 2010; Núñez-Olivera *et al*., 2006; Keller *et al*., 2003). However, total chlorophyll content per DW (but not per leaf area) significantly decreased under full solar UV-B in leaves of Malbec from grapevines grown at 1450 m (Berli *et al*., 2013), probably due to the increase in UV level with increasing altitude. In this line, chlorophyll content also decreased and xanthophyll index increased in leaves of Tempranillo exposed to near-ambient UV-B as compared to UV-B-deprived leaves, but at only 380 m altitude (Núñez-Olivera *et al*., 2006). These results suggest that chlorophyll and carotenoid response to solar UV exclusion in grapevine are slight and may depend on UV wavelength, UV level and genotype.

UV exclusion did not significantly modify the sclerophylly index of Graciano leaves, in line with findings in Viura and Tempranillo exposed to natural UV levels, although for shorter periods (Núñez-Olivera *et al*., 2006).

The bulk levels of MSPC and MIPC and most individual phenolic compounds did not respond to the radiation treatments, except *p*-caffeoyl-tartaric acid, *p*-coumaric acid, quercetin-3-*O*-glucuronide and *cis*-kaempferol-3-*O*-glucoside (Table 3.2).

Although UV exclusion did not affect the bulk level of MSPC in Graciano leaves, significant effects were found in Cabernet Sauvignon (Koyama *et al*., 2012; Keller *et al.*, 2003), Tempranillo and Viura (Núñez-Olivera *et al*., 2006), and Sangiovese (Pollastrini *et al*., 2011), where UV-exposed leaves exhibited significantly higher levels of MSPC than UV-deprived leaves. This may show a probable influence of genotype (Bidel *et al.*, 2007). UV exclusion did not either affect the bulk level of MIPC, but no literature data are available for comparison. However, the significant increase of *p*-caffeoyl-tartaric acid from the soluble fraction under UV radiation (Ambient and FUV+ samples) would suggest a role in UV protection. A similar although less clear role could be assigned to *cis*-kaempferol-3-*O*-glucoside, which was lower under FUV- than under FUV+. *p*-Coumaric acid (insoluble fraction) and quercetin-3-*O*-glucuronide (soluble fraction) were found in higher and lower concentrations respectively, in leaves of FUV+ and FUV- with regard to those of Ambient, thus showing the influence of filters through, probably, a temperature increase.

The slight responses of Graciano leaves found in this study suggest that this variety is well adapted to the current natural UV levels, as occurs in other grapevine varieties (Núñez-Olivera *et al*., 2006). In addition, it must be taken into account that the treatments were imposed at veraison, when the vegetative growth of grapevines stops, as the source-sink balance is mostly driven by berry ripening. Thus, the extent of the responses to the different treatments was more limited than if they had been imposed earlier in the season (at bud-break or flowering) (Berli *et al*., 2013; Pollastrini *et al*., 2011), given that the leaves could be adequately UV-protected at the onset of treatments through efficient excess energy dissipation mechanisms and the greater

synthesis of phenolic compounds that happens around veraison. Moreover, leaf samples were collected at harvest time, late in the season, and mature, pre-senescent leaves are less responsive to UV radiation than young leaves, as these are photosynthetically more active and prone to protect their photosynthetic apparatus (Majer and Hideg, 2012a,b). If UV enhancement rather than UV exclusion had been used, responses would presumably have been stronger in F_v/F_m (Majer and Hideg, 2012a,b; Pfündel, 2003), carotenoids (Majer and Hideg, 2012a,b), and phenolic compounds (Doupis *et al*., 2011), since damage could not have been counteracted by acclimation and protection mechanisms.

Confirming the lack of effect of the radiation regimes on the total chlorophyll and flavonoid contents (represented by the bulk level of MSPC, as flavonoid compounds mostly accumulate in the vacuoles), the chlorophyll- and flavonoid-related indices determined by the Dualex® 4 leaf clip sensor behaved similarly among UV treatments (Table 3.2). In this way, the regressions between the chlorophyll and flavonoids Dualex® indices and, respectively, the chlorophyll content and the bulk level of MSPC, expressed as per unit of leaf surface, showed high and significant coefficients of determination, indicating the suitability of this portable optical sensor to characterize these physiological variables (Figure 3.2). With regard to chlorophyll assessment, the use of optical chlorophyll sensors has been suggested to be more accurate and faster than depending on extractions because of the contrasted and verified reproducibility of these sensors (Cerovic *et al.,* 2012). In addition, the measurement of chlorophyll-fluorescence screening at 375 nm has proved to be a reliable means to assess the flavonoid content of the leaf epidermis on a per surface basis, as reported elsewhere (Cerovic *et al*., 2002). However, a higher coefficient of determination would have been expected between the Dualex® 4 flavonoid measurement and the bulk level of MSPC if this fraction would have been free of non-flavonoid compounds, like hydroxycinnamic acids.

Effects of UV radiation on berry skins

The bulk level of MSPC and the concentrations of 12 out of 22 soluble individual phenolic compounds (mostly glycosylated flavonols) responded significantly to the radiation regimes (Table 3.3). The bulk level of MSPC, one hydroxybenzoic acid (protocatechuic acid) and six flavonols (one kaempferol, three quercetins, one isorhamnetin and one syringetin, all of them glycosilated) showed smaller concentrations in FUV+ and FUV- berries as compared to Ambient ones. This suggests that the presence of filters was a more influencing factor than the presence of UV. This could be due to a higher temperature under the filters, which may favour the degradation of certain compounds (Berli *et al*., 2008). The lack of difference between FUV+ and FUV- samples was also reported in Malbec berries (Berli *et al.,* 2008), and thus higher solar UV doses might be necessary to induce a significant enhancement in both the bulk level of MSPC and individual soluble compounds.

Myricetin-3-*O*-glucoside, the predominant phenolic compound in Graciano berry skin, was much higher in the presence of UV (Ambient and FUV+ regimes) than in FUV-. Two additional flavonols (myricetin-3-*O*-glucuronide and kaempferol-3-*O*-galactoside) also responded to UV although less clearly, since their values in FUV- were significantly lower than either Ambient or FUV+ samples. Flavonols accumulation under UV radiation has previously been described in Silvaner and Cabernet Sauvignon cultivars (Koyama *et al.,* 2012; Kolb *et al*., 2003). The importance of flavonols in berry and wine quality is widely recognized, especially regarding wine aging potential and sensory properties (Kennedy *et al*., 2006). For this reason, viticultural practices affecting UV exposure of the fruits can influence the accumulation of certain flavonols in grapes, hence final grape and wine quality.

Other soluble phenolic compounds, such as protocatechuic acid and flavanols, hardly responded to the radiation regimes. Only one flavanol, catechin, accumulated in higher concentrations in the two filtered treatments (Table 3.3). The lack of response of flavanols was congruent with

previous studies (Kolb *et al*., 2001; Koyama *et al*., 2012), since these compounds would respond more strongly to photosynthetic than to UV radiation.

Concerning stilbenes, neither resveratrol nor its glucoside were affected by the radiation regime, in contrast to other studies where resveratrol increased in UV-exposed berries (Berli *et al*., 2008; Bonomelli *et al*., 2004; González-Barrio *et al.,* 2009; Pan *et al*., 2009; Petit *et al.,* 2009). Notwithstanding, each individual compound may also show a different response to UV radiation due to the influence of additional factors, such as cultivar, the developmental stage, the type of tissue and, in the case of stilbenes, the presence of fungal infections or water stress (Bonomelli *et al*., 2004; Deluc *et al*., 2011).

With respect to the insoluble fraction, and similarly to leaves, the bulk level of MIPC did not show any significant difference between the three radiation regimes (Table 3.3), showing that the insoluble fraction was less responsive than the soluble one. This was probably due to the fact that phenolic compounds are covalently linked to the cell wall polysaccharides, and new compounds could not be further transported from the protoplasts and deposited in the cell walls after veraison, irrespectively of the radiation regime imposed. However, the insoluble *p*-coumaric acid did react to the treatments, as occurred in leaves, and was higher in FUV+ than in Ambient and FUV- samples. This suggests it would be influenced not only by UV radiation but also by other factors (maybe temperature).

As in the case of leaves, stronger changes in the phenolic composition of berry skins could have been found if the treatments would have been imposed earlier than at veraison (Gregan *et al*., 2012).

CONCLUSIONS

Graciano cultivar seems to be well adapted to current UV levels, given that UV exclusion from veraison to harvest led to only subtle changes in the physiology and phenolic composition of leaves and berry skins. Only *p*-caffeoyl-tartaric acid from the soluble fraction of leaves and myricetin-3-*O*-glucoside from the soluble fraction of berry skin were significantly higher under UV radiation, and thus they could play a role in UV protection. Other compounds, such as *p*-coumaric acid from the insoluble fraction of leaves and several glycosylated flavonols from the soluble fraction of berry skins, were influenced by the presence of filters rather than by UV. Protocatechuic acid, flavanols and stilbenes did not respond to the radiation regimes. These results provide further understanding about the impact of solar UV on grapevine berry ripening, and may help support decision-making on viticultural practices which can improve grape and wine quality through manipulation of UV exposure of leaves and berries.

Higher solar UV doses (for example those received at higher altitudes) or treatment establishment well before veraison might be necessary to induce more significant changes in both leaf and berry skin physiology and phenolic composition.

TABLES AND FIGURES

Table 3.1. Doses of photosynthetically active radiation (PAR), ultraviolet-A (UV-A), B (UV-B) and biologically effective ultraviolet radiation (UV_{BE}) received by the plants of *Vitis vinifera* Graciano under each radiation regime (Ambient, no filter; FUV+, UV-transmitting filter; FUV-, UV-blocking filter) during the period of study (2 August to 14 October 2011; 73 days).

Radiation regime	PAR ($MJ\ m^{-2}$)	UV-A ($MJ\ m^{-2}$)	UV-B ($MJ\ m^{-2}$)	UV_{BE} ($MJ\ m^{-2}$)
Ambient	654	65	2.6	0.9
FUV+	616	46	2.1	0.7
FUV-	639	5.6	0.29	0.09

Table 3.2. Effects of the radiation treatment (Ambient, no filter; FUV+, UV-transmitting filter; FUV-, UV-blocking filter) on the maximal quantum yield of photosystem II (F_v / F_m), sclerophylly index, photosynthetic pigments (in µg cm^{-2}) and phenolic compounds in the insoluble and soluble fractions of leaves of *Vitis vinifera* Graciano sampled at harvest time. MIPC and MSPC, the bulk UV absorbances of methanol-insoluble and methanol-soluble phenolic compounds (respectively), measured as the area under the absorbance curve in the interval 280-400 nm ($AUC_{280-400}$) per unit of leaf surface. Individual phenolic compounds are expressed in µg cm^{-2}. Values are expressed as mean ± standard error (n = 9). Within each row, means with different letters are significantly different at least at $p < 0.05$.

Variable	Ambient	FUV+	FUV-
F_v / F_m	0.54 ± 0.03 **b**	0.59 ± 0 **b**	0.45 ± 0 **a**
Sclerophylly index (mg cm^{-2})	7.0 ± 0.3	6.9 ± 0.2	7.2 ± 0.3
Photosynthetic pigments			
Chl *a*	11 ± 1	10 ± 0	10 ± 1
Chl *b*	4.0 ± 0.5	4.4 ± 0.3	4.0 ± 0.5
Chl *a* + *b*	15 ± 1.70	15 ± 1	14 ± 2
Chlorophyll (DUALEX values)	28 ± 1	30 ± 1	29 ± 1
Chl *a* / Chl *b*	2.6 ± 0.1	2.5 ± 0.1	2.6 ± 0.1
β-Carotene	2.8 ± 0.3	2.8 ± 0.1	2.7 ± 0.2
Lutein	5.1 ± 0.6	5.3 ± 0.2	5.2 ± 0.6
Zeaxanthin	1.4 ± 0.2	1.5 ± 0.1	1.7 ± 0.2
Antheraxanthin	1.1 ± 0.2	1.0 ± 0.1	1.2 ± 0.2
Violaxanthin	1.4 ± 0.1	1.6 ± 0.3	1.9 ± 0.3
Neoxanthin	1.1 ± 0.2	1.1 ± 0.3	1.0 ± 0.1
Xanthophyll index (A+Z) /(A+Z+V)	0.62 ± 0.02	0.58 ± 0.02	0.59 ± 0.05
Total carotenoids	13 ± 1	13 ± 0	13 ± 1
Insoluble compounds			
MIPC ($AUC_{280-400}$ cm^{-2})	69 ± 5	69 ± 5	73 ± 3
p-Coumaric acid	0.68 ± 0.04 **a**	1.0 ± 0.1 **b**	1.0 ± 0.0 **b**
Ferulic acid	0.42 ± 0.07	0.30 ± 0.02	0.38 ± 0.04
Soluble compounds			
MSPC ($AUC_{280-400}$ cm^{-2})	235 ± 11	209 ± 18	240 ± 20
p-Coumaroyl-tartaric acid	1.3 ± 0.1	1.7 ± 0.2	1.4 ± 0.2
p-Caffeoyl-tartaric acid	77 ± 3 **b**	79 ± 4 **b**	63 ± 3 **a**
Feruloyl-tartaric acid	1.4 ± 0.1	1.8 ± 0.2	1.5 ± 0.1
Quercetin-3-*O*-glucopyranoside	22 ± 3	26 ± 6	17 ± 3
Quercetin-3-*O*-glucuronide	37 ± 2 **b**	32 ± 5 **a**	33 ± 3 **a**
Cis-kaempferol-3-*O*-glucoside	3.3 ± 0.4 **ab**	4.2 ± 0.6 **b**	2.4 ± 0.4 **a**
Trans-kaempferol-3-*O*-glucoside	3.9 ± 0.5	3.7 ± 0.2	3.0 ± 0.3
Flavonoids (DUALEX values)	1.4 ± 0.0	1.4 ± 0.0	1.4 ± 0.0

Table 3.3. Effects of the radiation treatment (Ambient, no filter; FUV+, UV-transmitting filter; FUV-, UV-blocking filter) on the phenolic compounds in the insoluble and soluble fractions of berry skins of *Vitis vinifera* Graciano sampled at harvest time. MIPC and MSPC, the bulk UV absorbances of methanol-insoluble and methanol-soluble phenolic compounds (respectively), measured as the area under the absorbance curve in the interval 280-400 nm ($AUC_{280\text{-}400}$) per unit of fresh weight (FW). Individual phenolic compounds are expressed in µg g^{-1} (FW). Values are expressed as mean ± standard error (n = 9). Within each row, means with different letters are significantly different at least at $p < 0.05$.

Variable	Ambient		FUV+		FUV-	
Insoluble compounds						
MIPC ($AUC_{280\text{-}400}$ mg^{-1}FW)	2.8 ± 0.3		2.5 ± 0.3		2.2 ± 0.2	
p-Coumaric acid	211 ± 22	a	354 ± 34	b	183 ± 31	a
Syringic acid	178 ± 23		175 ± 56		175 ± 33	
Soluble compounds						
MSPC ($AUC_{280\text{-}400}$ mg^{-1}FW)	16 ± 1	b	13 ± 0	a	12 ± 1	a
p-Coumaroyl-tartaric acid	99 ± 17		154 ± 27		181 ± 32	
p-Caffeoyl-tartaric acid	86 ± 17		55 ± 24		94 ± 26	
Protocatechuic acid	6.4 ± 0.5	b	4.1 ± 0.8	a	3.3 ± 0.7	a
Myricetin	77 ± 20		73 ± 10		44 ± 10	
Myricetin-3-*O*-glucoside	837 ± 76	b	710 ± 69	b	474 ± 25	a
Myricetin-3-*O*-glucuronide	35 ± 4	b	28 ± 2	ab	22 ± 1	a
Kaempferol-3-*O*-galactoside	22 ± 3	ab	30 ± 3	b	16 ± 2	a
Kaempferol-3-*O*-glucoside	113 ± 2	b	62 ± 8	a	46 ± 7	a
Quercetin-3-*O*-glucoside	39 ± 9		44 ± 7		27 ± 4	
Quercetin-3-*O*-galactoside	69 ± 4	b	39 ± 7	a	39 ± 3	a
Quercetin-3-*O*-glucopyranoside	436 ± 19	b	301 ± 25	a	254 ± 18	a
Quercetin-3-*O*-glucuronide	576 ± 13	b	429 ± 24	a	468 ± 57	a
Quercetin-3-*O*-rutinoside	11 ± 0	a	6.0 ± 1.0	b	11 ± 2	a
Isorhamnetin-3-*O*-glucoside	184 ± 16	b	135 ± 15	a	106 ± 9	a
Isorhamnetin-3-*O*-glucuronide	14 ± 2		11 ± 1		12 ± 2	
Syringetin-3-*O*-glucoside	104 ± 4	b	78 ± 3	a	72 ± 5	a
Catechin	32 ± 2	a	46 ± 4	b	44 ± 2	b
Epicatechin	7.1 ± 0.5		9.5 ± 0.9		8.3 ± 0.9	
Epigallocatechin	238 ± 21		305 ± 45		218 ± 13	
Procyanidin B1	32 ± 2		38 ± 2		37 ± 3	
Resveratrol	4.6 ± 0.6		5.5 ± 0.5		5.3 ± 0.5	
Resveratrol-3-*O*-glucoside	107 ± 10		123 ± 11		92 ± 8	

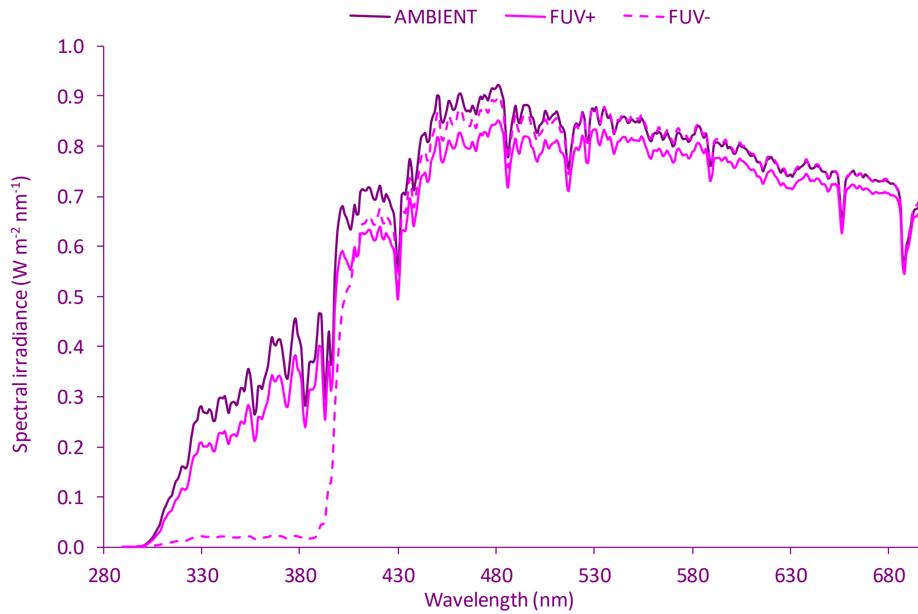

Figure 3.1. Spectral irradiances measured in the three treatments applied: no filter (Ambient), UV-transmitting filter (FUV+), and UV-blocking filter (FUV-).

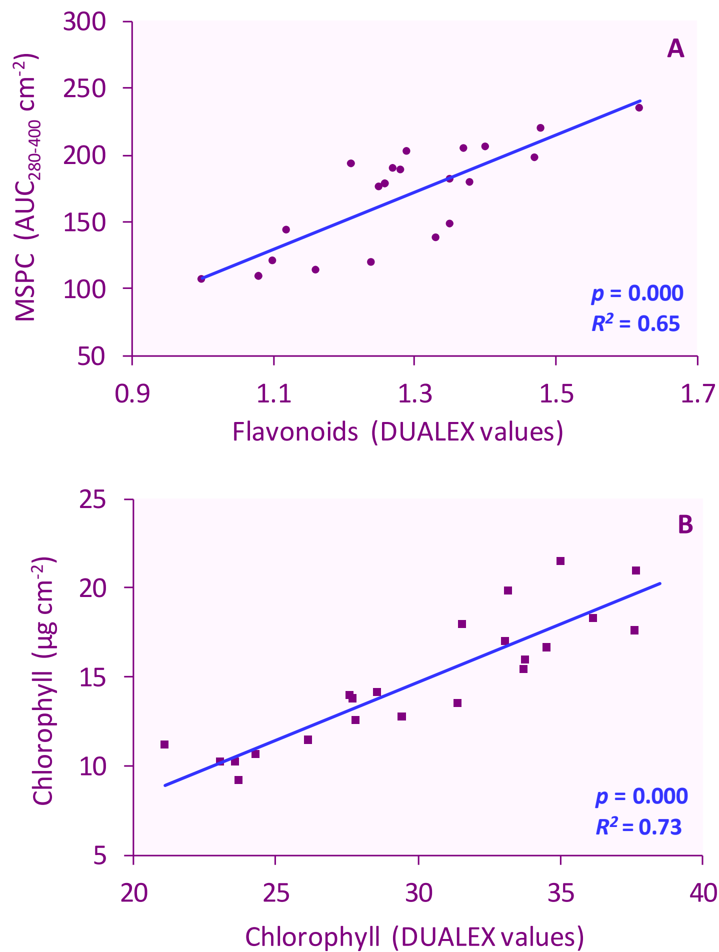

Figure 3.2. Linear regressions between variables measured by destructive and non-destructive methods in Graciano grapevine leaves. A, flavonoids estimated by Dualex® 4 instrument vs. the bulk UV absorbance of methanol-soluble phenolic compounds (MSPCs), measured as the area under the absorbance curve in the interval 280-400 nm ($AUC_{280-400}$) per unit of leaf surface. B, chlorophylls estimated by Dualex® 4 vs. chlorophyll concentration per unit of leaf surface measured by HPLC. Coefficients of determination and probability values are indicated.

Chapter 4. Effects of ambient solar UV radiation on grapevine leaf physiology and berry phenolic composition along one entire season under Mediterranean field conditions

Effects of ambient solar UV radiation on grapevine leaf physiology and berry phenolic composition along one entire season under Mediterranean field conditions

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ABSTRACT

In the present study we assessed the effects of ambient solar UV exclusion on leaf physiology, and leaf and berry skin phenolic composition, of a major grapevine cultivar (Tempranillo) grown under typically Mediterranean field conditions over an entire season. In general, the effects of time were stronger than those of UV radiation. Ambient UV caused a little stressing effect (eustress) on leaf physiology, with decreasing net photosynthesis rates and stomatal conductances. However, it was not accompanied by alterations in F_v/F_m or photosynthetic pigments, and was partially counterbalanced by the UV-induced accumulation of protective flavonols. Consequently, Tempranillo leaves are notably adapted to current UV levels. The responses of berry skin phenolic compounds were diverse, moderate, and mostly transitory. At harvest, the clearest response in UV-exposed berries was again flavonol accumulation, together with a decrease in the flavonol hydroxylation level. Contrarily, responses of anthocyanins, flavanols, stilbenes and hydroxycinnamic derivatives were much more subtle or nonexistent. Kaempferols were the only compounds whose leaf and berry skin contents were correlated, which suggests a mostly different regulation of phenolic metabolism for each organ. Interestingly, the dose of biologically effective UV radiation (UV_{BE}) was correlated with the leaf and berry skin contents of quercetins and kaempferols; relationships were linear except for the exponential relationship between UV_{BE} dose and berry skin kaempferols. This opens management possibilities to modify kaempferol and quercetin contents in grapevine through UV manipulation.

INTRODUCTION

Ultraviolet (UV) radiation is a minor fraction (around 8%) of the solar spectrum reaching the ground level in the Biosphere. At this level, it is composed by two types of wavelengths, being UV-A (315-400 nm) much more abundant than UV-B (280-315 nm). Traditionally, UV radiation (particularly UV-B) has been considered as a generic stressor for plants, inducing diverse damaging processes mainly affecting photosynthesis, DNA, membranes, proteins and hormones. Nowadays, however, there is consistent evidence that natural UV levels act rather as an environmental regulator, controlling gene expression, metabolism, and growth and development (Jansen and Bornman, 2012; Hideg *et al*., 2013). In crop plants, this new conception opens different management possibilities to improve agricultural products, conferring them an added value and quality differential through UV manipulation. Thus, research on the effects of UV on crop plants has notably increased in recent years (Wargent and Jordan, 2013).

In grapevine (*Vitis vinifera* L.), one of the main crops worldwide, UV radiation is a key factor regulating the contents of important healthy metabolites that determine berry and wine features, such as aroma, astringency, colour and stability. Additionally, UV radiation may increase tolerance to abiotic and biotic stressors, including pests and diseases (Jug and Rusjan, 2012). Thus, research on the effects of UV radiation on grapevine is strategically important because it offers enormous possibilities of management to improve both the production process and the quality of the final product. These objectives can be investigated through simple cultural practices modifying sun exposure of berries, such as defoliation (Pastore *et al*., 2013) or shading (Downey *et al*., 2004). Yet, this kind of methods cannot discriminate between the effects of the different wavelengths of the solar spectrum. To assess the specific effects of UV radiation, two basic manipulation approaches can be applied: UV enhancement using lamps and UV exclusion using filters. Both approaches have been used in grapevine, but many of these studies have focused on leaf physiology (Kolb *et al*., 2001; Pfündel, 2003; Núñez-Olivera *et al*., 2006;

Pollastrini *et al*., 2011; Majer and Hideg, 2012a,b; Berli *et al*., 2013; Alonso *et al*., 2015; Martínez-Lüscher *et al*., 2015; Grifoni *et al*., 2016). Obviously, the results of the studies conducted on leaves have a limited applicability to the production process. Many other studies have analyzed berry traits, particularly phenolic composition and gene expression, and their results may have a greater applicability. However, some of these studies have dealt with plants growing in pots under controlled conditions (Martínez-Lüscher *et al*., 2014a, 2014b), and thus their results cannot be directly extrapolated to the field. Other studies have been conducted on high-altitude vineyards (Berli *et al*., 2008, 2011), where plants are exposed to higher UV levels than those received in mid-altitude localities where most of worldwide grapevines are grown. Therefore, studies carried out under typical field Mediterranean environments are scarce (Gregan *et al*., 2012; Carbonell-Bejerano *et al*., 2014; Del-Castillo-Alonso *et al*., 2015; Liu *et al*., 2015) and badly needed.

Other aspects that remain underexplored in the research on UV and grapevine are, for example: (1) the relationship between phenolic compounds in leaves and berries (Del-Castillo-Alonso *et al*., 2015); (2) the temporal responses of phenolic compounds to UV radiation over the entire berry ripening process (Gregan *et al*., 2012), because responses may vary along the development of a specific organ (Wargent and Jordan, 2013); and (3) the cell compartmentalization of phenolic compounds between vacuoles and cell walls. This last point may be relevant for the physiology of the plant, because vacuolar and cell wall-bound compounds can represent different photoprotection modalities (Carbonell-Bejerano *et al*., 2014; Del-Castillo-Alonso *et al*. 2015), and also for enological purposes, because the different location of phenolic compounds may influence their extractability in the enological process. Furthermore, it must be pointed out that most studies on the effects of UV radiation on grapevine have been conducted using minor grapevine varieties.

The goal of this study was to assess, under typical mid-altitude Mediterranean field conditions, the effects of solar UV exclusion on grapevine physiology and phenolic composition in leaves

and berry skins of *Vitis vinifera* L. cv. Tempranillo at three different phenological stages along berry development (pea size, veraison and harvest). The accumulation of phenolic compounds was separately analyzed in the methanol-soluble (vacuoles) and -insoluble (cell walls) fractions in both leaves and berry skins. The study was carried out using a major grapevine cultivar, given that Tempranillo is the fourth most used cultivar worldwide, and the first world's fastest-expanding wine grape in the period 2000-2010 (Anderson, 2013). It occupies more than 232,000 ha in the world (5.05% of the total), mostly in Spain.

MATERIALS AND METHODS

Plant material and experimental design

This field experiment was conducted in the 2012 season on a commercial vineyard located in Mendavia (Navarra, northern Spain, 42º 27' N, 2º 14' W, 371 m altitude). *Vitis vinifera* L. cv. Tempranillo, grafted onto 110R rootstock and planted in 2007 on clay-loam soil with NE-SW row orientation, was used. The vines were spur-pruned (12 buds per vine) in a bilateral cordon and trained to a vertical shoot positioning trellis system. At pre-bloom (7 June 2012, seven days before flowering), all vines were partially defoliated by removing the first six main basal leaves to increase and homogenize the exposure of fruits to solar radiation. Vines were not irrigated during the growing season.

A completely randomized block design was set-up. Six blocks of nine vines each were divided into three experimental conditions (three vines per replicate): no filter (Ambient); UV-transmitting filter (FUV+); UV-blocking filter (FUV-). The two filtered treatments were established using colourless and transparent polymetacrylate filters (PMMA XT Vitroflex 295 and XT Vitroflex 395 Solarium Incoloro, Polimertecnic, Girona, Spain). PMMA XT Vitroflex 295 filter allowed for the transmission of UV radiation, whereas PMMA XT Vitroflex 395 filter blocked UV transmission. Filters (1.30 x 0.75 m) were placed at 45° from the vertical axis of the plant, on both sides of the canopy, covering the fruiting zone and the first 0.7 m of the canopy of each grapevine. Filters were installed right after defoliation and maintained until harvest (7 September 2012). Spectral irradiances below filters, and also under Ambient conditions, were measured regularly from the beginning of the experiment (Figure 4.1) using a spectroradiometer (Macam SR9910, Macam Photometrics Ltd, Livingstone, Scotland), to confirm the stability of their filtering characteristics. Ambient photosynthetic (PAR), UV-A, and UV-B irradiances were continuously recorded close to the experimental plot by broad band radiometers (Skye Quantum SKP 215, SKU 420 and SKU 430, respectively, Skye Instruments Ltd, Powys, UK). The

biologically effective UV irradiance (UV_{BE}) was estimated using the action spectrum of Flint and Caldwell (2003). At veraison, fruit and mid-upper canopy temperatures were determined by thermography in each replicate to check the influence of filters. Thermal images were taken at solar noon with a thermal camera (ThermaCAMP640, FLIR Systems, Sweden) as in Pou *et al*. (2014).

Physiological measurements

All the physiological measurements were carried out around noon (except gas exchange) on a sunny day at three different phenological stages: pea size, veraison and harvest. Secondary leaves of the mid-upper part of the shoot (one leaf per vine for each type of measurement), and berries from the basal position of a south-oriented shoot (10 berries per cluster and three clusters per vine), were used for measurements.

The *in vivo* chlorophyll fluorescence of photosystem II (PSII) was measured *in situ*, using a portable fluorimeter (MINI-PAM, Walz, Effeltrich, Germany). Maximum (F_m) and minimum (F_0) fluorescence values were determined in intact leaves, previously subjected to dark adaptation, and the maximum quantum yield of PSII (F_v/F_m) was determined, where $F_v = F_m – F_0$ (Núñez-Olivera *et al*., 2006).

Net photosynthesis rate (A_n) and stomatal conductance (g_s) were measured *in situ* in the morning using an infrared open gas exchange system (Li-6400, Li-Cor Inc., Lincoln, NE, USA). All measurements were conducted at ambient conditions of humidity and air temperature.

Chlorophylls and carotenoids were analyzed in leaf discs by HPLC (Del-Castillo-Alonso *et al*., 2015). Two leaf discs (3.98 cm^2 each) were sampled from the lamina, avoiding main leaf nerves. Frozen leaf discs were twice ground for 45 s at 30 Hz using a Tissue-Lyser (Qiagen, Hilden, Germany). Then, pigments were extracted using 100% acetone (with 0.5 g L^{-1} of $CaCO_3$) in darkness for 24 h at 4 °C. Identification and quantification were carried out on a HP1100 HPLC (Agilent Technologies, Palo Alto, CA, USA). Commercial standards of chlorophyll *a* and *b* (Fluka, Buchs, Germany) and carotenoids (CaroteNature, Ostermundigen, Switzerland) were used to

build calibration curves for quantification. Total chlorophylls (Chl *a* + *b*), Chl *a* / *b* ratio, *β*-carotene, and xanthophylls such as lutein (L), neoxanthin (N), violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z), were determined. The xanthophyll index was computed as the (A+Z) / (A+Z+V) ratio.

Phenolic compounds were analyzed in both leaves and berry skins, differentiating the methanol-soluble (MSPC) and -insoluble (MIPC) fractions, which are mainly located, respectively, in the vacuoles or bound to the cell walls (Del-Castillo-Alonso *et al*., 2015). The bulk levels of both MSPC and MIPC were measured by spectrophotometry and the concentrations of individual compounds by HPLC (leaves) or UPLC (berry skins), following Del-Castillo-Alonso *et al*., (2015). For these analyses, berry skins were removed from the flesh and seeds using a scalpel and then immediately submerged in liquid nitrogen and lyophilized. Lyophilized berry skins were ground in a UltraTurrax® T25 Basic (IKA Labortechnik, Staufen, Germany) and 50 mg of this material were ground in a TissueLyser to obtain a homogeneous powder for each measurement. Two leaf discs (3.98 cm^2 each) per measurement were frozen in liquid nitrogen and ground in a TissueLyser. Then, 4 mL of methanol: water: 7M HCl (70:29:1 v:v:v) for berry skin samples and 5 ml for leaf samples, was added for extraction (24 h at 4 °C in the dark) for each analytical sample. The extract was centrifuged at 6000 x *g* for 15 min and the supernatant and pellet were considered the source of, respectively, MSPC and MIPC. The pellet was then hydrolyzed with 1 mL of 1M NaOH for 3 hours in a water bath at 80 °C. Afterwards, 1 ml of HCl (5.6 N) was added and the sample was rinsed three times with ethyl acetate. The supernatant obtained from the rinsing process was then allowed to evaporate (Büchi R-200, Büchi Labortechnik, Flawil, Switzerland) at 40 °C, and the remaining material was resuspended in 100% methanol up to a final volume of 1 mL (for leaf samples) or 2 mL (for berry skin samples).

In both soluble and insoluble fractions, bulk levels of phenolic compounds were measured as the area under the absorbance curve in the interval 280–400 nm ($AUC_{280–400}$: Del-Castillo-Alonso *et al*., 2015) using a Perkin-Elmer λ35 spectrophotometer (Perkin-Elmer, Wilton, CT, USA).

Soluble and insoluble individual phenolic compounds were analyzed by either HPLC (leaves) or UPLC (berry skins). HPLC determinations were performed using an Agilent HP1100 HPLC system. UPLC analyses were performed using a Waters Acquity Ultra Performance LC system (Waters Corporation, Milford, MA, USA). The UPLC system was coupled to a micrOTOF II high-resolution mass spectrometer (Bruker Daltonik, Bremen, Germany) equipped with an Apollo II ESI/APCI multimode source and controlled by the Bruker Daltonics DataAnalysis software. A UV detector module was used at 520 nm for the anthocyanins and at 324 nm for the rest of compounds. The electrospray source was operated in negative mode, except for the anthocyanins which operated in positive mode. The capillary potential was set to 4 kV; the drying gas temperature was 200 °C and its flow 9 L min^{-1}; the nebulizer gas was set to 3.5 bar and 25 °C. Spectra were acquired between *m/z* 120 and 1505 in both modes.

The different phenolic compounds analyzed were identified and quantified using commercial pure compounds. In absence of the commercial standard, compounds with the same chromophore were used: stilbenes using *t*-resveratrol (Sigma-Adrich, St. Louis, USA); flavanols using catechin, epigallocatechin (Sigma-Adrich, St. Louis, USA) and procyanidin B1 (Fluka, Buchs, Germany); flavonols using kaempferol-glucoside, quercetin-3-*O*-glucuronide (Fluka, Buchs, Germany), myricetin, quercetin, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucopyranoside, quercetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside and syringetin-3-*O*-glucoside (Sigma-Adrich, St. Louis, USA); hydroxycinnamic acids and its derivatives using caffeic, *p*-coumaric and ferulic acids (Sigma-Adrich, St. Louis, USA); hydroxybenzoic acids using gallic acid, syringic acid (Sigma-Adrich, St. Louis, USA) and protocatechuic acid (Fluka, Buchs, Germany); and anthocyanins using malvidin-3-*O*-glucoside (Extrasynthese, Genay, France). For both berry anthocyanins and flavonols, different ratios were calculated on the basis of their substitutions and hydroxylation levels (Bogs *et al*., 2006; Jeong *et al*., 2006; Martínez-Lüscher *et al*., 2014a): hydroxylated / methoxylated, trisubstituted / disubstituted, and trihydroxylated / dihydroxylated. For flavonols, additional ratios were calculated: trihydroxylated /

monohydroxylated, dihydroxylated / monohydroxylated, and (trihydroxylated+dihydroxylated) / monohydroxylated.

Statistical analysis

Pearson correlation coefficient (r) was used to examine the relationships between selected variables. The effect of the treatment (radiation regime) and time (phenological stage) on the variables measured was tested using a 2-way analysis of variance (ANOVA) with repeated measures for time, once proved that the data met the assumptions of normality (Shapiro–Wilks's test) and homoscedasticity (Levene's test). In the case of significant differences, means were compared by Tukey's test. Non-parametric tests (Kruskal-Wallis) were used if the data did not meet those assumptions. In this case, and when significant differences occurred, means were compared by Mann-Whitney's test. All the statistical procedures were performed with SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Light and temperature climate

UV_{BE} doses received by the plants from the onset of treatments until harvest (93 days) were 1782, 1524 and 51 kJ m^{-2} for Ambient, FUV+ and FUV- conditions, respectively. Total photosynthetically active radiation (PAR) doses during the experiment were 784.8, 687.8, and 650.2 MJ m^{-2} for Ambient, FUV+ and FUV-, respectively. On a typical summer day (Figure 4.1), peak daily irradiances (W m^{-2}) were: for Ambient treatment, 1.45 UV-B and 44.6 UV-A; for FUV+ treatment, 1.2 and 39.1; and for FUV- treatment, 0.03 and 3.47.

Berry temperature was 30.5±0.2, 31.4±0.2 and 31.0±0.2 under Ambient, FUV+ and FUV- conditions, respectively. Temperature in FUV+ berries was significantly higher (p = 0.012) as compared to Ambient conditions. No difference due to UV exclusion was detected. Mid-upper canopy temperature was 27.6±0.2, 26.4±0.4 and 26.0±0.5 in Ambient, FUV+ and FUV- conditions, respectively. Canopy temperatures under FUV+ and FUV- were significantly lower (p<0.01) than in Ambient.

Leaf physiology

The values recorded for A_n, g_s and F_v/F_m for all three treatments across the season are shown in Figure 4.2. A_n, g_s and F_v/F_m were not significantly affected by the treatment at the pea size stage. However, at veraison, A_n increased significantly in the absence of UV (FUV-) with respect to Ambient, and differences remained at harvest, where FUV- plants showed higher A_n rates than Ambient and FUV+ plants. A_n was higher at pea size and veraison than at harvest. A similar behavior to A_n was observed for g_s. In contrast, F_v/F_m values were higher at harvest than at pea size and veraison stages, and differences between treatments were found only at veraison, where FUV- leaves exhibited lower values than Ambient and UV+ leaves.

Chlorophylls (*a*, *b* and *a* + *b*) increased almost two-fold from pea size to veraison, and then decreased until harvest in all three treatments (Table 4.1). The global effect of the radiation

regime was not significant, and only at harvest chlorophyll contents were lower in FUV- leaves than in Ambient leaves. The Chl *a/b* ratio increased from pea size stage to veraison and then remained stable until harvest, but the effect of radiation regime was almost negligible. Leaf carotenoids and the xanthophyll index were not affected by the radiation regime and their temporal changes were diverse (Table 4.1).

No significant differences were found for MSPC or MIPC due to the radiation regime in any of the phenological stages, but both variables (particularly MSPC) increased over the season (Table 4.1). The soluble fraction represented around 65% of the total phenolic content in leaves regardless the UV treatment, and was mostly composed by glycosilated flavonols and two hydroxycinnamic acid derivatives. In the insoluble fraction, only *p*-coumaric acid was identified. The radiation regime affected the content of most phenolic individual compounds, both insoluble and soluble. In general, FUV- leaves had lower contents of most flavonols and higher contents of *p*-coumaric acid and coumaroyl-tartaric acid than ambient and FUV+ leaves (Table 4.1). Regarding temporal changes, flavonols generally showed the lowest values at pea size, increased at veraison and remained stable or continued increasing at harvest. Soluble hydroxycinnamic acids did not show significant temporal changes, but the insoluble *p*-coumaric acid decreased over the season.

UV absorbing compounds in berry skins

MSPC or MIPC contents increased or decreased, respectively, over the season (Table 4.2). The soluble reservoir accounted for 54, 65 and 80% of total berry skin phenolics at pea size, veraison and harvest respectively, and it was composed by 40 phenolic compounds. Of these, anthocyanins, flavonols, and flavanols, were the most abundant compounds at veraison and harvest, followed by hydroxycinnamic acid derivatives (the most abundant compounds at pea size) and stilbenes (Table 4.2). A total of 15 anthocyanins were identified, comprising the glucosylated, acetyl and coumaroyl forms of cyanidin, delphinidin, malvidin, peonidin and petunidin. In addition, 14 flavonols, 6 flavanols, 3 hydroxycinnamic acid derivatives and 2

stilbenes, were also identified. Almost every soluble phenolic compound showed significant temporal changes. The total contents of anthocyanins, flavonols and stilbenes, as well as most of the individual compounds of these phenolic families, increased, whereas flavanols and hydroxycinnamic acids derivatives decreased, as the season progressed (Figure 4.3, Table 4.2). Anthocyanins were not detected at pea size, whereas they strongly increased between veraison and harvest. The anthocyanin profile significantly changed from veraison to harvest: the trisubstituted/disubstituted ratio increased whereas the hydroxylated/methoxylated and trihydroxylated/dihydroxylated ratios decreased (Figure 4.4).

The radiation regime did not affect MSPC or MIPC contents (Table 4.2). At the individual scale, only a few soluble compounds were globally affected by the radiation regime, whereas a greater number of them were affected at specific phenological stages (Table 4.2, Figure 4.3). At veraison, the total anthocyanins, as well as most malvidins, petunidins and delphinidins, had lower contents under both filter treatments than in Ambient regime. Cyanidins and peonidins contents did not show differences between treatments, or were lower only in FUV- than in Ambient. At harvest, most of these differences had disappeared, and only the contents of total anthocyanins and one malvidin showed significant differences between treatments. Remarkably, total anthocyanin content was higher under both filter treatments than in Ambient regime. The radiation regime did not affect the anthocyanin profile except the changes found in the ratio trisubstituted/disubstituted anthocyanins at veraison, with lower values in FU+ and FUV- plants than in Ambient (Figure 4.4).

The soluble flavonols were the phenolics most markedly affected by the radiation regime, and their total content was significantly lower at veraison and harvest in FUV- berries, in comparison to UV-exposed ones (Figure 4.3). The same behaviour was observed for most individual flavonols (Table 4.2), excluding kaempferol-3-*O*-galactoside, isorhamnetin-3-*O*-galactoside and syringetin-3-*O*-glucoside, which showed no differences between radiation regimes at any phenological stage. The three myricetins detected, together with quercetin-3-*O*-glucoside and

isorhamnetin-3-*O*-glucuronide, showed lower values under FUV- treatment at veraison, but these differences disappeared at harvest. The flavonol profile strongly changed as influenced by both radiation regime and time (Figure 4.4). The ratios between trisubstituted and disubstituted flavonols and between hydroxylated and methoxylated flavonols significantly increased and decreased, respectively, from veraison to harvest. At harvest, the ratios between trisubstituted and disubstituted flavonols and between hydroxylated and methoxylated flavonols significantly increased and decreased, respectively, in FUV- plants in comparison with FUV+ and Ambient plants. The remaining flavonol ratios changed similarly to the trisubstituted/disubstituted ratio (data not shown).

Of the remaining soluble phenolic compounds, the content of one hydroxycinnamic acid derivative (caffeoyl-tartaric acid) was higher at harvest in FUV- berries, whereas stilbenes and flavanols levels increased only in FUV+ berries, and significant differences were only found for resveratrol-3-*O*-glucoside and epicatechin (Figure 4.3, Table 4.2).

In the insoluble fraction, two hydroxycinnamic and three hydroxybenzoic acids were identified (Table 4.2). Except syringic acid, whose contents considerably increased at harvest, the content of individual phenolic acids decreased from pea size to harvest. The total content of hydroxycinnamic and hydroxybenzoic acids did not show clear temporal trends (Figure 4.3). The two hydroxycinnamic acids (*p*-coumaric and caffeic acids) contents were not clearly affected by the different UV treatments, whereas one hydroxybenzoic acid (syringic acid) was found to decrease in both FUV+ and FUV- berries with respect to Ambient berries at harvest (Table 4.2).

Correlation analysis

Correlations between phenolic compounds of leaves and berries are shown in Table 4.3. Kaempferols were the only compounds whose leaf and berry skin contents were significantly and positively correlated. Leaf kaempferols were also positively correlated with berry skin quercetins. Correlations between radiation variables (PAR and UV_{BE} doses) and phenolic compounds of leaves and berries were calculated (Table 4.3, Figure 4.5), taking advantage of the

different doses of PAR and UV_{BE} radiation received by the plants under the three different radiation regimes (Ambient, FUV+ and FUV-) at the three different phenological stages considered. UV_{BE} dose was positively correlated only with the leaf and berry skin contents of quercetins and kaempferols. The relationships between UV_{BE} dose and the contents of these compounds were linear except for the exponential relationship between UV_{BE} dose and berry skin kaempferols (Figure 4.5). PAR dose was positively correlated with several compounds, including the leaf contents of MSPC, kaempferol and quercetins, and the berry skin contents of MSPC, flavonols, isorhamnetins, myricetins and anthocyanins. However, PAR dose was not correlated with berry skin kaempferols and quercetins. Stilbene contents were not correlated with any radiation variable.

DISCUSSION

Leaf physiology and metabolites

A_n and g_s were higher in UV-deprived plants, despite the lower PAR levels received by these plants in comparison with those of Ambient and FUV+ treatments. The decrease in A_n and g_s in UV-exposed plants would indicate a UV-induced stress, although slight because this effect was not corroborated by alterations in F_v/F_m or photosynthetic pigment contents. Thus, the response of photosynthesis to UV radiation in our study seems to have been mainly governed by CO_2 exchange, confirming the results obtained for other grapevine varieties in similar ambient UV exclusion studies (Kolb *et al*., 2001; Berli *et al*., 2013) or even under UV-B supplementation (Doupis *et al*., 2012; Majer and Hideg, 2012a,b). In this regard, UV-B radiation has been reported to affect stomata by acting on the guard cells aperture control mechanism (Nogués *et al*., 1999). In contrast to these results, Pollastrini *et al*. (2011) and Alonso *et al*. (2015) found no differences in the stomatal conductance and/or net photosynthesis rates of grapevine leaves that had or not received ambient solar UV radiation. This discrepancy with our results can be attributed to the cultivar effect or to differences in experimental conditions, because their grapevines were cultured in pots (Pollastrini *et al*., 2011) or at high altitude (Alonso *et al*., 2015).

In our study, the decrease in A_n and g_s in UV-exposed plants was not found at pea size stage but was significant at veraison and harvest. Thus, this decrease was progressively stronger over time. This suggests that the plants had acclimated to natural UV levels during the initial development stages, from bud break until the filters were installed in the pre-flowering stage. Therefore, effects of UV exclusion were delayed with respect to the treatments imposition. This is not surprising because UV effects may be cumulative over time (Götz *et al*., 2010).

The slight stressing effect of ambient UV levels on CO_2 exchange was not associated to important alterations in the efficiency of PSII, given that F_v/F_m values remained around 0.80 for all treatments and sampling dates, indicating no stress. These results are in agreement with those

obtained in other UV filtering experiments conducted on Tempranillo (Núñez-Olivera *et al*., 2006) and Silvaner (Kolb *et al*., 2001). Besides, Martínez-Lüscher *et al*. (2015) did not observe changes in F_v/F_m in Tempranillo leaves under supplemental UV-B doses either. A decrease in F_v/F_m in grapevine leaves exposed to full sunlight in comparison with leaves receiving UV-deprived solar radiation has been found in other studies using different varieties, but these studies were performed on young (2-year old) plants (Pfündel, 2003; Pollastrini *et al*., 2011) or in high-altitude localities where solar UV levels are higher than those used in our study (Berli *et al*., 2013). These factors could have influenced the responses of those leaves in comparison with ours.

Regardless the UV treatment, the temporal changes of chlorophyll content in this work were coherent with the temporal pattern observed for A_n and g_s, which has been well documented (Palliotti *et al*., 2010). The decrease in chlorophyll contents in FUV- Tempranillo leaves with respect to Ambient ones only, suggests a combined effect of both UV and PAR reduction (caused by the filter), rather than a mere UV-induced effect. As occurs in other variables, the effects of UV radiation on the photosynthetic pigment contents in grapevines cannot be compared directly among works, due to differences in plant material and experimental conditions (Jug and Rusjan, 2012). For example, ambient or supplemental UV-B levels did not alter chlorophyll content in leaves of Cabernet Sauvignon (Keller *et al*., 2003), Malbec (Berli *et al*., 2010) and Chardonnay (Majer and Hideg, 2012a,b), but led to a decrease in leaves of Malbec (Berli *et al*., 2013) and Romeiko (Doupis *et al*., 2012). Chl *a/b* ratio, the content of different carotenoids and the xanthophyll index changed over the season showing different temporal patterns, but were not clearly affected by the radiation regime. This lack of effects is in agreement with Del-Castillo-Alonso *et al*. (2015), although increased UV levels usually lead to enhanced photoprotective carotenoid contents (Doupis *et al*., 2012; Berli *et al*., 2013). However, the relative amounts of individual compounds can be differentially altered, and various responses can occur depending on leaf age (Majer and Hideg, 2012a,b) and cultivar (Núñez-Olivera *et al*., 2006).

Regarding leaf phenolic compounds, the bulk levels of MSPC and MIPC were not affected by the radiation treatments in Tempranillo. This confirms previous data found for both variables in a filtering experiment performed on Graciano grapevines (Del-Castillo-Alonso *et al*., 2015). However, higher MSPC levels were found in UV-exposed leaves in comparison to UV-deprived leaves in Tempranillo and Viura (Núñez-Olivera *et al*., 2006), Sangiovese (Pollastrini *et al*., 2011) and Malbec (Berli *et al*., 2013; Alonso *et al*., 2015). The contrasting results found for global variables, such as MSPC and MIPC (and other similar variables), can be partially explained because each individual compound or phenolic group contributing to these global variables may respond to UV exclusion in a different manner, and these different responses may compensate each other and obscure the global response. Thus, complementary analysis of individual compounds is always recommendable to obtain more reliable and specific results. In this sense, most hydroxycinnamic acid derivatives increased and flavonols decreased in FUV- plants, which confirms previous results obtained in UV-filtering experiments using leaves of different grapevine varieties (Kolb *et al*., 2001; Berli *et al*., 2010; Grifoni *et al*., 2016). This contrasting behavior of hydroxycinnamic acid derivatives and flavonols would be caused by the greater availability of precursors for the synthesis of hydroxycinnamic acid derivatives in the absence of adequate UV stimulus for the synthesis of flavonols (Kolb *et al*., 2001).

Berry skin metabolites

As occurred in leaves, the bulk levels of MSPC and MIPC in berry skins were not influenced by ambient UV exclusion. As commented previously, these global variables may hide the responses of the different phenolic groups and individual compounds, particularly in such a phenolically diverse material as berry skins. In this sense, glycosilated flavonols were the most UV-responsive compounds, increasing in UV-exposed berry skins (Ambient and FUV+ treatments) both at veraison and harvest. In contrast, UV radiation had a lesser impact on anthocyanins, flavanols, stilbenes and hydroxycinnamic derivatives. These responses are consistent with previous works performed both on Tempranillo and other cultivars (Cortell and Kennedy, 2006; Koyama *et al*.,

2012; Carbonell-Bejerano *et al*., 2014). This diversity of responses is congruent with the high phenolic diversity of berry skins, and with the fact that not all the gene sequences (at least five in grapevine: Fujita *et al*., 2006) encoding the enzyme flavonol-synthase may be overexpressed by the UV doses received (Liu *et al*., 2015).

The response of flavonols was not surprising because their contents are well known to increase with increasing UV levels (particularly UV-B) in grapevine berry skins of different varieties and under diverse experimental conditions (Spayd *et al*., 2002; Downey *et al*., 2004; Berli *et al*., 2011; Koyama *et al*., 2012; Carbonell-Bejerano *et al*., 2014; Martínez-Lüscher *et al*., 2014a, 2014b; Liu *et al*., 2015). Thus, flavonol accumulation is probably the most reliable response of grape skins (and also leaves) to increasing UV radiation. The accumulation trend of flavonols visibly reflected the two triggering points of their synthesis, the first one around flowering and the second right after veraison (Downey *et al*., 2003).

Despite the increase in flavonol content in UV-exposed berries, the flavonol ratios (except the hydroxylated/methoxylated ratio) were significantly lower in these berries at harvest. This seems contradictory, because the antioxidant activity of flavonoids strongly depends on the number of hydroxyl groups bound to the aromatic B-ring (Sroka, 2005). Thus, the responses of flavonol ratios in UV-exposed berries would reduce their antioxidant capacity, being UV radiation a recognized environmental factor inducing oxidative stress (Hideg *et al*., 2013). Yet, it must be taken into account that antioxidant capacity of Tempranillo berry skins is benefited from a constitutive clear predominance of trihydroxylated (myricetins) and dihydroxylated (quercetins) over monohydroxylated (kaempferols, isorhamnetins and syringetins) flavonols, which relativizes the importance of not increasing the flavonol hydroxylation ratios under solar ambient UV levels. In addition, flavonoids may have other key roles in plants apart from being antioxidants (Agati *et al*., 2013).

It was already known that the flavonol hydroxylation ratios decreased with increasing UV radiation in Tempranillo (Carbonell-Bejerano *et al*., 2014; Martínez-Lüscher *et al*., 2014a) and

Sangiovese (Pastore *et al*., 2013). However, the ratio increased in Pinot Noir (Del-Castillo-Alonso *et al*., 2016). This diversity of results may be attributed to genotypic differences, probably based on the intricate regulation mechanism of the genes and enzymes involved in the synthesis of flavonols with different hydroxylation levels (Downey *et al*., 2004; Bogs *et al*., 2006; Martínez-Lüscher *et al*., 2014b). Indeed, hydroxylations in the positions 3' and 3',5' of the B-ring are catalyzed by the flavonoid 3'- and 3',5'-hydroxylases (F3'H and F3'5'H, respectively: Bogs *et al*., 2006), and light exposure (in particular, UV-B) differentially influences the activity of each enzyme (Koyama *et al*., 2012; Guan *et al*., 2016).

In contrast to flavonols, anthocyanin synthesis starts at veraison (as evidenced in this work), when the anthocyanin specific UDP-glucose flavonoid-3-*O*-glucosyltransferase (UFGT) enzyme starts to be expressed (Boss *et al*., 1996). Anthocyanin content increased by around 10-fold from veraison to harvest, whereas flavonol content only increased by around 2-fold (except the trihydroxylated myricetins, whose increase was similar to that of anthocyanins). The increase of both anthocyanins and flavonols at harvest, and the particular increase of trihydroxylated over dihydroxylated forms, agrees with the results obtained in Sangiovese by Pastore *et al*. (2013).

Anthocyanin accumulation in Tempranillo berry skins was hardly affected by UV exclusion, and most differences between treatments were found between Ambient and filtered samples. In addition, most of these differences disappeared as season progressed. Given that anthocyanin content is influenced by PAR levels in combination with temperature (Azuma *et al*., 2012), the slight differences in anthocyanin content that have been found in our study would be congruent with the modest differences in PAR and temperature between the treatments. The specific effect of UV radiation on anthocyanin content is quite variable, because it may increase (Berli *et al*., 2011; Martínez-Lüscher *et al*., 2014b), decrease (Cortell and Kennedy, 2006; Guan *et al*., 2016) or remain unaltered (Price *et al*., 1995; Spayd *et al*., 2002; Downey *et al*., 2004) with increasing UV. In particular, high UV doses might be necessary to induce significant changes in anthocyanins, as occurs in Malbec berries from high altitudes (Berli *et al*., 2008, 2011), and the

effects are likely to be genotype-dependent. The different UV response of flavonols and anthocyanins in our study is not strange, because the biosynthesis of the two groups of compounds is controlled by different regulation systems: flavonols and anthocyanins accumulate at different stages of development, and the transcription of biosynthetic genes, the activity of certain biosynthetic enzymes, and the hormonal regulation, is different for each type of compound (Fujita *et al.*, 2006). Consequently, optimization of flavonols accumulation may compromise the anthocyanins production (Mattivi *et al.*, 2006).

In our study, the anthocyanin ratios changed from veraison to harvest, but UV exclusion did not affect these ratios. The only differences related to radiation regime were between filtered and non-filtered berries, and these changes were slight because they occurred only at veraison and disappeared at harvest. Thus, these differences could again be attributed to the slight PAR or temperature changes between treatments. The anthocyanin profile has been suggested to be influenced by sunlight exposure, but the specific effects are far from clear. For example, with increasing total or UV radiation, the hydroxylation ratio of anthocyanins may increase in Merlot (Spayd *et al.*, 2002) and Pinot Noir (Cortell and Kennedy, 2006), but the opposite effect was observed in Malbec (Berli *et al*, 2011) and Tempranillo (Martínez-Lüscher *et al.*, 2014b). These responses even vary depending on the year of study (Spayd *et al.*, 2002; Downey *et al.*, 2004). This diversity of results may be explained by the complex regulation of the synthesis of differently hydroxylated anthocyanins in the different varieties (Jeong *et al.*, 2006).

The observed weak effect of UV exclusion on flavanol content may rely on the fact that flavanols are little responsive to changes in environmental factors, including radiation (Cortell and Kennedy, 2006; Sternad-Lemut *et al.*, 2013). Nevertheless, flavanol content decreases in shaded berries (Koyama *et al.* 2012), and also responds to temperature (Cohen *et al.*, 2012). Responses to temperature in our study were not solid, probably because of little temperature differences between treatments.

Most phenolic acids (hydroxycinnamic and hydroxybenzoic acids and their derivatives) in both the soluble and insoluble fractions decreased from pea size onwards, accordingly to Romeyer *et al*. (1983). Among all these compounds, the total content of hydroxycinnamic acid derivatives and the content of the predominant derivative (caffeoyl-tartaric acid) were higher in FUV- berry skins. This is consistent with the findings of Price *et al*. (1995) and Kolb *et al*. (2001) in other varieties, and could be related to the complementary tuning between the biosynthesis rates of hydroxycinnamic acids and flavonoids, which compete for the same precursors (Kolb *et al*., 2001). Some genotype-dependence may not be discarded either, as hydroxycinnamic levels were not altered by UV exposure in Malbec (Berli *et al*., 2008, 2011). In addition, each specific cinnamic acid may differently react to UV (Carbonell-Bejerano *et al*., 2014).

As for the stilbenes, increased accumulation was observed from veraison to harvest, coherently with the results obtained over three seasons in more than 73 grapevine cultivars, including Tempranillo, by Gatto *et al*. (2008). In our work, resveratrol-3-*O*-glucoside and the sum of stilbenes were greater under FUV+ than under Ambient, and a trend towards increased content in FUV- berries with respect to Ambient ones was observed. This result would suggest a multi-factorial influence in the final stilbene accumulation, rather than a simple effect caused by the differences in UV exposure only. In this regard, only high solar doses, as those existing at high altitudes, have been shown to induce resveratrol accumulation in berry skins of Malbec (Berli *et al*., 2008).

In our study, the temperature differences between the different radiation regimes were low, although in some cases significant. Temperature is a recognized environmental factor influencing the contents of certain phenolic compounds, such as anthocyanins or flavanols (Spayd *et al*., 2002; Azuma *et al*., 2012; Cohen *et al*., 2012), but more extreme temperatures than those recorded in this study are probably required to influence more clearly the phenolic content.

Correlation analysis

Correlations between the content of a specific phenolic group in leaves and the content of its homologous in berry skins were only detected for kaempferols. This would indicate that, mostly, the phenolic composition is differentially regulated in different organs, confirming the findings of Jeong *et al*. (2006). Nevertheless, this is the first time that a relationship between the leaf and berry skin contents of kaempferols has been demonstrated in grapevine under field conditions. With respect to the correlations between radiation variables and phenolic groups, the UV_{BE} dose was positively correlated with the contents of quercetins and kaempferols, both in leaves and berry skins. This is not surprising because both quercetins and kaempferols belong to flavonols and, as commented above, flavonols are the most UV-responsive phenolic compounds in grapevine. Interestingly, all the compounds correlated with UV_{BE} dose varied linearly with this radiation variable except berry skin kaempferols, which showed an exponential relationship. No previous literature data have been found on this specific point, despite the high number of studies carried out on the effects of UV radiation on grapevine berries. The different relationship of UV_{BE} dose with either quercetins or kaempferols suggests a different UV regulation of the synthesis of both types of compounds in berry skins, with kaempferols requiring a much higher UV_{BE} dose than quercetins to reach a significant level. This could be one of the reasons why kaempferol contents are much lower than quercetin contents in berry skins, as it has repeatedly been found (see for example Berli *et al*., 2010; Carbonell-Bejerano *et al*., 2014; Del-Castillo-Alonso *et al*., 2015; Liu *et al*., 2015). The exponential relationship between kaempferol content and UV_{BE} dose would open management possibilities to increase this content through a high UV exposure at specific stages of berry development.

PAR dose was positively correlated with a higher diversity of compounds (MSPC, kaempferol and quercetins in leaves, and MSPC, flavonols, isorhamnetins, myricetins and anthocyanins in berry skins) than UV_{BE} dose was, but it was not correlated with berry skin kaempferols and quercetins. Thus, these compounds were only dependent on UV_{BE} and not on PAR dose. Diverse studies

have demonstrated that PAR can influence the contents of some of the compounds which were correlated with PAR dose in our study (Price *et al*., 1995; Spayd *et al*., 2002; Azuma *et al*., 2012). Yet, it must be taken into account that some of these correlations could simply be based on the fact that PAR dose increased as season progressed, in parallel to the increase of the contents of those compounds during the process of grape maturation. Thus, correlations between PAR dose and the contents of some phenolic compounds could be spurious.

Concluding remarks

Ambient solar UV levels typical of mid-altitude Mediterranean conditions caused a modest physiological stress on Tempranillo leaves and moderate changes in their phenolic composition, mainly inducing flavonol accumulation, which could be interpreted as a protective regulatory response increasing both UV screening and antioxidant capacity. These processes can be circumscribed within the concept of eustress (“good stress”) rather than distress (“destructive stress”) (Hideg *et al*., 2013). Consequently, Tempranillo can be considered as a typical Mediterranean cultivar notably adapted to current UV levels (Núñez-Olivera *et al*., 2006; Carbonell-Bejerano *et al*., 2014), which represent a regulatory factor rather than a generic stressor (Jansen and Bornman, 2012).

Together with leaf changes, ambient solar UV levels moderately influenced the phenolic composition of Tempranillo berry skins. Responses of phenolic compounds to UV were diverse and were modulated by time along berry ripening. Most responses were transitory and only a few were conserved at harvest. As occurred in leaves, flavonol accumulation was the most reliable response of grape skins to ambient solar UV levels, as it has previously been pointed out in other studies. Among flavonols, the behaviour of kaempferols was particularly interesting, because their total contents in leaves and berry skins were positively correlated and their content in berry skins showed a peculiar exponential relationship with UV_{BE} dose. This opens management possibilities to modify kaempferol content in berry skins through UV manipulation.

Overall, this is the first study that, using a major grapevine cultivar grown under typical field Mediterranean conditions, connects leaf and berry characteristics under the influence of ambient solar UV radiation over a complete season. Yet, comparison with other studies may be complex, because results are influenced by genotype, experimental conditions, treatment periods, phenological stage, and plant age. In particular, studies using UV supplementation with lamps seem to have stronger effects than those using ambient UV levels, and thus UV exclusion and UV supplementation studies should be clearly differentiated. More research is needed to fully understand the influence of UV radiation on grapevine leaf and berry skin characteristics.

TABLES AND FIGURES

Table 4.1. Effects of the radiation treatment (Ambient, no filter; FUV+, UV-transmitting filter; FUV-, UV-blocking filter) on photosynthetic pigments and phenolic compounds in the insoluble and soluble fractions of leaves of *Vitis vinifera* L. Tempranillo sampled at three phenological stages: pea size, veraison and harvest. MIPC and MSPC, the bulk UV absorbances of methanol-insoluble and –soluble phenolic compounds, respectively. $AUC_{280\text{-}400}$, area under the absorbance curve in the interval 280-400 nm. Values are expressed as mean ± standard error (n=6). For each variable, significance levels for global effects of treatment (radiation regime) and time (phenological stage), are shown: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, non-significant. For each variable and phenological stage, different letters mean significant differences between treatments (at least at $p < 0.05$).

	Pea size			Veraison			Harvest			*Statistical significance*	
Variables	**Ambient**	**FUV+**	**FUV-**	**Ambient**	**FUV+**	**FUV-**	**Ambient**	**FUV+**	**FUV-**	*Treatment*	*Time*
Photosynthetic pigments											
Chl *a* (µg cm⁻²)	15 ± 1	17 ± 3	16 ± 2	31 ± 1	29 ± 1	30 ± 1	25 ± 1 a	23 ± 1 ab	22 ± 1 b	ns	***
Chl *b* (µg cm⁻²)	6.8 ± 0.4	7.5 ± 1.0	7.0 ± 1.0	13 ± 0	11 ± 1	11 ± 0	9.9 ± 0.3 a	9.0 ± 0.3 ab	8.4 ± 0.3 b	ns	***
Chl *a/b*	2.3 ± 0.1	2.3 ± 0.2	2.4 ± 0.1	2.5 ± 0.0 a	2.5 ± 0.0 a	2.6 ± 0.0 b	2.6 ± 0.0	2.5 ± 0.0	2.6 ± 0.0	ns	***
Chl *(a+b)* (µg cm⁻²)	22 ± 1	25 ± 4	23 ± 3	44 ± 1	40 ± 2	41 ± 1	35 ± 1 a	32 ± 1 ab	30 ± 3 b	ns	***
β-carotene (µg cm⁻²)	4.5 ± 0.3	4.7 ± 0.8	4.5 ± 0.6	6.0 ± 0.2	5.6 ± 0.2	6.0 ± 0.2	5.2 ± 0.2	4.9 ± 0.1	4.8 ± 0.2	ns	***
Neoxanthin (µg cm⁻²)	2.1 ± 0.2	2.3 ± 0.4	2.1 ± 0.3	3.8 ± 0.1	3.4 ± 0.1	3.3 ± 0.1	3.3 ± 0.1	3.4 ± 0.1	3.2 ± 0.1	ns	***
Violaxanthin (µg cm⁻²)	2.0 ± 0.3	2.0 ± 0.5	2.0 ± 0.4	4.1 ± 0.2	3.7 ± 0.2	4.0 ± 0.2	4.4 ± 0.2	4.0 ± 0.2	4.1 ± 0.2	ns	***
Antheraxanthin (µg cm⁻²)	0.54 ± 0.11	0.57 ± 0.16	0.63 ± 0.15	0.73 ± 0.05	0.70 ± 0.05	1.1 ± 0.1	0.41 ± 0.05	0.46 ± 0.07	0.72 ± 0.11	ns	***
Lutein (µg cm⁻²)	8.2 ± 0.4	8.3 ± 1.1	7.6 ± 1.0	12 ± 0	11 ± 0	11 ± 0	12 ± 0	12 ± 0	11 ± 0	ns	***
Zeaxanthin (µg cm⁻²)	0.27 ± 0.10	0.56 ± 0.16	0.40 ± 0.13	0.34 ± 0.03	0.30 ± 0.02	0.43 ± 0.03	0.23 ± 0.02	0.26 ± 0.03	0.26 ± 0.02	ns	***
Xanthophyll Index ((A+Z)/(V+A+Z))	0.80 ± 0.04	0.81 ± 0.04	0.79 ± 0.04	0.79 ± 0.06	0.82 ± 0.06	0.77 ± 0.06	0.84 ± 0.05	0.83 ± 0.05	0.77 ± 0.06	ns	***
Insoluble compounds											
MIPC ($AUC_{280\text{-}400}$ cm⁻²)	61 ± 7	71 ± 3	73 ± 6				78 ± 3	72 ± 4	81 ± 4	ns	*
p-Coumaric acid (µg cm⁻²)	0.71 ± 0.08 a	0.74 ± 0.07 a	1.0 ± 0.1 b				0.54 ± 0.04 a	0.57 ± 0.04 a	0.77 ± 0.04 b	***	***
Soluble compounds											
MSPC ($AUC_{280\text{-}400}$ cm⁻²)	103 ± 10	120 ± 7	110 ± 13	155 ± 7	137 ± 4	123 ± 5	150 ± 5	142 ± 7	149 ± 6	ns	*
Caffeoyl-tartaric acid (µg cm⁻²)	25 ± 2	26 ± 2	19 ± 4	32 ± 2	28 ± 2	27 ± 3	23 ± 2	21 ± 3	30 ± 3	ns	ns
Coumaroyl-tartaric acid (µg cm⁻²)	1.8 ± 0.3	1.9 ± 0.2	2.2 ± 0.3	1.7 ± 0.1 a	1.6 ± 0.1 a	2.6 ± 0.1 b	1.5 ± 0.1 a	1.6 ± 0.1 a	3.2 ± 0.8 b	***	ns
Kaempferol 3-*O*-glucoside (µg cm⁻²)	1.5 ± 0.3	1.3 ± 0.1	1.1 ± 0.2	2.3 ± 0.3 a	1.9 ± 0.3 a	1.1 ± 0.1 b	2.3 ± 0.2 a	2.3 ± 0.2 a	1.7 ± 0.2 b	***	**
Quercetin 3-*O*-galactoside (µg cm⁻²)	6.6 ± 1.5	8.5 ± 1.9	6.7 ± 1.1	16 ± 2 a	12 ± 1 b	9.1 ± 1.0 b	11 ± 1	11 ± 2	15 ± 3	ns	**
Quercetin 3-*O*-glucuronide (µg cm⁻²)	48 ± 7	54 ± 4	45 ± 5	75 ± 5 a	67 ± 5 ab	56 ± 5 b	73 ± 4 a	70 ± 5 a	61 ± 5 b	**	**
Quercetin 3-*O*-glucopyranoside (µg cm⁻²)	3.6 ± 0.7	4.5 ± 0.7	3.1 ± 0.7	13 ± 2 a	8.6 ± 1.6 b	5 ± 0.6 c	13 ± 1 a	12 ± 2 ab	10 ± 2 b	***	***

Table 4.2. Effects of the radiation treatment (Ambient, no filter; FUV+, UV-transmitting filter; FUV-, UV-blocking filter) on phenolic compounds in the insoluble and soluble fractions of berry skins of *Vitis vinifera* L. Tempranillo sampled at three phenological stages: pea size, veraison and harvest. MIPC and MSPC, the bulk UV absorbances of methanol-insoluble and –soluble phenolic compounds, respectively. $AUC_{280\text{-}400}$, area under the absorbance curve in the interval 280-400 nm. Values are expressed as mean ± standard error (n=6). For each variable, significance levels for global effects of treatment (radiation regime) and time (phenological stage), are shown: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, non-significant. For each variable and phenological stage, different letters mean significant differences between treatments (at least at $p < 0.05$).

	Pea size	Veraison			Harvest			Statistical significance	
Insoluble compounds	**Ambient**	**Ambient**	**FUV+**	**FUV-**	**Ambient**	**FUV+**	**FUV-**	*Treatment*	*Time*
MIPC ($AUC_{280\text{-}400}$ mg^{-1} DW)	29 ± 1	18 ± 2	17 ± 1	17 ± 1	13 ± 1	12 ± 1	12 ± 0	ns	***
Hydroxycinnamic acids (µg g^{-1} DW)									
Caffeic Acid	37 ± 3	22 ± 6	15 ± 4	17 ± 5	3.3 ± 0.3	3.1 ± 0.4	2.7 ± 0.6	ns	***
p-Coumaric acid	238 ± 18	122 ± 12	120 ± 17	167 ± 15	166 ± 8	128 ± 13	169 ± 15	*	***
Hydroxybenzoic acids (µg g^{-1} DW)									
Gallic Acid	514 ± 52	36 ± 6	36 ± 8	31 ± 9	24 ± 4	32 ± 13	27 ± 12	ns	***
Protocatechuic Acid	49 ± 4	7.7 ± 1.5	6.3 ± 1.5	5.6 ± 1.4	8.6 ± 1.2	5.3 ± 1.2	6.7 ± 1.1	ns	***
Syringic Acid		35 ± 9	22 ± 6	19 ± 4	121 ± 12 **a**	83 ± 10 **b**	85 ± 5 **b**	**	***
Soluble compounds									
MSPC ($AUC_{280\text{-}400}$ mg^{-1} DW)	35 ± 1	37 ± 3	31 ± 2	28 ± 2	49 ± 5	50 ± 3	48 ± 3	ns	***
Stilbenes (µg g^{-1} DW)									
Resveratrol					0.44 ± 0.14	1.9 ± 0.9	0.6 ± 0.3	ns	-
Resveratrol-3-*O*-glucoside	3.3 ± 0.5	0.64 ± 0.10 **a**	5.1 ± 1.5 **b**	3.9 ± 1.4 **ab**	3.3 ± 0.7 **a**	24 ± 8 **b**	11 ± 5 **ab**	**	**
Flavanols (µg g^{-1} DW)									
Catechin	983 ± 36	637 ± 61	810 ± 112	752 ± 126	53 ± 9	93 ± 17	68 ± 6	ns	***
Catechin gallate	521 ± 14	0.57 ± 0.08	0.37 ± 0.08	0.44 ± 0.08				ns	***
Epicatechin	20 ± 2	18 ± 2	15 ± 2	14 ± 2	8.5 ± 1.0 **a**	17 ± 2 **b**	11 ± 1 **a**	ns	***
Epigallocatechin gallate	19 ± 1							-	-
Procyanidin B1	603 ± 36	226 ± 30	275 ± 41	220 ± 33	92 ± 15	130 ± 22	93 ± 7	ns	***
Procyanidin C1	0.44 ± 0.02	0.07 ± 0.01	0.11 ± 0.02	0.10 ± 0.02	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	ns	***

Soluble compounds	Pea size: Ambient	Veraison: Ambient		Veraison: FUV+		Veraison: FUV-		Harvest: Ambient		Harvest: FUV+		Harvest: FUV-		*Statistical significance*: *Treatment*	*Time*
Flavonols (µg g^{-1} DW)															
Kaempferol-3-*O*-glucoside	23 ± 2	226 ± 29	a	31 ± 4	b	3.8 ± 1.0	c	584 ± 40	a	383 ± 56	b	27 ± 4	c	***	**
Kaempferol-3-*O*-galactoside								8.5 ± 0.8		11 ± 1		9.2 ± 1.2		ns	-
Myricetin		24 ± 7	a	12 ± 4	ab	6.3 ± 1.0	b	232 ± 15		236 ± 21		271 ± 25		ns	***
Myricetin-3-*O*-glucoside		164 ± 44	a	50 ± 14	b	25 ± 4	c	2545 ± 84		2568 ± 117		2169 ± 188		**	***
Myricetin-3-*O*-glucuronide	22 ± 1	72 ± 18	a	29 ± 4	b	15 ± 1	c	603 ± 37		614 ± 60		530 ± 45		*	***
Quercetin-3-*O*-glucoside	0.91 ± 0.08	51 ± 6	a	36 ± 6	ab	33 ± 3	b	91 ± 12		88 ± 9		102 ± 9		ns	***
Quercetin-3-*O*-galactoside	31 ± 4	64 ± 14	a	39 ± 13	ab	5.4 ± 1.1	b	276 ± 14	a	204 ± 26	a	35 ± 6	b	***	***
Quercetin-3-*O*-glucuronide	1553 ± 54	1331 ± 159	a	1098 ± 103	a	399 ± 39	b	1625 ± 62	a	1485 ± 132	a	703 ± 61	b	***	***
Quercetin-3-*O*- glucopyranoside	144 ± 16	341 ± 59	a	216 ± 52	ab	33 ± 5	b	901 ± 29	a	781 ± 51	a	248 ± 32	b	***	***
Quercetin-3-*O*-rutinoside	48 ± 6	82 ± 20	a	48 ± 12	ab	8.2 ± 1.4	b	67 ± 8	a	51 ± 13	a	11 ± 1	b	***	ns
Isorhamnetin-3-*O*-glucuronide	41 ± 2	17 ± 2	a	10 ± 2	a	3.6 ± 0.7	b	5.1 ± 1.3		2.9 ± 1.1		1.5 ± 1.0		**	***
Isorhamnetin-3-*O*-galactoside								114 ± 14		118 ± 25		161 ± 26		ns	-
Isorhamnetin-3-*O*-glucoside								30 ± 3	a	25 ± 1	a	7.0 ± 1.7	b	***	-
Syringetin-3-*O*-glucoside								51 ± 5		48 ± 3		48 ± 9		ns	-
Hydroxycinnamic acid derivatives (mg g^{-1} DW)															
Caffeoyl-tartaric acid	46 ± 2	2.6 ± 0.4		2.1 ± 0.2		3.2 ± 0.4		0.52 ± 0.06	a	0.52 ± 0.06	a	0.81 ± 0.07	b	ns	***
Coumaroyl-tartaric acid	6.3 ± 0.1	2.2 ± 0.3		2.2 ± 0.2		2.7 ± 0.3		0.29 ± 0.04		0.32 ± 0.03		0.44 ± 0.07		ns	***
Feruloyl-tartaric acid	0.82 ± 0.04	0.03 ± 0.00	a	0.04 ± 0.00	ab	0.05 ± 0.01	b	0.04 ± 0.00		0.05 ± 0.00		0.04 ± 0.00		ns	***
Anthocyanins (mg g^{-1} DW)															
Cyanidin-3-*O*-glucoside		0.94 ± 0.14		0.79 ± 0.19		0.60 ± 0.07		1.8 ± 0.2		2.1 ± 0.3		2.0 ± 0.3		ns	***
Delphinidin-3-*O*-glucoside		2.9 ± 0.3		1.9 ± 0.3		1.6 ± 0.1		7.4 ± 0.7		7.6 ± 0.7		8.3 ± 1.1		ns	***
Malvidin-3-*O*-glucoside		6.6 ± 0.6	a	3.8 ± 0.5	b	4.0 ± 0.5	b	43 ± 2		46 ± 4		52 ± 5		ns	***
Peonidin-3-*O*-glucoside		2.5 ± 0.3		1.8 ± 0.4		1.6 ± 0.2		6.1 ± 0.6		7.1 ± 0.9		7.5 ± 1.1		ns	***
Petunidin-3-*O*-glucoside		2.5 ± 0.2	a	1.6 ± 0.2	ab	1.5 ± 0.1	b	11 ± 1		12 ± 1		13 ± 1		ns	***
Cyanidin-3-*O*-(6´-acetyl)glucoside		0.06 ± 0.01		0.05 ± 0.01		0.03 ± 0.00		0.12 ± 0.01		0.13 ± 0.01		0.13 ± 0.02		ns	***
Delphinidin-3-*O*-(6´-acetyl)glucoside		0.13 ± 0.01	a	0.09 ± 0.01	ab	0.08 ± 0.00	b	0.54 ± 0.02		0.62 ± 0.04		0.62 ± 0.04		ns	***
Malvidin-3-*O*-(6´-acetyl)glucoside		0.49 ± 0.05	a	0.29 ± 0.03	b	0.29 ± 0.03	b	6.8 ± 0.4		8.0 ± 0.2		7.6 ± 0.5		ns	***
Peonidin-3-*O*-(6´-acetyl)glucoside		0.11 ± 0.01	a	0.08 ± 0.01	ab	0.07 ± 0.00	b	0.48 ± 0.03		0.57 ± 0.05		0.57 ± 0.06		ns	***
Petunidin-3-*O*-(6´-acetyl)glucoside		0.19 ± 0.02	a	0.12 ± 0.02	ab	0.10 ± 0.01	b	1.3 ± 0.0		1.5 ± 0.1		1.4 ± 0.1		ns	***
Cyanidin-3-*O*-(6´-*p*-coumaroyl)glucoside		0.55 ± 0.06	a	0.39 ± 0.06	ab	0.31 ± 0.03	b	1.3 ± 0.1		1.4 ± 0.1		1.4 ± 0.2		ns	***
Delphinidin-3-*O*-(6´-*p*-coumaroyl)glucoside		0.83 ± 0.10	a	0.43 ± 0.08	b	0.42 ± 0.05	b	4.9 ± 0.2		5.7 ± 0.5		5.6 ± 0.5		ns	***
Malvidin-3-*O*-(6´-*p*-coumaroyl)glucoside		2.1 ± 0.3	a	1.1 ± 0.1	b	1.3 ± 0.2	b	35 ± 1	a	41 ± 1	b	40 ± 3	ab	ns	***
Peonidin-3-*O*-(6´-*p*-coumaroyl)glucoside		0.69 ± 0.07		0.48 ± 0.06		0.57 ± 0.08		4.4 ± 0.2		5.1 ± 0.1		4.7 ± 0.5		ns	***
Petunidin-3-*O*-(6´-*p*-coumaroyl)glucoside		0.89 ± 0.10	a	0.43 ± 0.04	b	0.48 ± 0.06	b	7.2 ± 0.4		7.8 ± 0.7		8.2 ± 0.7		ns	***

Table 4.3. Significant correlations among phenolic compounds of leaves and berry skins of *Vitis vinifera* L. Tempranillo, together with significant correlations among all these compounds and radiation variables (PAR and UV_{BE} doses) (n = 9 for leaf variables and n = 7 for berry variables). MIPC and MSPC, the bulk UV absorbances of methanol-insoluble and –soluble phenolic compounds, respectively. HCAd, the sum of hydroxycinnamic acids and their derivatives.

		Radiation		Leaf phenolics			
		PAR	UV_{BE}	MSPC	MIPC	HCAd	Kaempferol
Leaf phenolics	MSPC	0.85					
	MIPC						
	HCAd						
	Kaempferol	0.73	0.92				
	Quercetins	0.82	0.76				
Berry skin phenolics	MSPC	0.78			0.84		
	MIPC	-0.94		-0.79			
	Stilbenes						
	Flavanols	-0.99		-0.83			
	Flavonols	0.79			0.78		
	Isorhamnetins	0.77			0.91	0.79	
	Myricetins	0.87			0.82		
	Quercetins		0.91				0.86
	Kaempferols		0.89				0.81
	Syringetin						
	HCAd	-0.84		-0.85			
	Anthocyanins	0.97			0.99	0.99	

$p < 0.001$

$p < 0.01$

$p < 0.05$

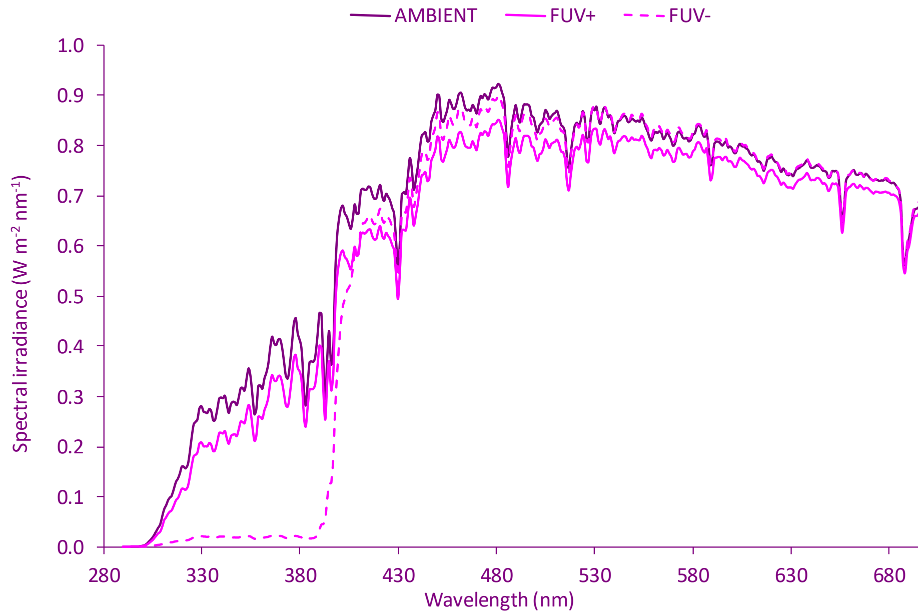

Figure. 4.1. Spectral irradiances measured around noon on a typical summer sunny day in each of the three experimental conditions used: Ambient, no filter (red garnet and solid line); FUV+, UV-transmitting filter (fuchsia and solid line); FUV-, UV-blocking filter (fuchsia and dashed line).

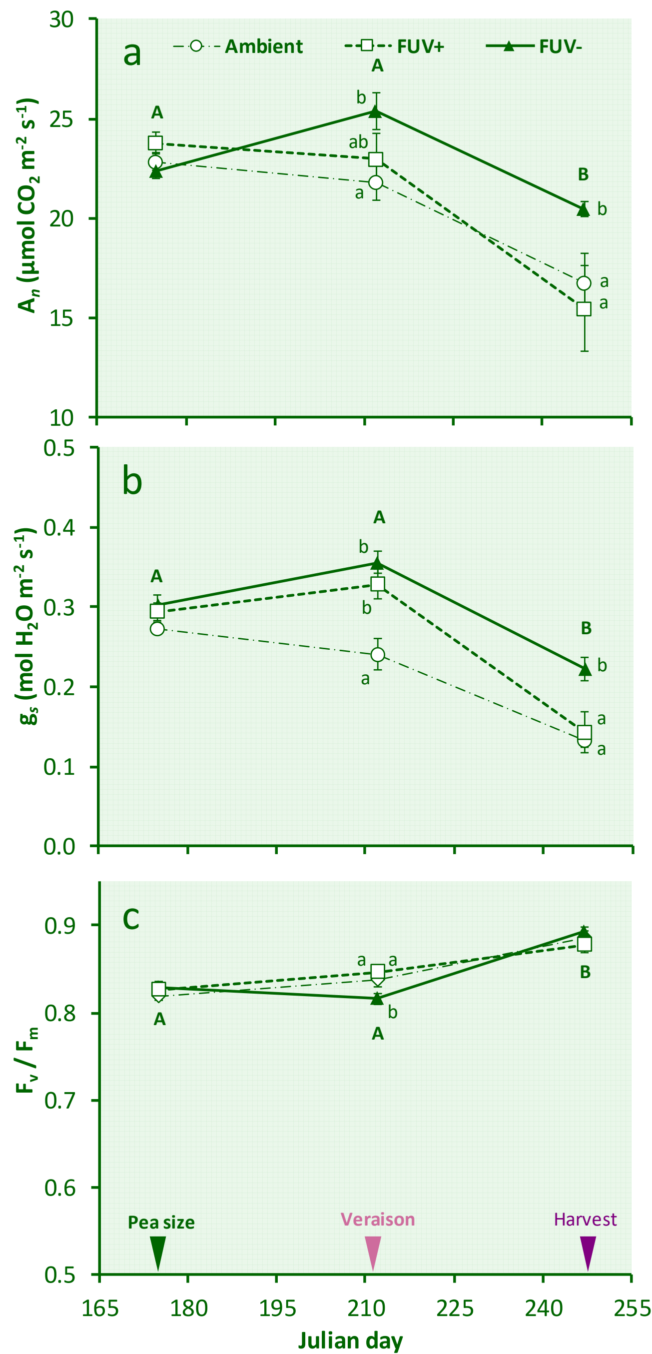

Figure. 4.2. Effects of the radiation treatment (Ambient, no filter; FUV+, UV-transmitting filter; FUV-, UV-blocking filter) on a) photosynthesis rates (A_n), b) stomatal conductance (g_s) and c) the maximal quantum yield of photosystem II (F_v/F_m) in *Vitis vinifera* L. Tempranillo leaves, measured at three phenological stages: pea size, veraison and harvest. Values are expressed as mean ± standard error (n=6). Different capital and lower case letters mean, respectively, significant differences between phenological stages and significant differences between treatments for each phenological stage (in both cases, at least at $p < 0.05$).

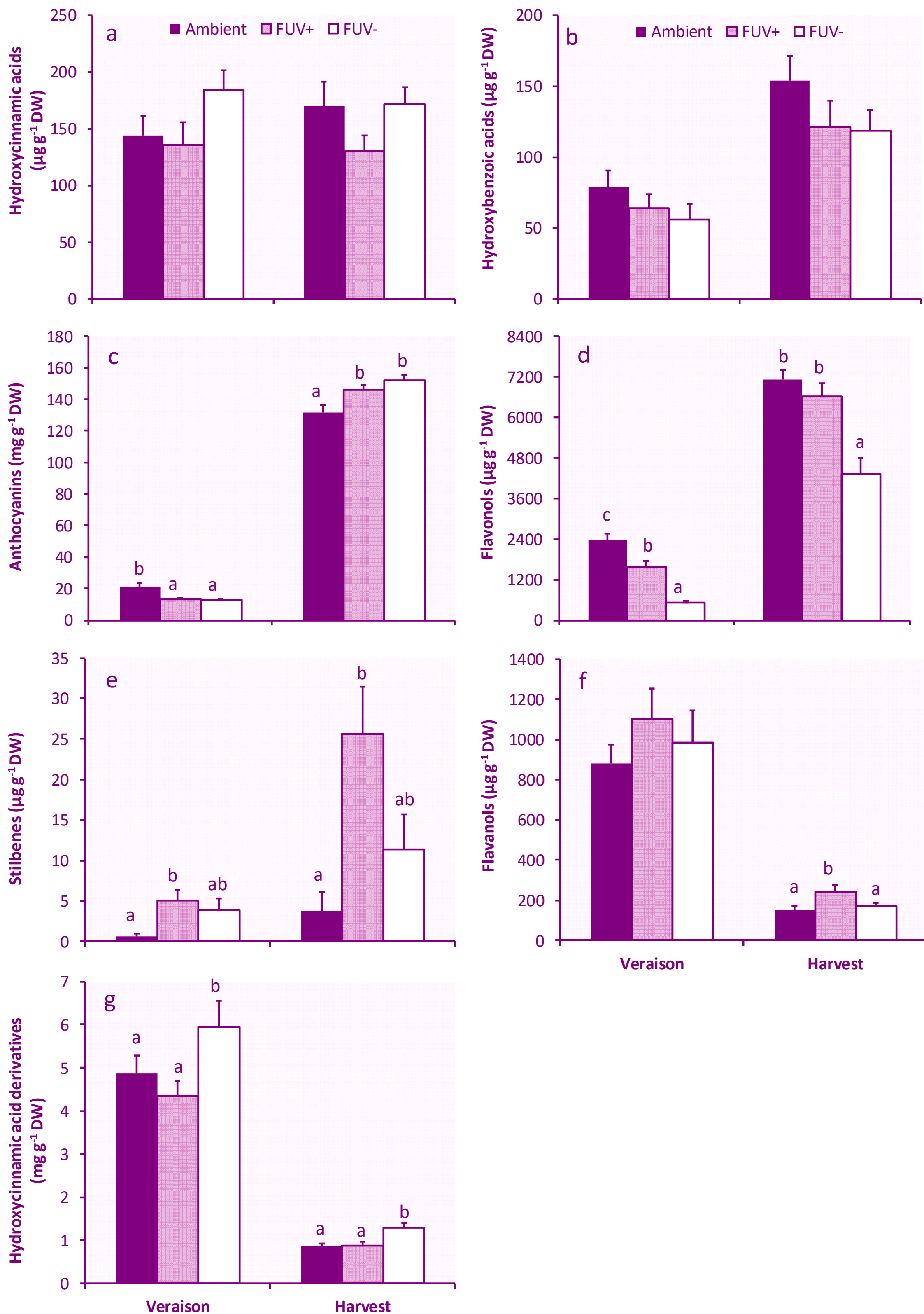

Figure. 4.3. Effects of the radiation treatment (Ambient, no filter; FUV+, UV-transmitting filter; FUV-, UV-blocking filter) on the total contents of different families of phenolic compounds in the insoluble (a-b) and soluble (c-g) fractions of berry skins of *Vitis vinifera* L. Tempranillo sampled at veraison and harvest. Values are expressed as mean ± standard error (n=6). For each phenological stage, different letters mean significant differences between treatments (at least at $p < 0.05$).

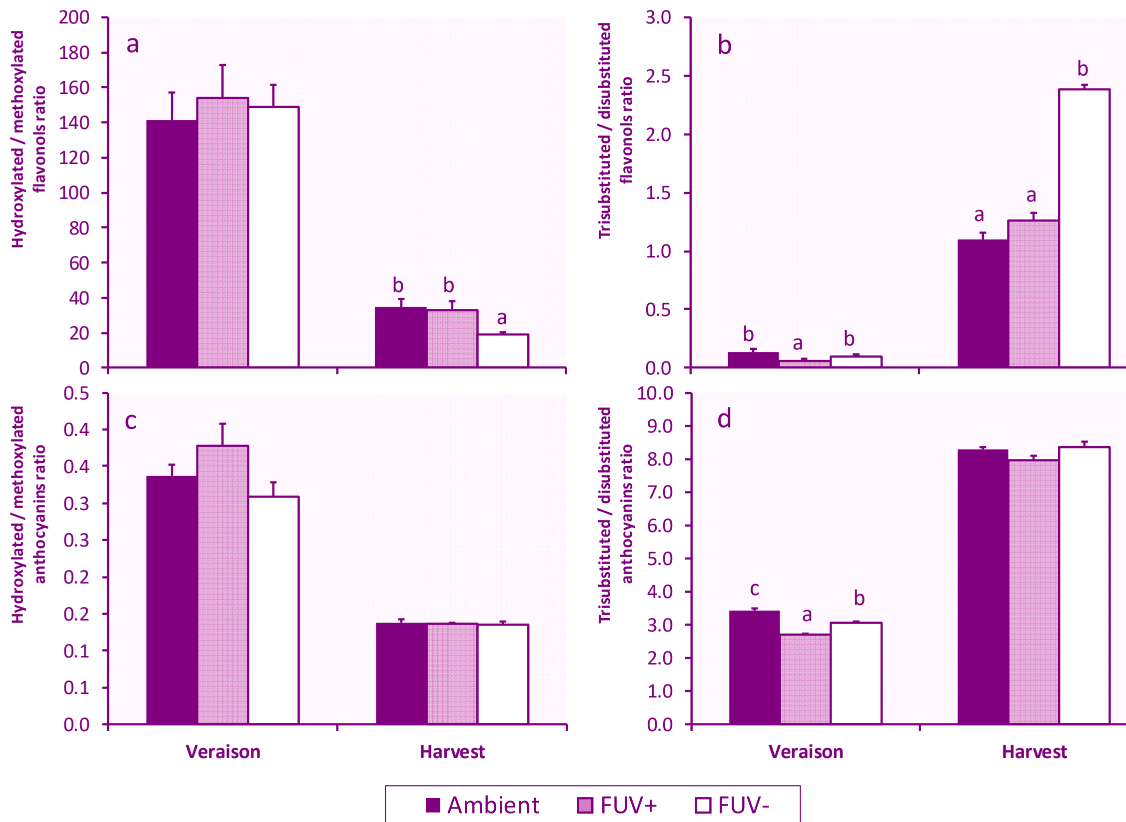

Figure. 4.4. Effects of the radiation treatment (Ambient, no filter; FUV+, UV-transmitting filter; FUV-, UV-blocking filter) on several ratios between differently hydroxylated flavonols and anthocyanins in berry skins of *Vitis vinifera* L. Tempranillo sampled at veraison and harvest. Values are expressed as mean ± standard error (n=6). For each phenological stage, different letters mean significant differences between treatments (at least at p <0.05).

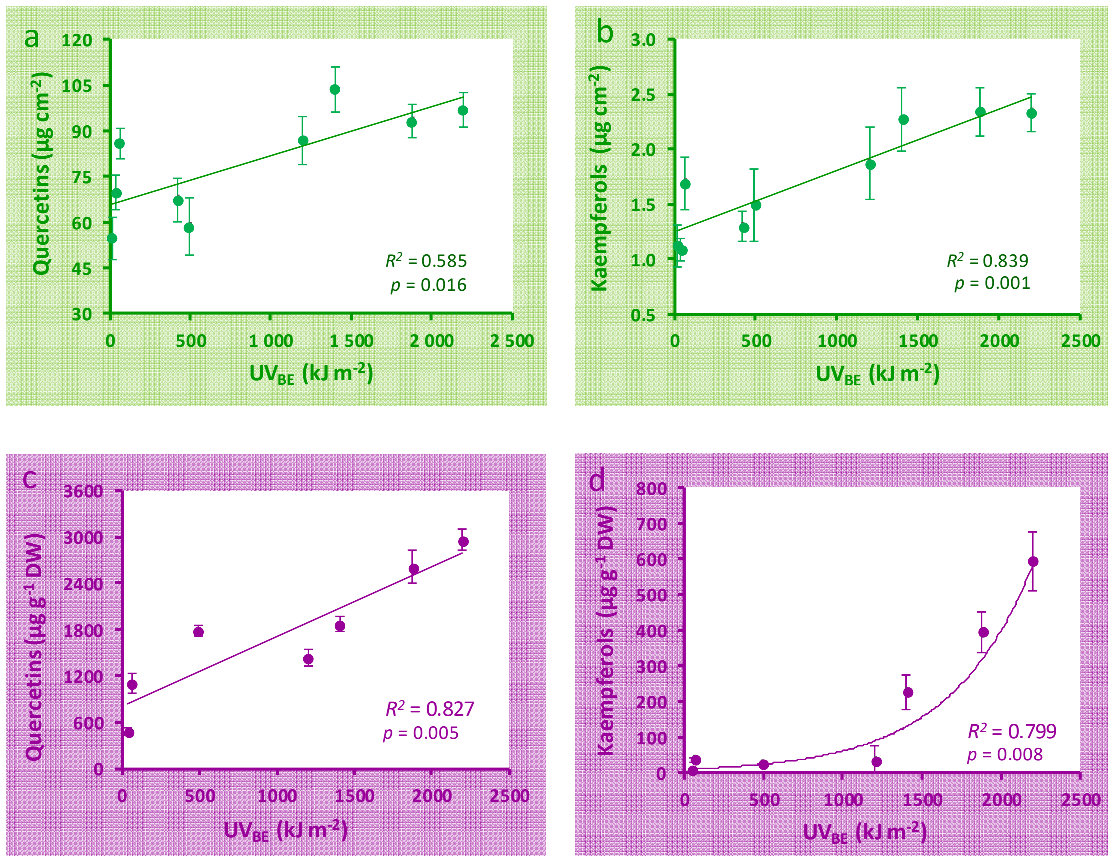

Figure. 4.5. Regressions between the total contents of kaempferols and quercetins in leaves and berry skins of *Vitis vinifera* L. Tempranillo and the doses of biologically effective UV radiation (UV_{BE}) received by the plants under the three different radiation regimes (Ambient, FUV+ and FUV-) at the different phenological stages in which samples were taken. Determination coefficients (R^2) and *p* values are shown.

Chapter 5. Environmental factors correlated with the metabolite profile of Vitis vinifera *cv. Pinot Noir berry skins along a European latitudinal gradient*

Environmental factors correlated with the metabolite profile of *Vitis vinifera* cv. Pinot Noir berry skins along a European latitudinal gradient

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ABSTRACT

Mature berries of Pinot Noir grapevines were sampled across a latitudinal gradient in Europe, from southern Spain to central Germany. Our aim was to study the influence of latitude-dependent environmental factors on the metabolite composition (mainly phenolic compounds) of berry skins. Solar radiation variables were positively correlated with flavonols and flavanonols and, to a lesser extent, with stilbenes and cinnamic acids. The daily means of global and erythematic UV solar radiation over long periods (bud break-veraison, bud break-harvest and veraison-harvest), and the doses and daily means in shorter development periods (5-10 days before veraison and harvest) were the variables best correlated with the phenolic profile. The ratio between trihydroxylated and monohydroxylated flavonols, which was positively correlated with antioxidant capacity, was the berry skin variable best correlated with those radiation variables. Total flavanols and total anthocyanins did not show any correlation with radiation variables. Air temperature, degree days, rainfall and aridity indices showed fewer correlations with metabolite contents than radiation. Moreover, the latter correlations were restricted to the period veraison-harvest, where radiation, temperature and water availability variables were correlated, making it difficult to separate the possible individual effects of each type of variable. The data show that managing environmental factors, in particular global and UV radiation, through cultural practices during specific development periods, can be useful to promote the synthesis of valuable nutraceuticals and metabolites that influence wine quality.

INTRODUCTION

Environmental factors, such as air temperature, ambient solar radiation (including UV) and photoperiod, vary with latitude. In turn, variations in these environmental factors may cause changes in physiological and/or biochemical characteristics of plants. Yet, this is not always the case as plant responses to latitudinal climatic conditions may be masked by, for example, local climatic factors, cultivational measures, or pest and diseases. Thus, there is a need for latitudinal studies that help to identify the environmental factors that impact most on plants, as well as the traits most affected. Such studies are important in terms of understanding ecological processes (especially in the context of climate change), but also have a direct relevance for the agricultural industry. A number of plant traits have been studied in relation to latitude, including plant height, seed production, growth, biomass production, photosynthesis rates, chlorophyll fluorescence, photosynthetic pigment composition, mineral nutrient contents and ratios, water relations and secondary metabolite contents (Núñez-Olivera *et al*., 1996; Llorens *et al* 2004; Martz *et al*., 2009; Jaakola and Hohtola, 2010; Jansen *et al*., 2010; De Frenne *et al*., 2011; Comont *et al*., 2012; Yang *et al*., 2013). Most of these traits have been measured in leaves, whereas only a few studies have used fruits. Latitude-related environmental variables that have been hypothesized to explain changes in plant traits include air temperature, degree days, rainfall, aridity indices, soil moisture, total solar radiation doses, and UV radiation doses. Most latitudinal studies have been carried out using wild species, while only a few studies have dealt with commercially interesting species, such as juniper (Martz *et al*., 2009), ryegrass (Comont *et al*., 2012) and currant (Yang *et al*., 2013). To our knowledge, no study has dissected the effects of latitudinal gradients, and the associated environmental parameters, on grapevine, although latitude is a recognized factor used, for example, to predict the suitability of territories for grapevine culture (Kenny and Shao, 1992).

Remarkably, the effects of latitude and associated environmental parameters on the phenolic composition of grapevine berries have not been studied, in spite of the fact that similar studies have been conducted on other species with less commercial impact (Martz *et al*., 2009; Jaakola and Hohtola, 2010; Jansen *et al*., 2010; Comont *et al*., 2012; Yang *et al*., 2013) . This omission is even more remarkable, given that the phenolic compounds synthesized in grapevine berries decisively determine wine characteristics and quality, including the presence of important nutraceuticals and nutritionally-desirable antioxidants (Sun *et al*., 2012; Calabrizo *et al*., 2016). Berry skin is the main source of many of these phenolic compounds, including anthocyanins, flavonols and stilbenes (Mattivi *et al*., 2006; Berli *et al*., 2011; Sternad Lemut *et al*., 2013).

The present study was conducted on Pinot Noir grapevines. This variety is the tenth most cultivated grapevine worldwide, and the seventh fastest-expanding winegrape variety in the period 2000-2010 (Anderson, 2013). Pinot Noir grapevines occupy more than 86,000 ha in the world (1.88% of the total grapevine acreage), especially in Europe, where it occupies 3% of the total acreage. Pinot Noir is especially adapted to cold climates, thus ascending to higher latitudes than other varieties. In fact, the European distribution of this cultivar ranges from southern Spain to central Germany. Given this wide ranging distribution, our aim was to identify the influence of latitude and associated environmental parameters (air temperature, global and UV radiation, rainfall and aridity) on the metabolite composition of berry skins of *Vitis vinifera* cv. Pinot Noir in Europe. This study will inform management of those environmental parameters that affect berry skin composition. In turn, a better understanding of the influence of these parameters can help improve wine quality.

MATERIALS AND METHODS

Collection sites and environmental variables

Berries of Pinot Noir grapevines (*Vitis vinifera* L.) were collected in 2013 from 11 localities in Spain, France, Italy, Hungary, Austria, Slovenia, the Czech Republic and Germany (Figure 5.1, Table 5.1). This represented a latitudinal gradient of almost 14º (36.7-50.0 ºN) and a linear distance of around 1,500 km, covering most of the commercial Pinot Noir growing latitudes in the Northern Hemisphere (35-55º) (Clarke and Rand, 2015). Vineyard age varied between 6 and 30 years, and vineyard soils were mostly calcareous and neutral-alkaline (pH between 7.0 and 8.5). No fertilization or irrigation had been applied to the vineyards.

In each locality, berry samples were collected from three separate plants (replicates) at commercial maturity, always around noon-time, and on a sunny day. Collection dates varied from 31 July to 22 October, depending on the location. Three clusters were collected for each replicate. As row orientation varied between vineyards, clusters were always picked from a SE-orientated shoot. In situ, every berry was separated from its cluster by cutting the pedicel. Subsequently, berry density was determined as floatability in a NaCl solution series, which allowed for harvesting berries of a similar ripeness using a non-destructive method (Rolle *et al.*, 2011; Carbonell-Bejerano *et al.*, 2014). To reduce the variability that is normally found within a cluster, berries with a density between 140-160 g NaCl L^{-1} were selected, rinsed in distilled H_2O and immediately transported to the laboratory in a portable icebox. In the laboratory, berries were frozen in liquid nitrogen and kept at -80ºC until further analyses.

Relevant environmental data were obtained for each locality. Daily values of mean temperature, rainfall and ground-station global radiation (GGR) were obtained for the period bud break-harvest from the nearest meteorological observatory to each vineyard. For most vineyards, meteorological stations were located less than 200 m from the actual vineyards. Remaining stations were located less than 20 km away, except in the case of Lednice (Czech Republic) where

the station for GGR measurement was located 50 km from the vineyard. In the latter cases, it was ascertained that meteorological stations were located at a similar latitude and altitude as the respective vineyards, which makes the assumption that data were homogeneous. Based on these data, two aridity indices were calculated: the ratio Rainfall/ETP, where ETP is the potential evapotranspiration computed according to Hargraves formula (based on solar global radiation and mean air temperature), and the Gaussen Index (the ratio between rainfall and twice the mean daily temperature). In addition, daily values of DSSF (Downward Surface Shortwave Flux) global radiation and TEMIS-derived erythematic UV radiation (T UVery) were obtained for the period bud break-harvest. Daily DSSF was calculated by integrating the 30 minutes of data downloaded from the LandSaf web page (http://landsaf.meteo.pt). The data in this archive take into account the differences in the day-length of the various locations. T UVery was downloaded from the ESA-TEMIS web page (http://www.temis.nl) and estimated on the basis of Meteosat data (to assess cloud cover), SCIAMACHY data (to assess O_3 column) and a radiative transfer model (Allaart *et al*., 2004). The degree days (using 10ºC as base temperature) and the daily doses of GGR, DSSF and T UVery were integrated over three different periods: bud break-veraison, bud break-harvest, and veraison-harvest. Additionally, DSSF and T UVery doses were integrated for 5 and 10 days before veraison, and for 5 and 10 days before harvest, because the periods around veraison and prior to harvest are important for the synthesis of phenolic compounds in grapevine berries and, thus, for their commercial quality (Downey *et al*., 2003; Bogs *et al*., 2006; Pastore *et al*., 2013).

Analysis of berries

Frozen berries were allowed to partially thaw and the skin was carefully removed from the flesh using a scalpel, and without rupturing the hypodermal cells. The content of total soluble solids (TSS) was measured in ºBrix in the flesh, using a digital refractometer. The skins were immediately submerged in liquid nitrogen, weighed and lyophilized. Lyophilized berry skins were

weighed and ground to obtain a homogeneous powder for each replicate. Then, all the samples were shipped to one laboratory for detailed analysis of metabolites.

For each analytical sample used for the analysis of phenolic compounds, 50 mg of skin powder was frozen in liquid nitrogen and ground again in a TissueLyser (Qiagen, Hilden, Germany). The total content of methanol-soluble phenolic compounds (MSPCs), mainly located in the vacuoles (Carbonell-Bejerano *et al*., 2014), was measured by spectrophotometry. For this analysis, 2 mL of a mixture of methanol: water: 7M HCl (70:29:1 v:v:v) was added for extraction (24 h at 4ºC in the dark). The extract was centrifuged at 6000 g for 15 min and the supernatant was selected for spectrophotometry. The level of MSPCs was measured as the area under the absorbance curve in the wavelength intervals between 280-315 and 280-400 nm ($AUC_{280–315}$ and $AUC_{280–400}$ respectively) and normalised per unit of dry weight (DW) (Del Castillo-Alonso *et al*., 2015), using a λ35 spectrophotometer (Perkin-Elmer, Wilton, CT, USA). Individual phenolic compounds were analysed by ultra-performance liquid chromatography (UPLC) using a Waters Acquity UPLC system (Waters Corporation, Milford, MA, USA) (Del Castillo-Alonso *et al*., 2015). Solvents were: A, water/formic acid (0.1%), and B, acetonitrile with 0.1% formic acid. The gradient program employed was: 0-7 min, 99.5-80% A; 7-9 min, 80-50% A; 9-11.7 min, 50-0% A; 11.7-15 min, 0-99.5% A. The UPLC system was coupled to a micrOTOF II high-resolution mass spectrometer (Bruker Daltonics, Bremen, Germany) equipped with an Apollo II ESI/APCI multimode source and controlled by the Bruker Daltonics DataAnalysis software. The electrospray source was operated in positive or negative mode. The capillary potential was set to 4 kV; the drying gas temperature was 200 ºC and its flow 9 L min^{-1}; the nebulizer gas was set to 3.5 bar and 25 ºC. Spectra were acquired between *m/z* 120 and 1505 in positive mode for anthocyanins and in negative mode for the remaining phenolic compounds. The different phenolic compounds analysed were identified according to their order of elution and the retention times of the following pure compounds: myricetin, quercetin, catechin, epicatechin, astilbin, *trans*-resveratrol, *p*-coumaric acid, caffeic acid and ferulic acid (Sigma, St. Louis, MO, USA); kaempferol-3-*O*-glucoside,

isorhamnetin-3-*O*-glucoside, syringetin-3-*O*-glucoside, procyanidin B1 and malvidin-3-*O*-glucoside (Extrasynthese, Genay, France); isorhamnetin, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucopyranoside, quercetin-3-*O*-glucuronide and quercetin-3-rutinoside (Fluka, Buchs, Germany). Quantification of compounds that were not commercially available was carried out using the calibration curves belonging to the most similar compound: myricetin for its glucosides; isorhamnetin for isorhamnetin-3-*O*-glucuronide; quercetin for quercetin-3-*O*-arabinoside; astilbin for taxifolin-3-*O*-glucoside; *trans*-resveratrol for its glucoside; *p*-coumaric acid for *p*-coumaroyl-tartaric acid; caffeic acid for *p*-caffeoyl-tartaric acid; ferulic acid for feruloyl-tartaric acid; and malvidin-3-*O*-glucoside for anthocyanins. Total contents of the different phenolic groups were obtained as the sum of the individual compounds. The ratios between trihydroxylated and dihydroxylated (3´,4´,5´-OH/3´,4´-OH) anthocyanins, and between trihydroxylated and monohydroxylated (3´,4´,5´-OH/4´-OH) and trihydroxylated and dihydroxylated (3´,4´,5´-OH/3´,4´-OH) flavonols, were also calculated.

For carotenoid and chlorophyll extraction (Heredia *et al*., 2010) ,[24] 6 mL of a mixture of methanol, acetone, and hexane (1:1:1 v:v:v) was added to a glass tube containing 50 mg of lyophilized skin powder. The mixture was vortexed for 30 s and then stirred for 30 min at 4°C in the dark. After the addition of 2 mL of MilliQ water the tube was vigorously shaken for 1 min and then centrifuged for 1 min at 1500 g. The non-polar phase containing carotenoids and chlorophylls was recovered. The extraction was repeated by adding 2 mL of hexane to the remaining mixture. The two extracts were pooled and the volume reduced to 1 mL by vacuum evaporation. The extract was filtered through 0.2-µm filters and immediately subjected to high-performance liquid chromatography (HPLC) analysis as follows. Separation was performed at room temperature by a Spectra System P4000 HPLC, equipped with a UV 6000 LP photodiode array detector (Thermo Fisher Scientific, Waltham, MA, USA) using a Zorbax ODS column (5 µm particle size, 250 x 4.6 mm, Agilent Technologies, Santa Clara, CA, USA). HPLC separation was carried out at a flow rate of 0.8 mL min^{-1} using the following linear gradient: 0 min, 82% A

(CH_3CN), 18% B (methanol/hexane/CH_2Cl_2 1:1:1 v:v:v); 20 min, 76% A, 24% B; 30 min, 58% A, 42% B; 40 min, 39% A, 61% B. The column was allowed to re-equilibrate in the starting solution (82% A, 18% B) for 5 min before the next injection. Different individual chlorophylls and carotenoids were detected by their absorbance at 445 nm.

The antioxidant capacity of berry skins was measured by generating the radical cation 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$) (Re *et al.*, 1999). The radical solution was diluted in ethanol to obtain an absorbance of 0.700 ± 0.020 at 734 nm (Perkin-Elmer λ35 spectrophotometer). After addition of 1 mL of diluted $ABTS^{\bullet+}$ solution to 100 µL of skin extract (250 µg of skin powder in 1 mL of a mixture of methanol: water: 7M HCl 70:29:1 v:v:v), the decrease in absorbance was monitored and compared to that of the Trolox standard (Sigma) exactly 4 min after initial mixing. Antioxidant capacity was expressed in terms of Trolox equivalent antioxidant capacity (TEAC) per g DW of skin.

DNA isolation from lyophilized berry skins was carried out using the ZenoGene40 Plant DNA Purifying Kit (Zenon Bio Kft., Szeged, Hungary). Concentration of the samples was measured with a Genova Nano Spectrophotometer (Jenway, Staffordshire, UK). DNA content per DW of berry skin (ng mg^{-1} DW) was calculated using the formula: mean of DNA concentration (ng μL^{-1}) multiplied by the volume of extraction (µL) and divided by the DW of the lyophilized sample (mg). This analysis served to calculate the metabolite concentrations on a DNA basis.

Statistical analysis

Pearson correlation coefficients (r) were used to examine the relationships between all the variables studied, both the environmental-geographical parameters and the traits analyzed in berry skins, including the total contents of the different groups of phenolic compounds. Correlations were considered significant when $p<0.05$. The sampling localities were ordinated by Principal Components Analysis (PCA), taking into account MSPCs and the total contents of the different groups of phenolic compounds. All the statistical procedures were performed with SPSS 19.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Variation in environmental variables

The latitudinal gradient used in this study was associated with substantial differences in several meteorological variables (Table 5.2). For the period from bud break to harvest, these differences were, amongst others, around 5ºC in mean daily temperature, 500 degree days, almost 300 mm in rainfall, almost 900 MJ m^{-2} in DSSF dose, and 241 kJ m^{-2} in T UVery dose. Interestingly, the parameters displaying the greatest differences were the DSSF and T UVery doses accumulated during the 10 days before harvest. For these variables, the differences between the maximum and the minimum values along the gradient were more than 80% of the maximum value. The highest and lowest values of temperature variables were usually recorded in Pécs and Rioja, respectively, except for the veraison-harvest period, in which they were recorded in Spanish localities (Jerez or Girona) and Lednice, respectively. The highest mean values of solar radiation (GGR, DSSF, T UVery) were always recorded in Jerez, and this included also the highest accumulated doses in the 5 or 10 days before veraison and before harvest. The highest accumulated doses over longer periods were recorded in Spanish localities (either Rioja, Girona or more rarely Jerez) or in Lednice, depending on the length of the period considered, because those periods were longer in Rioja, Girona or Lednice than in Jerez (see Table 5.1 for the length of the period bud break-harvest). The lowest values of radiation variables were generally recorded in Geisenheim or Lednice.

Variation in berries variables

Metabolite contents were obtained and normalized against both berry skin DW (Table 5.3) and DNA amount. The correlations between metabolites and environmental parameters were similar irrespective of the normalization approach, given that DNA amount and berry skin DW were significantly correlated ($r = 0.79$, $p < 0.01$, $n = 11$). Therefore, results are only described on a per berry skin DW basis. MSPC values varied between 9.7 and 40.3 (as $AUC_{280\text{-}315}$ mg^{-1} DW) and

between 17.1 and 74.3 (as $AUC_{280-400}$ mg^{-1} DW). Absorption levels in the two wavelength regions were strongly and positively correlated (Table 5.S1). The highest and lowest MSPC values were found in Girona and Lednice, respectively. We quantified 29 phenolic compounds: 24 flavonoids (14 flavonols, 5 anthocyanins, 3 flavanols –monomeric or dimeric tannins-, and 2 flavanonols) and 5 non-flavonoids (3 cinnamic acids and 2 stilbenes). Great differences in the concentrations of most groups of phenolic compounds were found between localities. Anthocyanins were the most abundant group, showing values between 18.9 (Bilje) and 110.1 (Girona) mg g^{-1} DW. In every locality, malvidin-3-*O*-glucoside was the major anthocyanin. Flavonols were the second most abundant group of flavonoids, ranging between 1.76 (Bilje) and 7.7 (Girona) mg g^{-1} DW. The major flavonol was quercetin 3-*O*-glucuronide. Flavanonols (between 0.18 and 1.14 mg g^{-1} DW, in Bilje and Jerez, respectively) and flavanols (between 0.21 and 0.99 mg g^{-1} DW, in Lednice and Bilje, respectively) were less abundant. Among non-flavonoids, cinnamic acids were the most abundant group, and also the group showing the greatest variability between localities, with values between 0.16 (Lednice) and 7.2 (Firenze) mg g^{-1} DW. Finally, the least abundant compounds were stilbenes, which also showed a great variability (between 14 and 928 µg g^{-1} DW, in Lednice and Girona, respectively).

The antioxidant capacity of berry skin extracts varied between 3592 (Lednice) and 9104 (Firenze) µM TE g^{-1} DW. Chlorophylls and all carotenoids showed the highest values in Rioja and the lowest in Pécs. β-Carotene was the most abundant carotenoid. The berry fresh weight varied between 1.1 (Girona and Bordeaux) and 2.1 g (Geisenheim), although most localities showed values between 1.1 and 1.3 g. TSS varied between 19.1 (Bilje) and 23.7 ºBrix (Jerez).

Correlations between variables

The correlations between all the environmental and plant response variables were determined (Table 5.S1). Unless otherwise stated, the correlations mentioned in this text were significant ($p<0.05$) and positive. With respect to the correlations between berry skin variables, MSPCs were correlated with the contents of most phenolic compounds (except flavanols) and

carotenoids. The total contents of flavonols, flavanonols, stilbenes and anthocyanins were correlated with one another, whereas the total content of cinnamic acids was only correlated with that of flavanonols. Total flavanol content was not correlated with the total content of any other phenolic group. The antioxidant capacity of berry skin extracts was correlated with anthocyanins, MSPCs, flavonols, the ratio 3´,4´,5´-OH/3´,4´-OH flavonols and, less significantly, with flavanonols, cinnamic acids, the ratio 3´,4´,5´-OH/4´-OH flavonols, and carotenoids. There was no correlation between the antioxidant capacity and contents of stilbenes or flavanols. Carotenoid and chlorophyll contents were correlated with each other, and carotenoid levels were also correlated with those of stilbenes.

Possible correlations between environmental-geographical parameters and berry skin variables were also explored. It was found that latitude was negatively correlated with MSPCs and the total contents of flavonols, flavanonols and stilbenes, but not flavanols, cinnamic acids, anthocyanins and carotenoids (Figure 5.2).

Correlations between temperature variables and berry variables were few for the periods bud break-veraison and bud break-harvest. The mean daily temperature and degree days in the period bud break-veraison (but not bud break-harvest) were correlated (negatively) with carotenoids, chlorophylls and TSS, only. Degree days in the period bud break-veraison were also correlated with flavanonols. No temperature variable in these two periods was correlated with the total content of any other phenolic group, although there were some correlations between temperature variables and individual compounds. For the period veraison-harvest, the mean daily temperature and degree days were correlated with MSPCs and the total contents of flavonols and flavanonols. In addition, the mean daily temperature was correlated with the ratios 3´,4´,5´-OH/4´-OH and 3´,4´,5´-OH/3´,4´-OH flavonols, and the degree days with the total content of anthocyanins.

Rainfall and aridity indices were hardly correlated with berry skin variables for the periods bud break-veraison and bud break-harvest. Only quercetin showed somewhat consistent (positive) correlations with rainfall, the Rainfall/ETP ratio and Gaussen Index (but only in the period bud break-harvest). For the period veraison-harvest, rainfall and aridity indices were negatively correlated with the total content of flavonols and flavanonols. In addition, Gaussen index was negatively correlated with MSPCs and the ratios 3´,4´,5´-OH/4´-OH and 3´,4´,5´-OH/3´,4´-OH flavonols.

Radiation variables, particularly DSSF and T UVery variables, correlated well with berry skin variables for the three periods considered. The daily means of DSSF and T UVery in the periods bud break-harvest and veraison-harvest, the DSSF doses in the 10 days before harvest, the daily mean of T UVery in the 5 and 10 days before veraison, and the T UVery doses in the 5 and 10 days before veraison were all correlated with MSPCs. The same variables, together with the T UVery doses in the 10 days before harvest and in the period bud break-harvest (in this last case, with a lower significance level), were correlated with the total contents of flavonols and flavanonols. Total stilbene content was only correlated with the DSSF and T UVery doses in the period bud break-harvest, and total cinnamic acid content only with the daily mean and the dose of T UVery in the 10 days before veraison. Total flavanol and anthocyanin contents were not correlated with any radiation variable. Regarding individual compounds, the strongest correlations were found between contents of several flavonols and flavanonols and the daily means of DSSF and T UVery in the periods bud break-harvest and veraison-harvest, as well as with the DSSF and T UVery doses in the periods of 5 or 10 days before veraison or harvest. Levels of two flavanols, one anthocyanin and the three cinnamic acids analyzed were also correlated with some of those T UVery expressions.

The ratio 3´,4´,5´-OH/3´,4´-OH anthocyanins was not correlated with any radiation or temperature variable. Yet, the ratios 3´,4´,5´-OH/4´-OH and 3´,4´,5´-OH/3´,4´-OH flavonols were the berry skin variables that displayed the strongest correlations with specific radiation

variables, such as the daily means of DSSF and T UVery in the periods bud break-harvest and veraison-harvest, and the accumulated doses in the 10 days before veraison and harvest. This correlation did, however, not extend to the accumulated doses in longer periods, as Figure 5.3 shows for the period bud break-harvest. Finally, the number of days from bud break to harvest and from veraison to harvest were negatively correlated with total and several individual flavanols.

Principal Components Analysis

The localities studied were ordinated by PCA using MSPCs and the different groups of phenolic compounds. The accumulated variance by the first three axes was 94.0% (67.3% for axis I, 17.3% for axis II and 9.4% for axis III). The plot using the first two axes, together with the loading factors and their significance, is shown in Figure 5.4. The total contents of all the phenolic groups, except flavanols, were significant loading factors for the positive part of axis I, which broadly ordinated the localities on the basis of their latitude, with southernmost localities situated towards the positive part of the axis and the northernmost ones towards the negative part. Total flavanols and total cinnamic acids were the only significant loading factors for the positive part of axis II, which separated localities 4, 6, 9, 7 and 1 from the remaining ones. No significant loading factor was found for the negative part of axes I and II.

DISCUSSION

Environmental-geographical gradients, such as those related to latitude, can be exploited to explore and predict the physiological and/or biochemical responses of plants by using a space-for-time substitution (De Frenne *et al*., 2011). This type of study cannot necessarily pinpoint the influence of one particular environmental parameter on a plant response, as can be done in controlled studies. However, the strength of latitudinal studies is that plant responses are studied under realistic conditions (i.e. commercial vineyards), where plants are exposed to a natural combination of ambient, environmental parameters. In this study a range of metabolites were measured in skins of Pinot Noir berries, originating from 11 vineyards along a latitudinal gradient of nearly 14º. The levels of the various metabolites measured in Pinot Noir berry skins were broadly in agreement with levels measured in other studies using this, or other cultivars (Mattivi *et al*., 2006; Carbonell-Bejerano *et al*., 2014; Del Castillo-Alonso *et al*., 2015).

Radiation is an important determinant of berry skin metabolite profile

A key finding of this study is that the contents of MSPCs, flavonols, flavanonols and stilbenes in Pinot Noir berry skins increased with decreasing latitudes. Previously, similar results were found for MSPC contents in leaves of *Lolium perenne* (Comont *et al*., 2012), but no comparative results existed for specific phenolic compounds nor for grapevine. It might be argued that negative correlations between latitude and the abovementioned phenolic groups are due to the longer berry maturation period at lower latitudes. However, we consider this unlikely because (1) latitude was not significantly correlated with the number of days from veraison to harvest, and (2) the latter variable was not correlated with the contents of those phenolic compounds. Rather, the correlations between latitude and contents of phenolic compounds were probably due to the negative correlation between latitude and radiation (both global and UV) variables. Radiation variables were strongly and positively correlated with the total contents of most phenolic groups, mainly flavonols and flavanonols, and to a lesser extent with stilbenes and

cinnamic acids, together with MSPCs. The relationship between radiation levels and the content of these phenolic compounds had previously been reported for berry skins of several red grapevine varieties, such as Pinot Noir, Merlot, Malbec and Cabernet Sauvignon (Price *et al*., 1995; Spayd *et al*., 2002; Berli *et al*., 2008; Koyama *et al*., 2012), although not in relation with latitudinal gradients.

Rather than radiation in general, the means of DSSF and T UVery over long periods (bud break-veraison, bud break-harvest and veraison-harvest) and the means or doses in important development periods (5-10 days before veraison and harvest) were the variables best correlated with phenolic compounds, particularly flavonols, flavanonols and cinnamic acids. This is related to the fact that the periods around veraison and prior to harvest are important for the synthesis of phenolic compounds (Downey *et al*., 2003; Bogs *et al*., 2006; Pastore *et al*., 2013). The stimulation of flavonol acumulation was expected because these compounds are radiation-reactive and concentrations are well known to increase with increasing levels of solar radiation (particularly UV-B) in grapevine berry skins (Berli *et al*., 2011; Carbonell-Bejerano *et al*., 2014; Spayd *et al*., 2002; Koyama *et al*., 2012; Downey *et al*., 2004; Martínez-Lüscher *et al*., 2014a; Liu *et al*., 2015; Malacarne *et al*., 2015).

It is not simply total flavonol levels that correlate with radiation parameters, the ratios 3´,4´,5´-OH/4´-OH and 3´,4´,5´-OH/3´,4´-OH flavonols were the berry skin variables best correlated with specific radiation variables, such as the mean values or doses of DSSF and T UVery radiation in critical periods (5-10 days before veraison and harvest), but not with the accumulated doses over long periods (Figure 5.3). Thus, higher solar radiation values (both total and UV) in those critical periods might increase the B-ring hydroxylation level of flavonols in Pinot Noir berry skins. Previously, it was shown that the hydroxylation level depends on both the grape variety (Mattivi *et al*., 2006) and environmental factors, such as the radiation level. The effect of radiation, in turn, may depend again on the variety considered: the hydroxylation ratios increased with increasing total or UV radiation in Pinot Noir (this study), but decreased with

increasing total or UV radiation in Sangiovese (Pastore *et al*., 2013) and Tempranillo (Carbonell-Bejerano *et al*., 2014; Martínez-Lüscher *et al*., 2014b). This complexity may be caused by the intricate regulation mechanism of the genes and enzymes involved in the synthesis of flavonols with different hydroxylation levels (Bogs *et al*., 2006; Downey *et al*., 2004; Martínez-Lüscher *et al*., 2014a. In petunia, the highest level of B-ring hydroxylation was caused by the specific effect of increased UV-B radiation (Ryan *et al*., 2002). The antioxidant activity of flavonoids strongly depends on the number of hydroxyl groups bound to the aromatic B-ring (Sroka, 2005). Given that the hydroxylation ratios were positively correlated with the antioxidant capacity in our study, flavonols may be important as both sunscreens and antioxidants in Pinot Noir berry skins, and their role as antioxidants would increase in those localities with higher radiation levels.

Flavanonols (dihydroflavonols) are bioactive compounds that contribute to tolerance to fungal infections and colour expression in some red wines (Fanzone *et al*., 2011). Given that flavanonols comprise a relatively small fraction of total wine flavonoids, their regulation by, and responses to, radiation were not clear. However, the results in this paper show that flavanonol levels were positively correlated with radiation. This observation is consistent with a previous study that reported increases in flavanonols in Malbec berry skins following exposure to higher solar radiation levels due to cluster thinning (Fanzone *et al*., 2011). Similarly, flavanonol levels were found to be elevated in berries exposed to ambient UV-B, in comparison with berries receiving no UV-B (Berli *et al*., 2011).

The reported data indicate positive correlations of cinnamic acid levels with radiation. Consistently, higher values of caffeoyl-tartaric acid were found in skins of Pinot Noir berries exposed to solar radiation when compared with shaded berries (Price *et al*., 1995). However, not all studies show increases in cinnamic acids with increasing radiation. Coumaroyl-tartaric acid levels showed no response to solar UV-B radiation exposure in Malbec berry skins (Berli *et al*., 2008). Probably, the synthesis of cinnamic acids in berries is more influenced by the radiation received prior to veraison, because contents are highest before berry maturation (Sternad

Lemut *et al*., 2013). Besides, there is some debate on whether cinnamic acids are predominantly present in pulp, rather than skin. Furthermore, the response of cinnamic acid levels to variations in radiation appears to be influenced by the specific year (Feng *et al*., 2015), and each specific cinnamic acid seems to react in a different way (Carbonell-Bejerano *et al*., 2014).

In contrast to flavonol and flavanonol content, the levels of total stilbenes were only correlated with the global and UV radiation doses over long periods (bud break-harvest). Both stilbenes and flavonoids derive from coumaroyl-coenzyme A in the general phenylpropanoid metabolism, but stilbenes are synthesized by stilbene synthase instead of chalcone synthase. Stilbene synthase is found in berry skins during all stages of fruit development (Fornara *et al*., 2008), which could explain the correlation of total stilbene contents with global and UV doses over long periods. Yet, similar to flavonols, stilbenes (resveratrol) were also found to be UV-induced, as was demonstrated in studies using Malbec berry skins (Berli *et al*., 2008).

It was found in this study that the total content of anthocyanins was not correlated with any radiation variable. This finding is congruent with previous findings on Pinot Noir berry skins, which showed that anthocyanin content was not affected by sun exposure (Price *et al*., 1995). The finding is also consistent with the fact that anthocyanin biosynthesis is controlled by a different system than that controlling flavonol biosynthesis (Fujita *et al*., 2006). In general, anthocyanins are accumulated under conditions of low temperature and high radiation levels (Yang *et al*., 2013; Azuma *et al*., 2012), but contradictory data have been reported in grape berries as a consequence of differences in cultivar, site, season, sampling and analytical techniques (Downey *et al*., 2006). In addition, it has often been difficult to separate the effects of light and temperature.

The ratio 3´,4´,5´-OH/3´,4´-OH anthocyanins was also not correlated with any radiation variable (unlike the hydroxylation ratio of flavonols). Previous studies had shown that the hydroxylation ratio of anthocyanins may increase (Guan *et al*., 2016) or decrease (Martínez-Lüscher *et al*., 2014a; Cortell and Kennedy, 2006) with increasing (total or UV) radiation in different grapevine

varieties, and even the responses may vary depending on the year of study (Spayd *et al*., 2002; Downey *et al*., 2004). These diverse responses to radiation may be due not only to a complex regulation of the synthesis of differently hydroxylated anthocyanins in the different varieties (as occurred with respect to the hydroxylation ratios of flavonols), but also to the specific responses of each individual anthocyanin. For example, in our study the trisubstituted malvidin-3-*O*-glucoside was the only anthocyanin (positively) correlating with radiation variables, thus affecting the response of the ratio to radiation.

Total flavanol levels were not correlated with any radiation variable nor with levels of any other phenolic group. A likely explanation for this observation is that flavanols are synthesized during the early stages of berry development and that their levels remain fairly stable during subsequent berry growth. Several authors have reported that flavanol levels are stable, and show little responsiveness to changes in radiation or other environmental parameters (Sternad Lemut *et al*., 2013; Cortell and Kennedy, 2006; Hanlin and Downey, 2009). Nevertheless, there is no consensus on this point, as solar UV exclusion has been reported to decrease flavanol content (Koyama *et al*., 2012), and responses to temperature and water availability have also been reported (Hanlin and Downey, 2009; Pastor del Rio and Kennedy, 2006).

Thus, it is concluded that radiation is strongly correlated with Pinot Noir berry skin phenolic profile. Radiation-related changes in phenolic profile are highly specific. Radiation appears to affect one class of metabolites, while other compounds are not affected. Such specific regulatory interactions offer scope to precision manipulation of berry skin metabolite profiles, in order to increase berry and wine quality.

Effects of temperature and water supply on berry skin metabolic profile

Along the latitudinal gradient studied, the effect of temperature on overall phenolic composition of Pinot Noir berry skins was weaker than the effect of radiation, because temperature variables were correlated with phenolic composition only when they were calculated for the period veraison-harvest. In this case, MSPCs, flavonols, flavanonols, anthocyanins, and the ratios

3´,4´,5´-OH/4´-OH and 3´,4´,5´-OH/3´,4´-OH flavonols, were positively correlated with the mean daily temperature and/or degree days. These correlations might be due to the fact that temperature and radiation variables were also correlated for that period (Table 5.S1), and it may be difficult to differentiate radiation and temperature effects (Downey *et al*., 2006). It may not be surprising that the effects of temperature were more clear in the most important period for berry maturity (veraison-harvest) (Downey *et al*., 2003), particularly in the case of anthocyanins, which increase strongly in that period (Downey at al., 2003; Bogs *et al*., 2006; Pastore *et al*., 2013). Anthocyanins are known to be influenced by specific temperature conditions, such as ambient temperatures recorded after veraison (Spayd *et al*., 2002; Azuma *et al*., 2012; Cohen *et al*., 2012; Nicholas *et al*., 2011). Results are also congruent for flavonols because, although more influenced by radiation, these compounds can also respond to temperature (Jaakola and Hohtola, 2010). Flavanols are known to be influenced by specific temperature conditions, but in this study effects of a limited range of temperatures were tested, and it is possible that more extreme temperatures are required to impact on these phenolics. With respect to cinnamic acids, their synthesis in the first stages of berry development and the strong decrease in concentrations after veraison (Downey *et al*., 2003) may mask the influence of temperature on their content at harvest, thus concealing any correlation between temperature parameters and cinnamic acid concentrations.

Rainfall and aridity indices showed a similar behavior as temperature variables, and were correlated with some phenolic compounds only when the period veraison-harvest was considered. In this period, water availability variables were correlated with temperature and radiation variables, and thus the individual effect of each variable could not be differentiated. Water availability typically shows strong relationships with different plant traits (Moles *et al*., 2014), but direct effects on the contents of grape skin phenolic compounds are considered to be relatively minor (Kennedy *et al*., 2002; Cadot *et al*., 2011). This could be due to the fact that the effects of water availability on berry skin composition are mainly mediated by changes in

berry size which subsequently affect the proportion of skin in relation to total berry, or by changes in photosynthesis rates modifying source-sink relationships (Downey *et al*., 2006). Nevertheless, changes in anthocyanins, flavonols and stilbenes caused by water deficit or excess have been described, sometimes in contradictory ways (Downey *et al*., 2006; Kuhn *et al*., 2014), and drought conditions have been reported to increase the expression of different genes involved in the biosynthesis of phenolic compounds (Martínez-Lüscher *et al*., 2014a; Kuhn *et al*., 2014). Overall, correlations between water availability and phenolic composition were not conclusive in our study.

In summary

PCA was used to summarize the results described above. Axis I mostly represented a latitude gradient, and was determined by nearly all different groups of phenolic compounds that are present in berry skins (flavonols, flavanonols, anthocyanins, stilbenes and cinnamic acids, together with MSPCs). Thus, Pinot Noir berry skins from southern localities were more enriched in most phenolics than those from northern latitudes. This is congruent with the general variation in phenolic compounds (except anthocyanins) with latitude (Jaakola and Hohtola, 2010). Changes in phenolic composition can influence wine quality and will contribute to wine genuineness in each locality. Given that, in our study, latitude was more often correlated with radiation variables than with temperature or water availability variables, radiation appeared to be the most important factor contributing to the differentiation of berry skin composition at the localities studied. Nevertheless, in the most important period for phenolic ripeness (veraison-harvest), latitude and radiation, temperature and water availability variables were correlated with one another, and the effect of each type of variable was difficult to separate. Thus, apart from the effect of radiation in every period considered, the interaction of radiation, temperature and water availability in the period veraison-harvest was strongly correlated with the phenolic composition of berry skins along the latitudinal gradient considered. Flavanols and cinnamic acids were the only phenolic compounds that define axis II of the PCA, thus contributing to the

differentiation of berry skins from some localities, in particular those situated to the positive part of the axis II, such as Bilje, Firenze, Retz, Potoče and Jerez.

Genetic and environmental factors (other than radiation, temperature and water availability) have not been considered in our study, but may also affect the metabolite composition of berry skins. In particular, a clone effect cannot be excluded. However, this effect has been demonstrated to be relatively minor and/or non-significant in previous studies using both Pinot Noir (Nicholas *et al*., 2011; Lee and Skinkis, 2013) and other grapevine cultivars (Van Leeuwen *et al*., 2004). On the other hand, additional environmental factors related to the so-called "terroir" and not analyzed in detail in our study, such as soil type or mineral nutrition, could have influenced metabolites composition (Van Leeuwen *et al*., 2004; Schreiner *et al*., 2014), although it is doubtful whether the impacts of such variables would have been correlated with latitude. Overall, in spite of having used different clones, plant ages and soils, a significant relationship between metabolites composition and the latitude-dependent environmental changes in radiation, temperature and water availability was found. It is likely that this environmental influence masked the possible effects of genetic factors and other non-considered environmental variables.

Particularly relevant is the finding that skin phenolic composition was correlated with the DSSF and T UVery means and doses in relatively short development periods (5-10 days before veraison and harvest). Thus, increasing the total and/or UV radiation received by the clusters in those periods through management practices, such as leaf removal or supplemental UV exposure, could promote the synthesis of valuable phenolic metabolites. This may eventually contribute to improved wine quality because of the notable contribution of phenolic compounds to wine flavor and also by increasing the amount of nutraceuticals and healthy antioxidants, such as flavonols, flavanonols, stilbenes and cinnamic acids (Sun *et al*., 2012; Calabriso *et al*., 2016). Among others, UV radiation has been demonstrated to be an important factor correlated with berry skin composition in our study. Although some of the effects observed, such as the

increase in MSPCs, flavonols and cinnamic acids, have been repeatedly attributed to UV (particularly UV-B) radiation (Berli *et al*., 2011; Carbonell-Bejerano *et al*., 2014; Koyama *et al*., 2012; Martínez-Lüscher *et al*., 2014a), more specific manipulative experiments are needed to prove the specific effects of this fraction of solar radiation across the latitudinal gradient considered.

It is concluded that radiation in several development periods, and an interaction between radiation, temperature and water availability in the period veraison-harvest, were the environmental factors most correlated with the phenolic composition of Pinot Noir berry skins along a latitudinal gradient in Europe. In addition, it was demonstrated that effects of environmental variables may be different for different compounds and that some compounds were more responsive (for example, flavonols) than others (flavanols).

TABLES AND FIGURES

Table 5.1. Geographic location (latitude, longitude and altitude) of the 11 European sampling localities used in this study, together with the number of days from bud break to harvest.

	Sampling site	Country	Latitude (ºN)	Longitude (ºE)	Altitude (m)	Days from bud break to harvest
1	Jerez de la Frontera	Spain	36.7	-6.2	40	141
2	Girona	Spain	41.8	2.6	150	174
3	La Rioja	Spain	42.5	-2.3	342	175
4	Firenze	Italy	43.9	11.2	280	131
5	Bordeaux	France	44.8	-0.6	22	176
6	Bilje	Slovenia	45.9	13.6	70	143
7	Potoče	Slovenia	45.9	13.8	120	140
8	Pécs	Hungary	46.1	18.1	200	152
9	Retz	Austria	48.8	15.9	324	172
10	Lednice	Czech Republic	48.8	16.8	176	183
11	Geisenheim	Germany	50	8	95	170

Table 5.2. Ranges of the environmental variables in the 11 European sampling localities used in this study, together with the localities in which each extreme value was recorded (between brackets). ETP, potential evapotranspiration. GGR, Ground-station Global Radiation. DSSF, Downward Surface Shortwave Flux. T UVery, TEMIS-derived erythematic UV. The different variables were calculated along three periods: bud break-veraison (white background), bud break-harvest (light pink background) and veraison-harvest (dark pink background). In addition, DSSF doses were calculated in the 10 days before harvest, and T UVery (mean values and total doses) in different periods.

	Min	Max
Mean daily temperature (°C)	16.4 (3)	21.2 (8)
Mean daily temperature (°C)	16.6 (10)	21.1 (8)
Mean daily temperature (°C)	13.1 (10)	24.4 (1)
Degree days (°C)	936 (3)	1367 (8)
Degree days (°C)	1197 (3)	1703 (8)
Degree days (°C)	113 (10)	381 (2)
Rainfall (mm)	155 (4)	439 (5)
Rainfall (mm)	196 (4)	481 (5)
Rainfall (mm)	0 (1)	103 (10)
Rainfall/ETP	0.31 (4)	0.80 (5)
Rainfall/ETP	0.28 (1)	0.82 (9)
Rainfall/ETP	0 (1)	0.9 (9,10)
Gaussen Index	4.0 (4)	12.8 (5)
Gaussen Index	4.9 (4)	13.7 (5)
Gaussen Index	0 (1)	4.7 (10)
GGR (mean) (MJ m^{-2} d^{-1})	12.7 (9)	24.2 (1)
GGR (mean) (MJ m^{-2} d^{-1})	11.2 (9)	24.9 (1)
GGR (mean) (MJ m^{-2} d^{-1})	8.1 (9)	28.6 (1)
GGR (dose) (MJ m^{-2})	1487 (9)	3035 (3)
GGR (dose) (MJ m^{-2})	1939 (9)	3718 (2)
GGR (dose) (MJ m^{-2})	370 (4)	759 (10)
DSSF (mean) (MJ m^{-2} d^{-1})	18.3 (11)	23.8 (1)
DSSF mean (MJ m^{-2} d^{-1})	15.9 (11)	24.5 (1)
DSSF mean (MJ m^{-2} d^{-1})	10.1 (11)	28.4 (1)
DSSF (dose) (MJ m^{-2})	2201 (11)	2908 (2)
DSSF (dose) (MJ m^{-2})	2684 (11)	3542 (2)
DSSF (dose) (MJ m^{-2})	384 (4)	695 (10)
T UVery (mean) (kJ m^{-2} d^{-1})	3.0 (11)	3.8 (1)
T UVery (mean) (kJ m^{-2} d^{-1})	2.4 (11)	4.0 (1)
T UVery (mean) (kJ m^{-2} d^{-1})	1.5 (11)	4.8 (1)

	Min	Max
T UVery (dose) (kJ m^{-2})	254 (11)	483 (3)
T Uvery (dose) (kJ m^{-2})	329 (11)	570 (3)
T Uvery (dose) (kJ m^{-2})	49 (4)	114 (1)
DSSF (10-days-before-harvest dose) (MJ m^{-2})	56.6 (11)	284 (1)
T Uvery (5-days-before-veraison mean (kJ m^{-2} d^{-1})	2.0 (10,11)	5.1 (1)
T Uvery (10-days-before-veraison mean (kJ m^{-2} d^{-1})	2.4 (10,11)	5.0 (1)
T Uvery (5-days-before-veraison dose) (kJ m^{-2})	9.9 (10)	25.3 (1)
T Uvery (10-days-before-veraison dose) (kJ m^{-2})	23.8 (10)	50.2 (1)
T Uvery (10-days-before-harvest dose) (kJ m^{-2})	6.9 (11)	47.4 (1)

Table 5.3. Values (means ± SE) of the variables analyzed in Pinot Noir berries in the 11 European sampling localities used in this study. MSPC, methanol-soluble phenolic compounds. AUC, area under curve. TSS, total soluble solids.

	Jerez	Girona	La Rioja	Firenze	Bordeaux	Bilje	Potoče	Pécs	Retz	Lednice	Geisenheim
Total content of MSPC											
$AUC_{280-315}$ mg^{-1} DW	39 ± 2	40 ± 1	31 ± 3	32 ± 1	22 ± 1	15 ± 0	13 ± 0	32 ± 0	32 ± 5	10 ± 0	24 ± 1
$AUC_{280-400}$ mg^{-1} DW	71 ± 4	74 ± 3	55 ± 5	58 ± 2	41 ± 2	25 ± 0	23 ± 0	57 ± 1	56 ± 10	17 ± 0	41 ± 2
Flavonols (μg g^{-1} DW)											
Myricetin	139 ± 20	153 ± 8	112 ± 24	234 ± 27	39 ± 6	7.3 ± 2.8	13 ± 3	74 ± 9	164 ± 31	2.5 ± 0.8	15 ± 2
Myricetin-3-*O*-glucoside	1066 ± 137	1041 ± 62	864 ± 86	918 ± 112	487 ± 37	157 ± 17	277 ± 45	473 ± 38	535 ± 92	61 ± 16	272 ± 30
Myricetin-3-*O*-glucuronide	391 ± 50	355 ± 54	183 ± 32	368 ± 21	117 ± 11	62.5 ± 6.8	86.1 ± 7.5	267 ± 23	68.5 ± 9.1	22 ± 6	47.4 ± 8.6
Kaempferol-3-*O*- glucoside	177 ± 37	273 ± 61	78.5 ± 9.9	109 ± 30	106 ± 7	22 ± 5	44 ± 8	41 ± 5	145 ± 36	48 ± 21	106 ± 36
Isorhamnetin 3-*O*- glucoside	319 ± 31	433 ± 49	324 ± 33	274 ± 25	252 ± 16	84 ± 8	109 ± 11	234 ± 6	252 ± 27	138 ± 39	283 ± 21
Isorhamnetin 3-*O*- glucuronide	73 ± 8	92 ± 7	42 ± 3	80 ± 6	50 ± 3	22 ± 5	28 ± 1	67 ± 2	27 ± 5	77 ± 15	52 ± 5
Syringetin 3-*O*- glucoside	171 ± 26	130 ± 15	139 ± 16	88 ± 12	132 ± 8	62 ± 4	69 ± 5	156 ± 5	66 ± 8	57 ± 11	106 ± 7
Quercetin	4.3 ± 0.4	5.6 ± 0.7	3.9 ± 0.7	2.8 ± 0.3	7.3 ± 3.2	1.3 ± 0.2	1.3 ± 0.1	3.5 ± 0.3	5.8 ± 2.4	2.3 ± 0.3	3.4 ± 0.5
Quercetin 3-*O*-glucoside	105 ± 12	160 ± 21	159 ± 26	133 ± 13	51 ± 3	18 ± 2	23 ± 4	93 ± 9	181 ± 26	28 ± 5	94 ± 11
Quercetin 3-*O*-galactoside	240 ± 33	400 ± 68	174 ± 11	228 ± 32	187 ± 14	40 ± 9	51 ± 3	106 ± 3	133 ± 30	51 ± 9	120 ± 24
Quercetin-3-*O*-glucopyranoside	1075 ± 100	1361 ± 122	849 ± 47	973 ± 90	825 ± 45	260 ± 47	447 ± 41	629 ± 19	599 ± 107	300 ± 51	622 ± 100
Quercetin-3-*O*-arabinoside	25 ± 3	22 ± 2	17 ± 2	15 ± 2	18 ± 2	3.6 ± 1.1	11 ± 2	8.6 ± 1.4	10.7 ± 2	5.7 ± 1	13 ± 2
Quercetin 3-*O*-glucuronide	2726 ± 177	3121 ± 128	1951 ± 103	3014 ± 108	2119 ± 89	995 ± 132	1211 ± 19	2900 ± 44	1430 ± 253	1454 ± 259	1656 ± 156
Quercetin-3-*O*-rutinoside	272 ± 35	170 ± 23	76 ± 10	279 ± 22	114 ± 10	28 ± 5	51 ± 3	144 ± 3	107 ± 38	49 ± 14	57 ± 6
Flavanols (μg g^{-1} DW)											
Catechin	126 ± 9	110 ± 8.7	111 ± 14	224 ± 19	82 ± 7	355 ± 25	188 ± 48	66.4 ± 1.8	162 ± 23	78 ± 6	102 ± 5
Epicatechin	8.8 ± 1.3	5.1 ± 0.6	8.4 ± 0.7	13 ± 1	5.9 ± 0.7	7.2 ± 1.2	4.5 ± 0.6	3.3 ± 0.3	9.2 ± 1	1.8 ± 0.2	2.7 ± 0.1
Procyanidin B1	331 ± 27	324 ± 35	266 ± 23	467 ± 40	208 ± 18	633 ± 40	384 ± 59	173 ± 7	323 ± 40	130 ± 6	168 ± 10
Flavanonols (μg g^{-1} DW)											
Astilbin	715 ± 61	591 ± 68	629 ± 59	511 ± 40	568 ± 45	163 ± 12	265 ± 35	476 ± 17	493 ± 43	299 ± 58	257 ± 43
Taxifolin-3-*O*-glucoside	429 ± 64	114 ± 14	194 ± 37	250 ± 19	168 ± 38	22 ± 8	75 ± 19	138 ± 11	141 ± 21	11 ± 2	27 ± 6

	Jerez	Girona	La Rioja	Firenze	Bordeaux	Bilje	Potoče	Pécs	Retz	Lednice	Geisenheim
Stilbenes (µg g^{-1} DW)											
Resveratrol	55 ± 7	123 ± 28	105 ± 29	34 ± 12	31 ± 5	22 ± 9	6.4 ± 1.4	41 ± 4	57 ± 19	12 ± 7	15 ± 1
Resveratrol-3-*O*-glucoside	395 ± 62	805 ± 77	385 ± 52	117 ± 32	120 ± 27	54 ± 28	18 ± 6	243 ± 32	303 ± 19	2.2 ± 0.6	29 ± 8
Hydroxycinnamic Acids (µg g^{-1} DW)											
Coumaroyl-tartaric acid	876 ± 142	221 ± 14	215 ± 37	1016 ± 143	208 ± 54	73 ± 32	89 ± 25	72 ± 50	824 ± 114	15 ± 10	49 ± 15
Caffeoyl-tartaric acid	4943 ± 716	2101 ± 427	1763 ± 214	6195 ± 809	1870 ± 497	894 ± 282	1047 ± 244	1597 ± 296	5855 ± 967	144 ± 108	947 ± 315
Feruloyl-tartaric acid	5.7 ± 0.4	5.1 ± 0.6	2.3 ± 0.2	5.9 ± 0.7	5.8 ± 0.8	3.6 ± 0.4	5.0 ± 0.8	5.7 ± 2.4	4.0 ± 0.3	1.8 ± 0.3	2.1 ± 0.4
Anthocyanins (mg g^{-1} DW)											
Delphinidin-3-*O*-glucoside	1.7 ± 0.2	2.9 ± 0.3	3.0 ± 0.5	2.9 ± 0.2	0.80 ± 0.10	0.20 ± 0.00	0.20 ± 0.00	1.4 ± 0.0	3.7 ± 0.5	0.30 ± 0.00	2.6 ± 0.3
Cyanidin-3-*O*-glucoside	0.90 ± 0.10	4.4 ± 0.1	1.6 ± 0.2	1.0 ± 0.3	0.90 ± 0.00	0.20 ± 0.00	0.10 ± 0.00	1.8 ± 0.3	1.7 ± 0.2	0.30 ± 0.00	1.5 ± 0.1
Petunidin-3-*O*-glucoside	5.0 ± 0.9	6.4 ± 0.8	4.3 ± 0.0	5.7 ± 0.7	1.8 ± 0.3	0.70 ± 0.10	0.90 ± 0.10	2.7 ± 0.0	4.8 ± 2.0	1.0 ± 0.1	2.8 ± 0.2
Peonidin-3-*O*-glucoside	15 ± 2	35 ± 1	21 ± 1	14 ± 1	12 ± 1	5.7 ± 0.8	3.2 ± 0.2	26 ± 0	17 ± 3	5.8 ± 0.8	8.1 ± 0.8
Malvidin-3-*O*-glucoside	55 ± 1	62 ± 1	40 ± 4	44 ± 0	26 ± 4	12 ± 0	17 ± 0	40 ± 1	36 ± 4	13 ± 1	27 ± 0
Other variables											
Antioxidant capacity (µM TE g^{-1} DW)	8013 ± 942	8639 ± 408	8637 ± 216	9104 ± 212	5576 ± 654	4134 ± 308	5111 ± 600	6330 ± 730	8212 ± 902	3592 ± 685	8424 ± 595
Lutein (µg g^{-1} DW)	66.2 ± 0.8	55.5 ± 5.2	67.7 ± 1.2	32.9 ± 1.6	32.3 ± 1.2	24.1 ± 1.0	31.8 ± 1.3	16.1 ± 1.3	48.4 ± 0.6	20.2 ± 1.6	52.0 ± 10.1
Zeaxanthin (µg g^{-1} DW)	8.6 ± 0.4	8.4 ± 0.0	9.2 ± 0.7	3.7 ± 0.3	5.5 ± 0.4	3.7 ± 0.5	4.9 ± 0.3	2.1 ± 0.0	6.7 ± 0.4	2.6 ± 0.1	9.2 ± 0.4
β-Carotene (µg g^{-1} DW)	171 ± 7	165 ± 6	195 ± 2	96 ± 4	112 ± 11	83 ± 4	69 ± 8	57 ± 5	129 ± 9	66.8 ± 4.8	148 ± 19
Chlorophylls (*a*+*b*) (µg g^{-1} DW)	438 ± 22	424 ± 44	525 ± 14	227 ± 6	290 ± 32	188 ± 16	182 ± 9	117 ± 10	360 ± 16	135 ± 5	480 ± 51
Fresh weight per berry (g)	1.4 ± 0.2	1.1 ± 0.1	1.3 ± 0.0	1.3 ± 0.1	1.1 ± 0.1	1.2 ± 0.2	1.2 ± 0.0	1.4 ± 0.1	1.7 ± 0.1	1.5 ± 0.1	2.1 ± 0.0
TSS (°Brix)	24 ± 0	20 ± 0	22 ± 0	21 ± 0	21 ± 0	19 ± 0	20 ± 1	20 ± 2	23 ± 0	21 ± 0	22 ± 0

Figure 5.1. Geographic location of the 11 European sampling localities used in this study. 1, Jerez de la Frontera (Spain); 2, Girona (Spain); 3, La Rioja (Spain); 4, Firenze (Italy); 5, Bordeaux (France); 6, Bilje (Slovenia); 7, Potoče (Slovenia); 8, Pécs (Hungary); 9, Retz (Austria); 10, Lednice (Czech Republic); 11, Geisenheim (Germany).

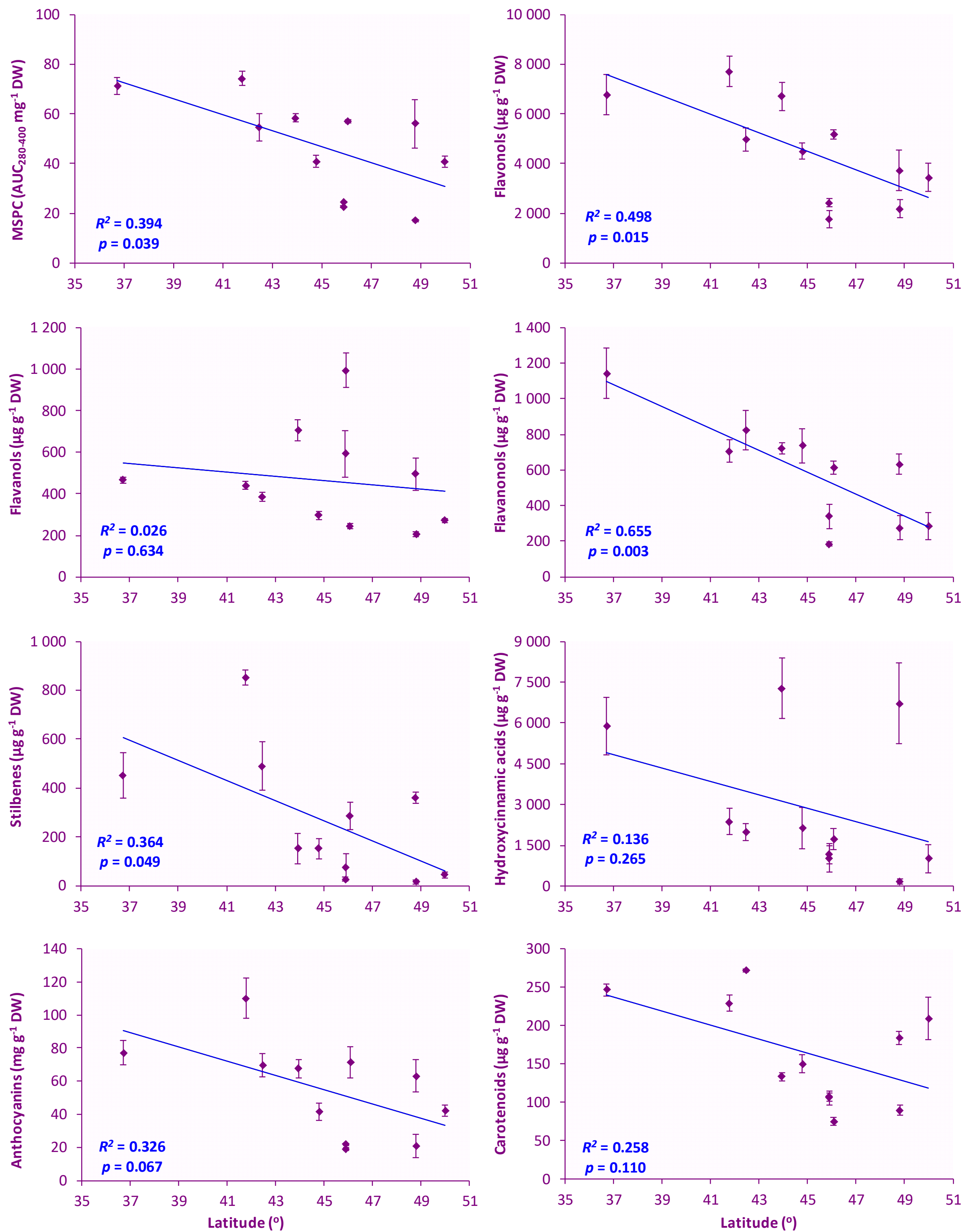

Figure 5.2. Regressions between selected berry variables, including carotenoids and the different groups of phenolic compounds, and latitude. Determination coefficients (R^2) and *p* values are shown.

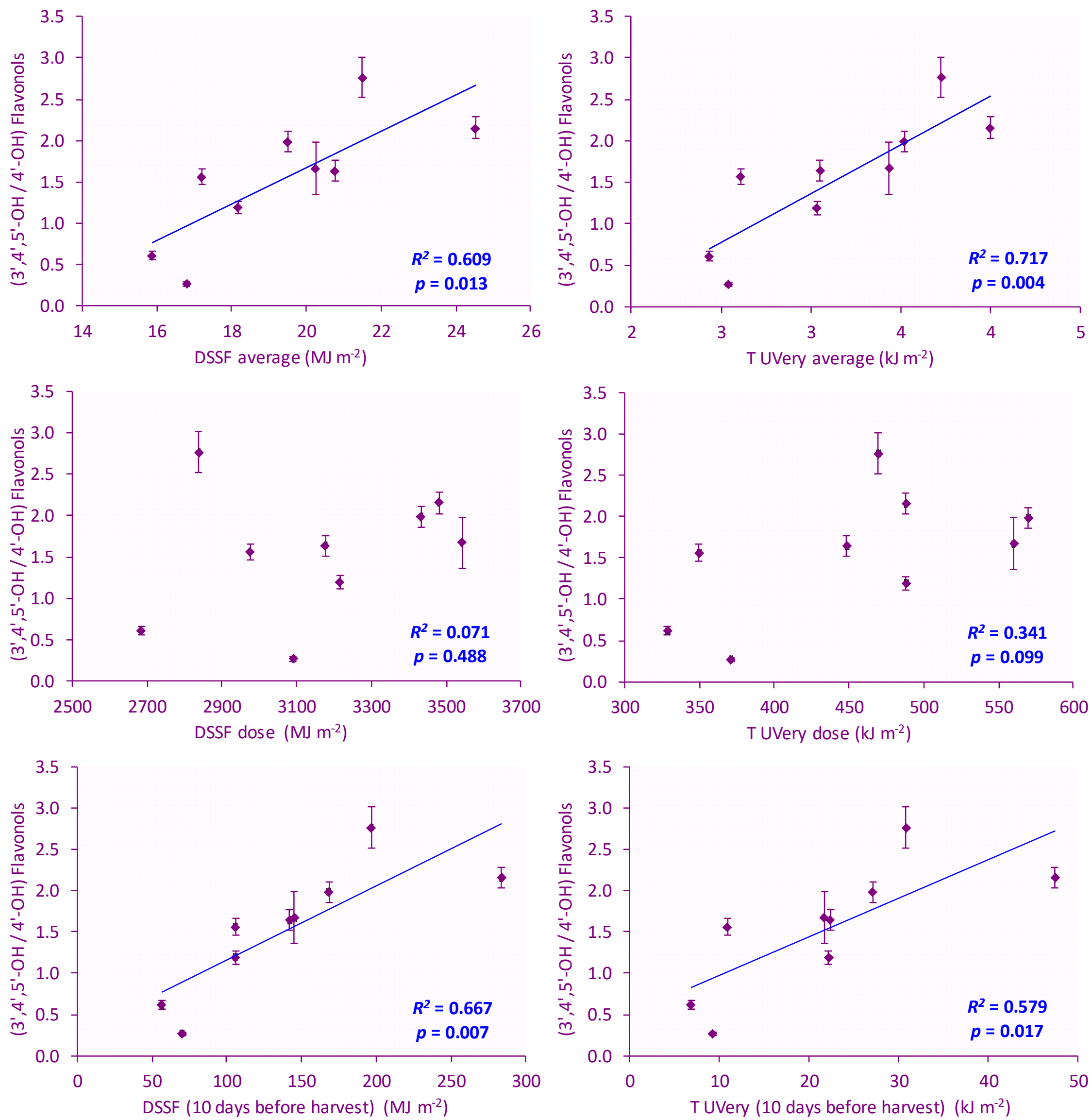

Figure 5.3. Regressions between the ratio trihydroxylated / monohydroxylated flavonols and selected radiation variables. DSSF, Downward Surface Shortwave Flux. T UVery, TEMIS-derived erythematic UV. For both variables, the daily mean in the period budbreak-harvest, and the accumulated dose in the same period and in the 10 days before harvest, were used for calculations. Determination coefficients (R^2) and *p* values are shown.

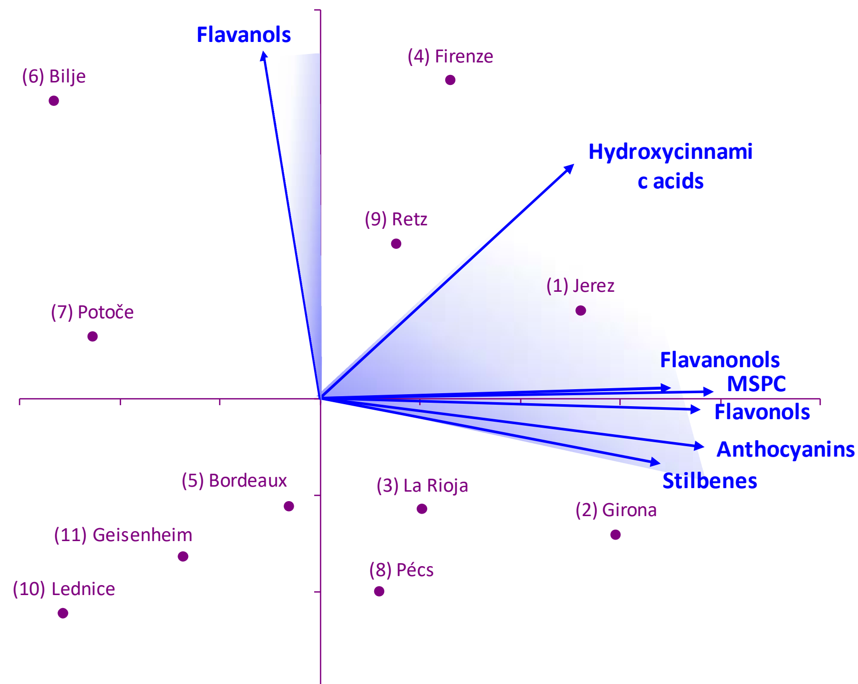

Figure 5.4. Ordination, through Principal Components Analysis (PCA), of the 11 sampling localities used in this study, taking into account the total content of methanol-soluble phenolic compounds (MSPC) and the total concentrations of the different groups of phenolic compounds. Significant loading factors for the positive and negative parts of each axis, together with their corresponding significance levels, are shown (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$). Axis 1 is the horizontal one, and axis 2 is the vertical one. Each mark on the axes represents 0.5 units.

		Latitude	Longitude	Altitude	Mean daily temperature bb-v	Mean daily temperature bb-h	Degree days bb-v	Degree days bb-h	Rainfall bb-v	Rainfall bb-h	Rainfall/ETP bb-v
	Longitude	.755									
	Altitude	.178	.268								
	Mean daily temperature bb-v	.232	.745	-.061							
	Mean daily temperature bb-h	-.376	.173	-.007	.783						
	Degree days bb-v	.375	.672	-.272	.868	.567					
	Degree days bb-h	.008	.381	-.252	.741	.747	.836				
	Rainfall bb-v	-.054	-.120	-.500	.054	-.213	.320	.365			
	Rainfall bb-h	.127	-.191	-.351	-.423	-.362	-.056	.121	.947		
	Rainfall/ETP bb-v	.221	.084	-.236	.040	-.327	.176	.203	.833	.809	
	Rainfall/ETP bb-h	.485	.186	-.003	-.230	-.438	-.104	-.050	.616	.748	.945
	Gaussen Index bb-v	-.109	-.283	-.480	-.166	-.301	.126	.222	.973	.932	.810
	Gaussen Index bb-h	.206	-.201	-.323	-.542	-.536	-.163	-.049	.900	.980	.800
MEAN	GGR bb-v	-.685	-.588	-.587	-.086	.330	.043	.174	.048	-.188	-.707
	GGR bb-h	-.834	-.630	-.375	-.070	.429	-.068	.164	-.001	-.233	-.551
DOSE	GGR bb-v	-.640	-.652	-.358	-.356	.073	-.103	.084	.325	.103	-.502
	GGR bb-h	-.625	-.675	-.411	-.430	-.026	-.153	.060	.358	.218	-.264
MEAN	DSSF bb-v	-.697	-.097	-.129	.477	.758	.188	.400	-.126	-.509	-.314
	DSSF bb-h	-.897	-.445	-.118	.229	.708	-.091	.266	-.135	-.409	-.394
DOSE	DSSF bb-v	-.762	-.535	-.192	-.143	.237	-.065	.250	.420	.238	.022
	DSSF bb-h	-.764	-.600	-.139	-.308	.115	-.287	.176	.468	.278	.102
MEAN	T Uvery bb-v	-.797	-.404	.083	.054	.363	-.159	-.021	.048	-.361	-.219
	T Uvery bb-h	-.938	-.625	-.016	-.061	.508	-.336	.041	-.006	-.292	-.323
DOSE	T Uvery bb-v	-.633	-.407	-.012	-.001	.329	.070	.276	.451	.137	.050
	T Uvery bb-h	-.780	-.652	.035	-.313	.255	-.277	.148	.430	.198	-.027
	DSSF 10 days before harvest	-.913	-.540	-.033	.064	.549	-.316	.042	-.184	-.467	-.362
MEAN	T Uvery 5 days before veraison	-.891	-.526	-.036	.039	.548	-.190	.196	.157	-.148	.024
	T Uvery 10 days before veraison	-.878	-.487	.079	.060	.592	-.213	.157	-.013	-.306	-.124
DOSE	T Uvery 5 days before veraison	-.891	-.526	-.036	.039	.548	-.190	.196	.157	-.148	.024
	T Uvery 10 days before veraison	-.878	-.487	.079	.060	.592	-.213	.157	-.013	-.306	-.124
	T Uvery 10 days before harvest	-.941	-.636	-.180	-.011	.529	-.307	.055	-.056	-.359	-.329
	Days from budbreak to veraison	-.201	-.492	-.096	-.576	-.402	-.244	-.105	.533	.666	.270
	Days from budbreak to harvest	.348	-.119	.141	-.663	-.787	-.418	-.377	.038	.685	.167
	MSPC ($AUC_{280-315}$)	-.597	-.486	.265	-.263	.549	-.443	.039	-.321	-.130	-.266
	MSPC ($AUC_{280-400}$)	-.627	-.507	.241	-.274	.557	-.457	.035	-.297	-.094	-.257
	Antioxidant Capacity	-.344	-.440	.438	-.400	.181	-.546	-.260	-.432	-.250	-.298
FLAVONOLS	TOTAL FLAVONOLS	-.705	-.553	.138	-.280	.616	-.442	.015	-.251	-.038	-.358
	3',4',5'-OH / 4'-OH	-.667	-.323	.368	.078	.619	-.234	.061	-.189	-.398	-.219
	3',4',5'-OH / 3',4'-OH	-.701	-.526	.408	-.228	.343	-.489	-.112	-.143	-.238	-.078
	Myricetin	-.467	-.238	.544	-.134	.472	-.496	-.185	-.408	-.305	-.250
	Myricetin glucoside	-.809	-.668	.234	-.372	.409	-.575	-.137	-.202	-.121	-.274
	Myricetin glucuronide	-.802	-.450	.067	-.008	.745	-.235	.167	-.254	-.272	-.448
	Kaempferol glucoside	-.481	-.530	-.022	-.464	.028	-.543	-.155	.015	.383	.062
	Isorhamnetin glucoside	-.494	-.634	.199	-.598	.129	-.616	-.189	-.234	.242	-.248
	Isorhamnetin glucuronide	-.376	-.223	-.033	-.172	.427	-.264	-.051	-.399	-.040	-.605
	Syringetin glucoside	-.675	-.681	-.168	-.322	.462	-.279	.166	-.155	-.019	-.365
	Quercetin	-.208	-.496	-.004	-.604	-.123	-.584	-.182	.110	.770	.293
	Quercetin glucoside	-.249	-.286	.659	-.401	.109	-.584	-.245	-.413	-.109	-.170
	Quercetin galactoside	-.628	-.619	.039	-.457	.243	-.516	-.084	.001	.333	-.098
	Quercetin glucopyranoside	-.704	-.689	.050	-.455	.338	-.535	-.084	-.073	.197	-.187
	Quercetin arabinoside	-.772	-.861	-.139	-.580	.129	-.644	-.243	-.023	.166	-.136
	Quercetin glucuronide	-.588	-.367	.085	-.093	.821	-.234	.184	-.320	-.098	-.483
	Quercetin rutinoside	-.678	-.380	.061	-.027	.719	-.369	-.045	-.404	-.382	-.436
FLAVANOLS	TOTAL FLAVANOLS	-.162	.130	-.041	.426	.329	.294	.173	.348	-.326	.308
	Catechin	.007	.247	-.048	.455	.199	.354	.139	.313	-.419	.304
	Epicatechin	-.449	-.266	.395	-.072	.279	-.420	-.295	-.187	-.364	-.052
	Procyanidin B1	-.248	.067	-.042	.400	.375	.257	.187	.357	-.274	.299
FLAVANONOLS	TOTAL FLAVANONOLS	-.809	-.714	.125	-.417	.406	-.645	-.195	-.245	-.148	-.254
	Astilbin	-.735	-.695	.211	-.510	.342	-.690	-.214	-.222	.033	-.224
	Taxifolin glucoside	-.824	-.636	.022	-.228	.465	-.536	-.197	-.315	-.401	-.337
STILBENES	TOTAL STILBENES	-.604	-.508	.238	-.379	.179	-.421	.064	.035	.269	-.012
	Resveratrol	-.530	-.523	.391	-.494	.026	-.491	-.064	.017	.266	-.036
	Resveratrol glucoside	-.592	-.485	.189	-.348	.193	-.383	.100	.065	.301	.008
CINNAMIC ACIDS	TOTAL CINNAMIC ACIDS	-.368	-.166	.363	-.032	.386	-.471	-.264	-.404	-.395	-.111
	Cutaric Acid	-.430	-.224	.314	-.066	.346	-.514	-.338	-.425	-.448	-.175
	Caftaric Acid	-.349	-.145	.394	-.014	.417	-.449	-.242	-.438	-.430	-.146
	Fertaric Acid	-.515	-.132	-.224	.390	.819	.197	.540	.228	.036	.156
ANTHOCYANINS	TOTAL ANTHOCYANINS	-.571	-.440	.305	-.282	.497	-.408	.084	-.239	.050	-.250
	3',4',5'-OH / 3',4'-OH	-.050	.024	-.325	.278	-.077	.224	-.011	.200	-.465	.133
	Delphinidin glucoside	-.108	-.246	.636	-.428	-.028	-.581	-.339	-.462	-.152	-.186
	Cyanidin glucoside	-.217	-.248	.239	-.299	.151	-.230	.173	.008	.448	.009
	Petunidin glucoside	-.503	-.416	.443	-.349	.329	-.587	-.212	-.386	-.152	-.277
	Peonidin glucoside	-.404	-.270	.331	-.194	.452	-.209	.280	-.097	.254	-.143
	Malvidin glucoside	-.678	-.524	.217	-.282	.534	-.457	.024	-.279	-.075	-.306
OTHER VARIABLES	CAROTENOIDS	-.509	-.804	.134	-.777	-.364	-.753	-.496	-.115	.058	-.098
	Lutein	-.523	-.766	.149	-.705	-.326	-.729	-.490	-.142	-.039	-.101
	Zeaxanthin	-.361	-.738	.002	-.729	-.440	-.631	-.433	-.014	.141	.020
	β- carotene	-.507	-.813	.134	-.798	-.372	-.761	-.496	-.109	.091	-.102
	Chlorophyll *a*	-.327	-.726	.111	-.776	-.448	-.686	-.482	-.105	.118	-.048
	Chlorophyll *b*	-.464	-.806	.073	-.766	-.420	-.727	-.539	-.106	-.013	-.081
	FW per berry	.493	.228	.158	-.093	-.275	-.121	-.298	-.651	-.406	-.348
	TSS (°Brix)	-.285	-.511	.229	-.609	-.356	-.836	-.710	-.484	-.312	-.196

Table 5.S1. Correlation coefficients among environmental-geographic and berry variables. Significant correlations are indicated in different colors depending on the significance level: red garnet, $p < 0.001$; fuchsia, $p < 0.01$; light pink, $p < 0.05$. Bb, bud break; v, veraison; h, harvest; see the remaining abbreviations in the Table 2 and 3 legends.

Chapter 6. Phenolic characteristics acquired by berry skins of Vitis vinifera *cv. Tempranillo in response to close-to-ambient solar ultraviolet radiation are mostly reflected in the resulting wines*

Phenolic characteristics acquired by berry skins of *Vitis vinifera* cv. Tempranillo in response to close-to-ambient solar ultraviolet radiation are mostly reflected in the resulting wines

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ABSTRACT

Ultraviolet (UV) radiation influences the phenolic composition of grape skins, but it is unknown if this influence is reflected in the resulting wines. In this study, Tempranillo grapevine plants were exposed or non-exposed to close-to-ambient solar UV levels by using appropriate filters, and the phenolic profile of skins and wine was analyzed in detail. In UV-exposed skins, flavonols and (less clearly) anthocyanins increased, whereas flavanols and hydroxybenzoic acids showed no significant change. These characteristics were conserved in the resulting wines. However, for stilbenes, hydroxycinnamic acid derivatives, and antioxidant capacity, the effect of UV was different in skin and wine, probably mostly due to changes during winemaking. Overall, the effects of solar UV on skin phenolic composition could predict, to some extent, the phenolic composition of the resulting wines. In addition, manipulating the UV radiation received by grape skins can improve wine quality, in terms of color stability and healthy properties.

INTRODUCTION

Solar ultraviolet (UV) radiation is divided into UV-C (100–280 nm), UV-B (280–315 nm) and UV-A (315–400 nm) bands, but only wavelengths greater than 290 nm reach the Biosphere. UV radiation represents a minor fraction of total solar radiation, with UV-A and UV-B photons being equivalent to around 5% and 0.33% of photosynthetically active radiation photons (PAR, 400–700 nm) at ground level (Robson *et al*., 2019). However, UV causes significant effects on the morphology, physiology and gene expression of plants. Although traditionally considered as a stressor, UV radiation (particularly UV-B) may lead to both damage and acclimation responses, which depend on the irradiance and dose received, and can be notably different under controlled and field conditions (Robson *et al*., 2019). High UV doses, frequently artificial and unrealistic, may cause DNA damage and alterations in the photosynthetic machinery, leading to plant distress (permanent damage), but real ambient UV levels rather cause acclimation responses (eustress) including DNA repairing, growth modulation, and induction of antioxidant defenses and potentially protecting secondary metabolites (Hideg *et al*., 2013; Jordan, 2017). Thus, UV radiation is conceived nowadays as a general regulator of plant metabolism and, indeed, some of its impacts on plants are commercially desirable, such as the increase in secondary metabolites, a more compact architecture and an increased resistance to pests and diseases (Robson *et al*., 2019). As a consequence, UV radiation has an increasing agronomic importance as a biotechnological tool which, adequately handled, can improve crop quality (Wargent, 2017).

Grapevine has been one of the most widely investigated woody crops regarding the effects of UV radiation, from genes to the field (Robson *et al*., 2019; Teixeira *et al*., 2013; Alonso *et al*., 2016). Although UV effects on leaf physiology have notably been studied (Pfündel, 2003; Núñez-Olivera *et al*., 2006; Grifoni *et al*., 2016; Castagna *et al*., 2017; Csepregi *et al*., 2019), the research performed on berries is more meaningful for agricultural applications. Studies of this type have assessed the effects of both ambient solar and artificially enhanced UV levels, by using specific

cut-off filters (Berli *et al*., 2008; Koyama *et al*., 2012; Carbonell-Bejerano *et al*., 2014; Del Castillo-Alonso *et al*., 2015; Song *et al*., 2015; Del Castillo-Alonso *et al*., 2016b; Liu *et al*., 2018; Marfil *et al*., 2019) or lamps providing supplemental UV (Liu *et al*., 2018; Doupis *et al*., 2011; Zhang *et al*., 2013; Martínez-Lüscher *et al*., 2014b; Loyola *et al*., 2016), respectively. In addition, the effects of UV natural gradients have occasionally been evaluated (Del Castillo-Alonso *et al*., 2016a). The induction of nutraceuticals (mainly the stilbene resveratrol) in UV-C-irradiated post-harvest grapes has been another interesting applied research topic (Guerrero *et al*., 2010). Several conceptually different studies have examined the effects of decreasing or increasing the exposure of grapes to the full solar spectrum through shading (Koyama *et al*., 2012; Price *et al*., 1995; Cortell and Kennedy, 2006; Friedel *et al*., 2016) or leaf removal (Kemp *et al*., 2011; Diago *et al*., 2012), respectively. Obviously, in these cases, the specific effects of UV solar wavelengths could not be determined.

Among the diverse variables measured to analyze the UV effects on grapes, their phenolic composition has been by far the most widely assessed. Flavonols, anthocyanins, flavanols, stilbenes, and hydroxycinnamic and hydroxybenzoic acids, are the main phenolic compounds accumulated in grape skins and/or seeds (Teixeira *et al*., 2013). These compounds play important roles in the plant as UV screens, antioxidants, herbivore deterrents, antifungal agents, etc. The most common responses of phenolic compounds to UV radiation are the increase in flavonols and, to a lesser extent, in anthocyanins, whereas the responses of the remaining compounds are more subtle or nonexistent (Jordan, 2017). Phenolic compounds are essential to determine the quality of both grapes and wines, contributing for example to wine color (anthocyanins, flavonols) and mouthfeel (flavanols). In addition, important nutraceutical and pharmacological properties have been attributed to grape and wine phenolic compounds (Teixeira *et al*., 2013). However, only a few studies to our knowledge have assessed to what extent the phenolic composition of UV-exposed or non-exposed grapes is reflected in the resulting wines (Berli *et al*., 2008; Song *et al*., 2015). Moreover, in these studies, only global

variables such as total anthocyanins and total phenolics were measured, without differentiating the diverse phenolic families and individual compounds. Paralleling studies conducted on only grapes, other investigations analyzed in detail the phenolic composition of both grapes and the resulting wines after exposing grapes to different levels of solar radiation, either by shading or leaf removal (Price *et al*., 1995; Kemp *et al*., 2011; Diago *et al*., 2012; Ristic *et al*., 2007). As occurred in the studies performed on only grapes, the specific effects of the UV band could not be established using these experimental designs.

Our aim was to study, under field commercial conditions, to what extent the effects that close-to-ambient solar UV radiation caused on the phenolic composition of grape skins were conserved in the resulting wines. If conserved, the effects of solar UV on wine phenolic composition could be predicted by simply analyzing the composition of grapes and, in addition, wine phenolic composition and associated sensorial characteristics could be somewhat controlled by manipulating the UV exposure of grapes in the field. This manipulation could contribute to improve the quality of both grapes and wine. The study was carried out on grapevines of the variety Tempranillo, one of the most used and fastest-expanding cultivars in recent years (Anderson, 2013).

MATERIALS AND METHODS

Plant material and experimental design. This field experiment was conducted in the 2015 season on an experimental vineyard located in the University of La Rioja (Logroño, La Rioja, northern Spain, 42º 27’ N, 2º 25’ W, 373 m elevation). Plants of *Vitis vinifera* L. cv. Tempranillo (clone 261), grafted onto 110R rootstock and planted in 2011 on loamy soil with N-S row orientation, were used. The plant spacing was 2.7 m between rows and 1.0 m between plants within rows. The vines were spur-pruned (12 buds per vine) in a bilateral cordon and trained to a vertical shoot positioning trellis system. In 2015, the annual precipitation was 398.4 mm, and the average annual temperature was 13.4 ºC. Vines were not irrigated during the growing season.

A completely randomized block design was set-up. Six blocks of two vines each were divided into two experimental conditions (three replicates per treatment): UV-transmitting filter (FUV+) and UV-blocking filter (FUV-). The treatments were established using colourless and transparent polymetacrylate filters (PMMA XT Vitroflex 295 and XT Vitroflex 395 Solarium Incoloro, Polimertecnic, Girona, Spain), which allowed for and blocked, respectively, the transmission of ambient solar UV radiation. The spectral characteristics of these filters were previously published and compared to ambient conditions (Del Castillo-Alonso *et al.*, 2016b). Filters (1.30 x 0.75 m) were placed at 45° from the vertical axis of the plant, on both sides of the canopy, covering the fruiting zone and the first 0.7 m of the canopy of each grapevine. Filters were installed after budbreak (29 April 2015) and maintained until harvest (14 September 2015). Spectral irradiances under the filters were measured regularly from the beginning of the experiment using a spectroradiometer (Macam SR9910, Macam Photometrics Ltd, Livingstone, Scotland) to confirm the stability of their filtering characteristics. Ambient photosynthetic (PAR), UV-A, and UV-B irradiances were continuously recorded close to the experimental plot by broad band radiometers (Skye Quantum SKP 215, SKU 420 and SKU 430, respectively, Skye Instruments Ltd, Powys, UK). The biologically effective UV irradiance (UV_{BE}) received by the plants was estimated using an action spectrum (Flint and Caldwell, 2003). Temperature under the filters was measured using a digital thermometer.

Berry sampling and berry skin analysis

For each treatment and replicate, berries on commercial maturity were collected from two different plants around noon on a sunny day. Berries used for skin analysis were immediately frozen in liquid nitrogen, transported to the laboratory and kept at -80ºC until analysis. The remaining collected berries were used for vinification.

Frozen berries were allowed to partially thaw and skin was carefully removed from the flesh using a scalpel without rupturing the hypodermal cells. The skins were immediately submerged in liquid nitrogen, lyophilized and ground (UltraTurrax® T25 Basic homogenizer, IKA

Labortechnik, Staufen, Germany). For each analytical sample, 50 mg of this material was subsequently ground in a TissueLyser (Qiagen, Hilden, Germany) to obtain a homogeneous powder. Then, 4 mL of methanol:water:7M HCl (70:29:1 v:v:v) was added for extraction (24 h at 4 °C in the dark). The extract was centrifuged at 6000 g for 15 min and the supernatant was considered the source of phenolic compounds.

The bulk level of phenolic compounds was measured as the area under the absorbance curve in the interval 280–400 nm ($AUC_{280–400}$) per unit of dry weight (DW) (Del Castillo-Alonso *et al.*, 2016a), using a Perkin-Elmer λ35 spectrophotometer (Perkin-Elmer, Wilton, CT, USA). Individual phenolic compounds were analyzed by UPLC using a Waters Acquity Ultra Performance LC system (Waters Corporation, Milford, USA) (Del Castillo-Alonso *et al.*, 2016a). The UPLC system was coupled to a micrOTOF II high-resolution mass spectrometer (Bruker Daltonik, Germany) equipped with an Apollo II ESI/APCI multimode source and controlled by the Bruker Daltonics DataAnalysis software. A UV detector module was used at 520 nm for anthocyanins and at 324 nm for the remaining compounds. The electrospray source was operated in negative mode, except for the anthocyanins which operated in positive mode. The capillary potential was set to 4 kV; the drying gas temperature was 200 °C and its flow 9 L min^{-1}; the nebulizer gas was set to 3.5 bar and 25 °C. Spectra were acquired between *m/z* 120 and 1505 in both modes. The different phenolic compounds were identified and quantified using specific commercial pure compounds or, in their absence, compounds with the same chromophore: *t*-resveratrol, catechin, epigallocatechin, procyanidin B2, kaempferol-3-*O*-glucoside, quercetin-3-*O*-glucuronide, myricetin, quercetin, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, syringetin-3-*O*-glucoside, caffeic acid, *p*-coumaric acid, gallic acid, protocatechuic acid, and malvidin-3-*O*-glucoside (Sigma-Aldrich, St. Louis, MO, USA; Fluka, Buchs, Germany; Extrasynthese, Genay, France). Total contents of the different phenolic groups (stilbenes, flavanols, flavonols, hydroxybenzoic acids, hydroxycinnamic acids, and anthocyanins) were obtained as the sum of the respective individual

compounds. The ratios between trihydroxylated and dihydroxylated (3´,4´,5´-OH/3´,4´-OH) anthocyanins, and between trihydroxylated and monohydroxylated (3´,4´,5´-OH/4´-OH) and trihydroxylated and dihydroxylated (3´,4´,5´-OH/3´,4´-OH) flavonols, were also calculated. The antioxidant capacity of berry skins was measured by generating the radical cation 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) as previously described (Del Castillo-Alonso *et al*., 2016a), and was expressed in terms of Trolox equivalent (TE) antioxidant capacity.

Vinification and wine analysis

For each treatment and replicate, grapes were destemmed and crushed and the alcoholic fermentation was carried out (Sampaio *et al*., 2007). Around 3 kg of pomace (must, seed, and skin) were introduced into 2.5 L glass bottles. Potassium metabisulfite (0.09 g kg^{-1}) was added to the samples to give a final total SO_2 concentration of 50 mg L^{-1} and then musts were inoculated with 0.2 g kg^{-1} of commercial *Saccharomyces cerevisiae* r.f. *bayanus* (Enartis, Trecate, Italy). The must was fermented at a controlled temperature of 25 ºC. The alcoholic fermentation finished when reducing sugars were below 2.5 g L^{-1} (two weeks after yeast inoculation). Then, wine was separated from seeds and skins by pressing, and wine analysis was performed. The bulk level of phenolic compounds, individual phenolic compounds, ratios between trihydroxylated, dihydroxylated and monohydroxylated compounds, and antioxidant capacity were analyzed following the same procedures as in berry skins. Enological parameters (alcoholic degree, pH, total acidity, malic acid, color intensity, and total polyphenol index (TPI)) were analyzed according to official methods (EEC, 1990). Total phenols were determined using the Folin–Ciocalteu reagent and data were expressed as gallic acid equivalents (GAE) (Villaño *et al*., 2004).

Statistical analysis

Data normality and homoscedasticity were tested using Shapiro–Wilks's and Levene's tests, respectively. For each variable measured, the effect of the filter (FUV+ *vs.* FUV- samples) was

tested using a Student's *t* test. The statistical procedures were performed with SPSS 24.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Radiation and temperature conditions

The PAR, UV-A, UV-B and UV_{BE} doses received by the FUV+ plants during the studied period were 740 MJ m^{-2}, 79382 kJ m^{-2}, 1759 kJ m^{-2}, and 1956 kJ m^{-2}, respectively. The respective doses received by the FUV- plants were 699 MJ m^{-2}, 2513 kJ m^{-2}, 56 kJ m^{-2}, and 66 kJ m^{-2}. Thus, plants under the FUV+ filter received close-to-ambient solar UV radiation levels (Del Castillo-Alonso *et al*., 2016b), whereas FUV- plants only received around 3% of the doses of UV_{BE} and unweighted UV-A and UV-B received by FUV+ plants. Both FUV+ and FUV- plants received similar PAR doses, and temperature under the FUV+ and FUV- filters was similar along the experiment.

Phenolic compounds and antioxidant capacity of berry skins and wines

A total of 47 phenolic compounds were identified in Tempranillo berry skins and the resulting wines, among which 16 were flavonols, 15 anthocyanins, 8 flavanols, 3 stilbenes, 3 hydroxycinnamic acid derivatives, and 2 hydroxybenzoic acids (Table 6.1). The same compounds were found in both skins and wines, except caffeic acid ethyl ester (only found in wines) and two flavonols, quercetin-3-*O*-rutinoside and the aglycone kaempferol (only found in skins). In skins, the most abundant phenolic group was anthocyanins, followed by flavonols, hydroxycinnamic acid derivatives, flavanols, hydroxybenzoic acids, and stilbenes. In wines, the order was similar but hydroxycinnamic acid derivatives and flavonols switched places in the ranking.

Flavonols were the only phenolic group whose total content increased significantly in FUV+ samples of both skins and wines (Table 6.1). Total contents of anthocyanins in skins and stilbenes in wines also increased significantly under the FUV+ treatment. The total content of hydroxycinnamic acid derivatives in skins showed the opposite behavior, with significantly higher values in FUV- than in FUV+ samples. The total contents of the remaining compounds (anthocyanins and hydroxycinnamic acid derivatives in wines, stilbenes in skins, and flavanols and hydroxybenzoic acids in both skins and wines) did not respond to ambient solar UV.

Regarding the individual compounds, flavonols and anthocyanins showed the clearest responses to UV radiation, particularly in skins (Table 6.1). A total of 13 flavonols (out of 16) in skins and 8 (out of 14) in wines, together with 11 anthocyanins (out of 15) in skins and 6 (out of 15) in wines, significantly increased in FUV+ samples in comparison with FUV- samples. The three stilbenes (resveratrol and two derivatives) also increased significantly under the FUV+ treatment, but only in wines. Two hydroxicinnamic acid derivatives (caffeoyl-tartaric and coumaroyl-tartaric acids) responded to ambient solar UV, but only in skins and showing lower values in FUV+ than in FUV- samples. The contents of one flavanol (gallocatechin) in skins and another one (epigallocatechin gallate) in wines were also lower in FUV+ than in FUV- samples. Finally, the individual hydroxybenzoic acids were not affected by ambient solar UV radiation, neither in skins nor in wines.

The different groups of flavonols and anthocyanins whose contents increased under the FUV+ treatment showed different levels of response (Figure 6.1). Regarding flavonols, increases were relatively stronger in skins than in wines. Kaempferols were the flavonols showing the greatest increase under the FUV+ treatment, both in skins (42-fold) and wines (8-fold). Quercetins also showed a notorious increase in skins (8-fold) and wines (4-fold), while the increase in isorhamnetins was more modest (1.7- and 1.5-fold, respectively) but very significant. Myricetins increased in skins (2-fold) but not in wines, and syringetins did not respond to ambient solar UV neither in skins nor in wines. Overall, mono- and dihydroxylated flavonols (mainly kaempferols and quercetins, respectively) increased stronger than trihydroxylated flavonols in FUV+ samples, both in skins and wines, and thus the ratios 3´,4´,5´-OH/4´-OH and 3´,4´,5´-OH/3´,4´-OH decreased. With respect to anthocyanins, the increases in FUV+ samples were smaller than those found in flavonols, and these increases were similar in skins (1.3- to 2-fold) and wines (1.3- to 2.3-fold). All the types of anthocyanins increased significantly in FUV+ samples except malvidins, and the highest increases were found in disubstituted (cyanidins and peonidins) than in trisubstituted (delphinidins and petunidins) compounds.

The antioxidant capacity in wines was significantly higher in FUV+ than FUV- samples, whereas in skins, although there was a similar trend, differences were not significant (Table 6.1).

Enological parameters of wines

No enological parameter showed significant differences between FUV+ and FUV- samples (Table 6.2). However, there was a trend for higher values of color intensity, TPI and total phenols in FUV+ than FUV- samples.

DISCUSSION

In the present study, we demonstrate for the first time to our knowledge that the influence of (close-to-ambient) solar UV radiation levels on the detailed phenolic composition of grape skins is reflected, to a great extent, in the resulting wines. Previously, only a few studies have investigated the phenolic composition of wines elaborated with grapes exposed or non-exposed to UV radiation, but only analyzing global variables such as total phenols (Berli *et al*., 2008; Song *et al*., 2015). Other studies have analyzed the different phenolic families and individual compounds in both grapes and the resulting wines, but without differentiating the specific effects of the UV band, just exposing grapes to different levels of solar radiation (either by shading or leaf removal) (Price *et al*., 1995; Kemp *et al*., 2011; Diago *et al*., 2012; Ristic *et al*., 2007). Thus, our study is the first showing that the specific effects of UV radiation are notably conserved in the whole way from grape skins to wine.

Responses of phenolic compounds and antioxidant capacity in grape skins

In grape skins, flavonols showed the strongest response to UV radiation, increasing in FUV+ samples in comparison to FUV- samples. This was expected and had previously been found in a number of studies carried out under different experimental conditions in both white and red grapevine varieties, including Tempranillo (Teixeira *et al*., 2013; Koyama *et al*., 2012; Carbonell-Bejerano *et al*., 2014; Del Castillo-Alonso *et al*., 2015, 2016b; Liu *et al*., 2018; Martínez-Lüscher *et al*., 2014b; Downey *et al*., 2004). Flavonols increase was surely favored by the UV-induced up-regulation of phenylpropanoid pathway genes (Jordan, 2017). Anthocyanins response to UV radiation was almost as consistent as that of flavonols, mostly showing higher values in FUV+ than in FUV- samples. Responses of anthocyanins to UV are complex because they generally depend on the interaction of additional internal (variety, developmental stage of the berry) and environmental (temperature) factors (Jordan, 2017; Del Castillo-Alonso *et al*., 2016b; Martínez-Lüscher *et al*., 2014b; Berli *et al*., 2011; Verzera *et al*., 2016). In some cases, anthocyanins may

even not respond to radiation (Alonso *et al*., 2016; Cortell and Kennedy, 2006) or may only respond in interaction with low temperatures (Mori *et al*., 2007). Thus, in our study, responses of anthocyanins to UV were clearer than usual. The hydroxylation level of both flavonols and anthocyanins decreased under UV radiation. The change towards less hydroxylated flavonol forms in skin grapes under UV radiation is a common finding in several grapevine varieties (Carbonell-Bejerano *et al*., 2014; Del Castillo-Alonso *et al*., 2015, 2016b; Liu *et al*., 2018; Martínez-Lüscher *et al*., 2014b; Berli *et al*., 2011; Liu *et al*., 2015). Regarding anthocyanins, the picture may be more complex because their hydroxylation level can be affected not only by radiation but also by temperature and hormonal factors (Alonso *et al*., 2016; Spayd *et al*., 2002; Downey *et al*., 2006).

Hydroxybenzoic acids, stilbenes, and most flavanols in grape skins did not respond to UV, whereas hydroxycinnamic acid derivatives and the flavanol gallocatechin showed an opposite response to that of most flavonols and anthocyanins, increasing in FUV- samples in comparison to FUV+ samples. This lack of induction of all these compounds by UV was consistent with previous studies performed on Tempranillo and other varieties (Koyama *et al*., 2012; Carbonell-Bejerano *et al*., 2014; Del Castillo-Alonso *et al*., 2016b). Stilbenes are synthesized by grapes in response to both biotic and abiotic stressors, including pathogen attacks, and they also vary along berry development (Jug and Rusjan, 2012; Flamini *et al*., 2013). Regarding UV radiation, they mainly respond to UV-C (González-Barrio *et al*., 2009; Hasan and Bae, 2017), whereas their response to other UV wavelengths is more subtle or nonexistent (Del Castillo-Alonso *et al*., 2016b), unless the UV levels received are considerably high (Berli *et al*., 2008). Flavanols are little responsive to environmental factors (Teixeira *et al*., 2013), and, when responding, they may preferentially be influenced by photosynthetic than by UV radiation (Jordan, 2017). The contrasting response of hydroxycinnamic acid derivatives in comparison with that of most flavonols and anthocyanins would be due to the lack of an adequate UV stimulus for the synthesis of more complex phenolic compounds in FUV- samples, given that the biosynthesis of

hydroxycinnamic acids and flavonoids compete for the same precursors (Del Castillo-Alonso *et al.*, 2016b).

The bulk level of phenolic compounds in grape skins was not significantly affected by UV radiation, as occurred in other similar studies (Berli *et al.*, 2008; Del Castillo-Alonso *et al.*, 2015, 2016b). This was probably due to the fact that this variable encompasses many different compounds which may respond differently to UV (see above). This lack of response makes this variable not particularly recommendable to analyze the effects of UV radiation on the phenolic composition of grape skins.

As occurred with the bulk level of phenolic compounds, the antioxidant capacity of grape skins was not significantly affected by UV radiation. In previous studies using also close-to-ambient UV levels, the antioxidant capacity slightly increased with increasing UV exposure (Alonso *et al.*, 2016; Berli *et al.*, 2015). These nonexistent or only slight responses may be logical because, in UV-adapted plants such as grapevine, ambient UV levels do not induce a strong oxidative stress but only eustress (Hideg *et al.*, 2013), and thus antioxidant mechanisms would be activated only modestly or not activated at all. Moreover, the antioxidant capacity is a global variable comprising the antioxidant properties of many different compounds which may increase, decrease or remain unaffected in response to UV radiation, compensating in some way the specific response of each compound. In our study, the lack of response could be partly related with the proportionally lower values of trihydroxylated forms of flavonols and anthocyanins which were found in FUV+ samples in comparison with FUV- samples, given that the antioxidant capacity generally decreases in compounds with lower hydroxylation levels (Heim *et al.*, 2002; Csepregi *et al.*, 2016). Nevertheless, the antioxidant capacity of flavonoids depends on other structural features (types of substitutions, glycosylation, etc.) and the diverse mechanisms of action of the different molecules (Heim *et al.*, 2002), together with the method used for its determination (Csepregi *et al.*, 2016). In addition, phenolic compounds can act not only as antioxidants but also as UV screens (Agati and Tattini, 2010), which further complicates the

interpretation of results. Thus, more studies are needed to better understand the effects of UV radiation on the antioxidant capacity of grape skins.

Responses of phenolic compounds and antioxidant capacity in wines

Among the 65 variables measured (Table 6.1, Figure 6.1), 36 (55%) showed the same response to UV radiation in both grape skins and wine. Among these 36 variables, 19 had significantly been induced by UV in grapes, whereas 17 had shown no response to UV. Thus, the UV-induced changes found in grape skins were notably reflected in the resulting wines and some of their phenolic characteristics (particularly, flavonols) could be predicted from those of grape skins. Conversely, other phenolic compounds in grape skins would change or would be produced *de novo* during vinification (for example, stilbenes and hydroxycinnamic acid derivatives), and hence their contents in wine could not be predicted on the basis of skin composition. Our results are hardly comparable with those obtained in other studies because the specific effects of UV radiation on grape skins have rarely been further assessed in the resulting wines (Berli *et al*., 2008; Song *et al*., 2015). In the vast majority of related studies, full solar radiation (and hence not only the UV wavelengths) has been considered, which limits the significance of any comparison and makes our results quite unique.

Flavonols were the phenolic compounds more reliably conserved from grape skins to wine. The contents of total flavonols, kaempferols, quercetins, and isorhamnetins, together with the contents of seven individual flavonols (out of 14), increased in FUV+ wine samples in comparison with FUV- samples, showing the same response as in grape skins. In addition, syringetins showed a similar lack of response to UV in grape skins and wines. Flavonols in general, and particularly quercetins, have been found to increase in wines elaborated with grapes exposed to full solar radiation, without differentiating the specific effect of UV wavelengths (Price *et al*., 1995; Diago *et al*., 2012). Our results suggest that the UV fraction of solar radiation would be responsible for these increases. The strong increase in flavonols in wine would improve its quality through

several aspects, such as their role in copigmentation (stabilizing anthocyanins and wine color) and their importance as bioactive healthy compounds (Price *et al.*, 1995; Flamini *et al.*, 2013).

The wine contents of all the main types of anthocyanins and six individual anthocyanins (out of 15) paralleled the responses to UV found in grape skins, showing mostly positive reactions. However, the contents of total and nine individual anthocyanins showed opposite responses to UV to those found in skins. Thus, anthocyanins were worse predictors than flavonols for the UV-induced changes occurred from skins to wine. This was probably due to the diverse anthocyanin changes that may take place during winemaking (Gao *et al.*, 1997; Squadrito *et al.*, 2010; Dimitrovska *et al.*, 2015). The increase in wine anthocyanins in response to close-to-ambient solar UV radiation was less consistent than in the case of flavonols. Given that anthocyanins are crucial compounds for the red color of wine, the influence of UV radiation on this parameter seemed to be rather limited in our study. This was in line with the not significant increase in color intensity in wines made from UV-exposed grapes. However, in Pinot noir, bunch exposure to ambient levels of UV substantially increased wine anthocyanins in comparison with bunches non-exposed to UV (Song *et al.*, 2015). Similar results were found in wines made from Pinot noir and Shiraz grapes exposed to the full solar spectrum (not only UV) as compared with wine made from shaded grapes (Price *et al.*, 1995; Ristic *et al.*, 2007). Differences in the variety and the experimental conditions could explain these discrepancies.

The three individual stilbenes analyzed (resveratrol and two derivatives), together with the total content of stilbenes, increased significantly in wines under the FUV+ treatment. A similar increase was found in Tempranillo wines when elaborated with grapes exposed to solar radiation through leaf removal (Diago *et al.*, 2012). More studies are needed to confirm that this increase can be attributed to solar UV wavelengths. This would be important because resveratrols are considered healthy compounds (Flamini *et al.*, 2013). In our study, resveratrols increased but not significantly in skins, and thus their contents in wine could not be reliably predicted on the basis of skin contents. This finding was apparently contradictory because

resveratrols are mainly located in skins (Sun *et al*., 2006), but it can be justified because these compounds can change (even increase) during winemaking, as has been reported in other red varieties (Sun *et al*., 2006; Mattivi *et al*., 1995).

As occurred in grape skins, the contents of total and most individual flavanols in wine did not respond to ambient solar UV. However, Pinot noir bunch exposure to ambient UV levels increased substantially wine tannin content (Song *et al*., 2015), This apparent discrepancy could be due to varietal differences, and also to the fact that tannins include compounds ranging from small oligomeric forms to large polymers (Downey *et al*., 2006), representing a different variable to that analyzed in our study. Different studies have reported that the wines produced from grapes exposed to the full spectrum of solar radiation can have higher (Kemp *et al*., 2011; Ristic *et al*., 2007) or lower (Price *et al*., 1995) flavanol contents than the wines produced from shaded grapes. Clearly, more studies are needed to solve these inconsistencies, because flavanols are important in wine, contributing to body, mouthfeel, and color stability (Downey *et al*., 2006). In addition, flavanols are healthy compounds due to their antioxidant and anticarcinogenic properties (Heim *et al*., 2002).

Hydroxybenzoic acids in wines were not affected by ambient solar UV radiation, as occurred in grape skins. However, hydroxycinnamic acid derivatives showed different responses in skins and wine, increasing in FUV- skins but not showing any influence of UV in wines. To our knowledge, there is no comparative study analyzing the specific influence of UV exposure of grapes on the contents of these compounds in wines. Regarding the effects of total solar radiation, early defoliation (and a consequently higher sun exposure of grapes) in Tempranillo led to wines with higher levels of hydroxycinnamic acids (Diago *et al*., 2012), but in Pinot noir these compounds in wine were inversely related to grape sun exposure because of reactions produced during vinification (Price *et al*., 1995). As discussed for other compounds, further research is needed on this topic.

Although the antioxidant capacity of grape skins was not influenced by UV, that of wines was higher in FUV+ than FUV- samples. This occurred despite the relative decrease of trihydroxylated forms of flavonols and anthocyanins, in comparison with di- or monohydroxylated forms, in FUV+ wine samples. Consequently, 1) the antioxidant capacity of grape skins could not predict that of wines, possibly because of the chemical changes occurring during vinification; and 2) the antioxidant capacity of wines did not strongly depend on the hydroxylation level of flavonols and anthocyanins. In addition, it should be taken into account that there exist different methods to measure the antioxidant capacity, and each method may lead to different conclusions (Csepregi *et al*., 2016). To our knowledge, the effect of the UV exposure of grapes on the antioxidant capacity of the resulting wines has not previously been studied, despite the healthy effects derived from a potential increase of this variable (Heim *et al*., 2002).

Responses of other enological parameters of wines

Although close-to-ambient levels of solar UV radiation greatly influenced the phenolic composition of grape skins and the resulting wines, they did not affect significantly other enological parameters of these wines, such as alcoholic degree, pH, total acidity, malic acid, color intensity, TPI, and total phenols. However, there was a trend for higher values of the three last variables in FUV+ than FUV- samples, in line with the significant results found in wine that have been described above. In this regard, previous studies show inconclusive results. In Malbec, exposure of grapes to UV radiation did not influence TPI and color intensity in the resulting wines (Berli *et al*., 2008), but in Pinot noir color and total phenols increased while malic acid decreased (Song *et al*., 2015). These inconsistencies have also been found in wines made from grapes that had been differently exposed to the full solar spectrum through leaf removal or shading. In wines made from exposed grapes, the alcoholic degree increased (Diago *et al*., 2012; Ristic *et al*., 2007) or remained unaltered (Kemp *et al*., 2011; Vilanova *et al*., 2012); total phenols and color increased (Diago *et al*., 2012; Ristic *et al*., 2007), but not always (Price *et al*., 1995); pH decreased (Verzera *et al*., 2016) or remained unchanged (Kemp *et al*., 2011; Diago *et al*., 2012; Ristic *et al*.,

2007; Vilanova *et al*., 2012), acidity increased (Verzera *et al*., 2016), decreased (Ristic *et al*., 2007), or did not vary (Kemp *et al*., 2011; Diago *et al*., 2012; Vilanova *et al*., 2012); and malic acid decreased (Ristic *et al*., 2007). The variability of these responses could be due to differences in the grapevine variety, the experimental conditions applied to the grapes, and the vinification process used.

CONCLUSIONS

To our knowledge, this is the first study in which the effects of close-to-ambient solar UV radiation on phenolic composition have been assessed from grape skins to wine. We have demonstrated that the phenolic profile of UV-exposed grape skins could predict, to a great extent, the phenolic profile of the resulting wines. In both skins and wines, the UV influence was positive for flavonols and, to a lesser extent, anthocyanins, and neutral for flavanols, hydroxybenzoic acids, and the bulk level of phenolic compounds. However, for stilbenes, hydroxycinnamic acid derivatives, and antioxidant capacity, the effect of UV was different in skin and wine, probably due to changes during vinification. In addition, the enological parameters of wine were not significantly influenced by the UV exposure of grapes, although there was a trend for increasing color intensity, TPI, and total phenols in wines elaborated from UV-exposed grapes. The significant increases of flavonols, anthocyanins, stilbenes, and antioxidant capacity in wines produced from UV-exposed grapes suggested that UV improved wine quality by positively influencing color stability and healthy properties. Overall, in the context of our study, wine quality depended on both the phenolic composition of grape skins and the changes occurring during vinification. Thus, it is important to know how UV management could contribute to improve both factors, and further research is needed to better understand these processes.

TABLES AND FIGURES

Table 6.1. Influence of the treatments received by the Tempranillo berry skins on the phenolic composition and antioxidant capacity of both skins and the resulting wines. FUV+, UV-transmitting filter. FUV-, UV-blocking filter. DW, dry weight. TE, Trolox equivalent. AUC280–400, area under the absorbance curve in the interval 280–400 nm. Values are means ± standard errors (n=3). For each variable, significant differences between FUV+ and FUV- samples (Student's t) are shown: ***, *p* < 0.001; **, *p* < 0.01; *, *p* < 0.05; ns, not significant.

	Berry skin		Wine		Statistical significance	
	FUV+	FUV-	FUV+	FUV-	Berry skin	Wine
Antioxidant capacity	(µmol TE g^{-1} DW)		(mM TE)			
	706 ± 15	685 ± 18	5.9 ± 0.2	3.6 ± 0.3	ns	**
Bulk level of phenolic compounds	($AUC_{280-400}$ mg^{-1} DW)		($AUC_{280-400}$)			
	57 ± 2	50 ± 4	1052 ± 69	1177 ± 42	ns	ns
Stilbenes	(µg g^{-1} DW)		(mg L^{-1})			
Resveratrol	1.6 ± 0.2	3.0 ± 0.4	0.04 ± 0.00	0.02 ± 0.00	ns	**
cis Resveratrol-3-*O*-glucoside	24 ± 3	13 ± 1	0.97 ± 0.11	0.46 ± 0.01	ns	*
trans Resveratrol-3-*O*-glucoside	28 ± 4	17 ± 5	0.59 ± 0.04	0.26 ± 0.01	ns	***
Total stilbenes	**54 ± 6**	**33 ± 7**	**1.60 ± 0.14**	**0.74 ± 0.02**	ns	**
Flavanols	(µg g^{-1} DW)		(mg L^{-1})			
Catechin	89 ± 8	91 ± 3	11.1 ± 0.7	11.3 ± 0.8	ns	ns
Epicatechin	9.9 ± 0.6	9.1 ± 0.0	5.6 ± 0.5	4.6 ± 0.3	ns	ns
Catechin gallate	10 ± 1	8.6 ± 1.0	0.0 ± 0.0	0.04 ± 0.01	ns	ns
Epigallocatechin	3.0 ± 0.2	3.6 ± 0.1	12 ± 1	13 ± 1	ns	ns
Gallocatechin	34 ± 1	40 ± 1	1.2 ± 0.1	1.4 ± 0.1	*	ns
Epigallocatechin gallate	13 ± 1	14 ± 1	0.02 ± 0.00	0.03 ± 0.00	ns	*
Procyanidin B1	116 ± 9	133 ± 5	5.4 ± 0.3	5.7 ± 0.3	ns	ns
Procyanidin B2	14 ± 1	13 ± 0	1.1 ± 0.1	0.9 ± 0.0	ns	ns
Total flavanols	**289 ± 21**	**312 ± 17**	**37 ± 3**	**37 ± 3**	ns	ns
Flavonols	(µg g^{-1} DW)		(mg L^{-1})			
Kaempferol	0.28 ± 0.05				***	
Kaempferol-3-*O*-glucoside	57 ± 7	1.4 ± 0.4	0.08 ± 0.01	0.01 ± 0.00	*	*
Kaempferol-3-*O*-galactoside	11.3 ± 1.6	0.23 ± 0.04	0.03 ± 0.00	0.00 ± 0.00	*	**
Myricetin	69 ± 3	58 ± 5	3.1 ± 0.2	1.5 ± 0.0	ns	*
Myricetin-3-*O*-glucoside	2557 ± 152	1286 ± 9	3.5 ± 0.5	2.6 ± 0.2	**	ns
Myricetin-3-*O*-glucuronide	234 ± 4	145 ± 10	1.7 ± 0.2	1.0 ± 0.1	**	*
Laricitrin-3-*O*-glucoside	199 ± 9	141 ± 7	0.81 ± 0.08	0.65 ± 0.05	**	ns
Quercetin	1.4 ± 0.1	0.29 ± 0.04	1.9 ± 0.2	0.57 ± 0.03	**	ns
Quercetin-3-*O*-galactoside	154 ± 18	31 ± 6	14 ± 1	1.7 ± 0.1	*	*
Quercetin-3-*O*-glucoside	788 ± 65	60 ± 2	7.9 ± 0.5	1.6 ± 0.2	**	*
Quercetin-3-*O*-glucuronide	922 ± 87	147 ± 17	8.5 ± 1.0	2.0 ± 0.2	*	**
Quercetin-3-*O*-rutinoside	64 ± 8	18 ± 2			*	
Isorhamnetin-3-*O*-glucoside	76 ± 1	48 ± 0	0.10 ± 0.00	0.03 ± 0.00	***	***
Isorhamnetin-3-*O*-glucuronide	10 ± 1	2.0 ± 0.3	2.0 ± 0.3	1.3 ± 0.3	*	ns
Syringetin	4.0 ± 0.4	3.3 ± 0.0	0.20 ± 0.02	0.20 ± 0.01	ns	ns
Syringetin-3-*O*-glucoside	65 ± 3	62 ± 2	2.4 ± 0.2	2.2 ± 0.1	ns	ns
Total flavonols	**5213 ± 274**	**2003 ± 71**	**46 ± 3**	**15 ± 1**	***	**

	Berry skin		Wine		Statistical significance	
	FUV+	FUV-	FUV+	FUV-	Berry skin	Wine
Hydroxybenzoic acids	($\mu g\ g^{-1}$ DW)		($mg\ L^{-1}$)			
Protocatechuic acid	77 ± 4	75 ± 1	0.75 ± 0.03	0.60 ± 0.08	ns	ns
Gallic acid	15 ± 1	14 ± 1	15 ± 1	15 ± 1	ns	ns
Total hydroxybenzoic acids	**92 ± 4**	**89 ± 1**	**16 ± 1**	**15 ± 1**	ns	ns
Hydroxycinnamic acid derivatives	($\mu g\ g^{-1}$ DW)		($mg\ L^{-1}$)			
Caffeoyl tartaric acid	279 ± 6	469 ± 19	39 ± 2	35 ± 4	*	ns
Caffeic acid ethyl ester			0.21 ± 0.01	0.20 ± 0.03		ns
Coumaroyl tartaric acid	104 ± 6	171 ± 9	11 ± 1	10 ± 0	**	ns
Total hydroxycinnamic acids	**383 ± 6**	**640 ± 11**	**50 ± 3**	**46 ± 4**	*	ns
Anthocyanins	($mg\ g^{-1}$ DW)		($mg\ L^{-1}$)			
Cyanidin-3-*O*-glucoside	2.2 ± 0.4	1.1 ± 0.1	1.7 ± 0.1	0.5 ± 0.1	ns	*
Cyanidin-3-*O*-(6'-acetyl)glucoside	0.05 ± 0.00	0.03 ± 0.00	1.3 ± 0.1	0.6 ± 0.1	*	ns
Cyanidin-3-*O*-(6'-*p*-coumaroyl)glucoside	0.02 ± 0.00	0.00 ± 0.00	3.0 ± 0.4	0.7 ± 0.0	ns	*
Peonidin-3-*O*-glucoside	7.8 ± 0.8	5.2 ± 0.4	17 ± 1	8.2 ± 0.8	*	**
Peonidin-3-*O*-(6'-acetyl)glucoside	0.18 ± 0.00	0.15 ± 0.00	1.6 ± 0.1	1.4 ± 0.1	**	ns
Peonidin-3-*O*-(6'-*p*-coumaroyl)glucoside	4.2 ± 0.1	3.0 ± 0.2	2.2 ± 0.2	1.4 ± 0.2	*	*
Delphinidin-3-*O*-glucoside	17 ± 1	11 ± 1	43 ± 4	24 ± 2	*	*
Delphinidin-3-*O*-(6'-acetyl)glucoside	0.25 ± 0.01	0.21 ± 0.00	1.4 ± 0.2	1.1 ± 0.1	ns	ns
Delphinidin-3-*O*-(6'-*p*-coumaroyl)glucoside	8.7 ± 0.3	5.5 ± 0.5	11 ± 1	8.4 ± 0.9	**	ns
Petunidin-3-*O*-glucoside	18 ± 1	13 ± 1	50 ± 4	32 ± 4	**	*
Petunidin-3-*O*-(6'-acetyl)glucoside	0.57 ± 0.03	0.52 ± 0.01	3.3 ± 0.4	3.6 ± 0.5	ns	ns
Petunidin-3-*O*-(6'-*p*-coumaroyl)glucoside	12 ±1	7.5 ± 0.6	4.6 ± 0.5	4.0 ± 0.5	**	ns
Malvidin-3-*O*-glucoside	74 ± 3	58 ± 3	244 ± 17	231 ± 23	*	ns
Malvidin-3-*O*-(6'-acetyl)glucoside	14 ± 1	9.3 ± 1.3	22 ± 2	18 ± 0	*	ns
Malvidin-3-*O*-(6'-*p*-coumaroyl)glucoside	51 ± 1	39 ± 2	25 ± 4	20 ± 2	*	ns
Total anthocyanins	**210 ± 7**	**154 ± 10**	**432 ± 35**	**364 ± 37**	*	ns

Table 6.2. Influence of the treatments received by the Tempranillo berry skins on the enological parameters of the resulting wines. FUV+, UV-transmitting filter. FUV-, UV-blocking filter. GAE, gallic acid equivalents. Values are means ± standard errors (n=3). For each variable, significant differences between FUV+ and FUV- samples (Student's t) are shown (ns, not significant).

Enological parameters	FUV+	FUV-	Statistical significance
Alcoholic degree (% v/v)	11 ± 0	12 ± 0	**ns**
pH	3.9 ± 0.0	4.0 ± 0.0	**ns**
Total acidity (g L^{-1} tartaric acid)	5.3 ± 0.1	4.9 ± 0.0	**ns**
Malic acid (g L^{-1})	3.4 ± 0.0	3.1 ± 0.1	**ns**
Color intensity	11 ± 1	9.1 ± 0.9	**ns**
Total polyphenol index (TPI)	25 ± 1	22 ± 1	**ns**
Total phenols (GAE, g L^{-1})	1.3 ± 0.1	1.2 ± 0.0	**ns**

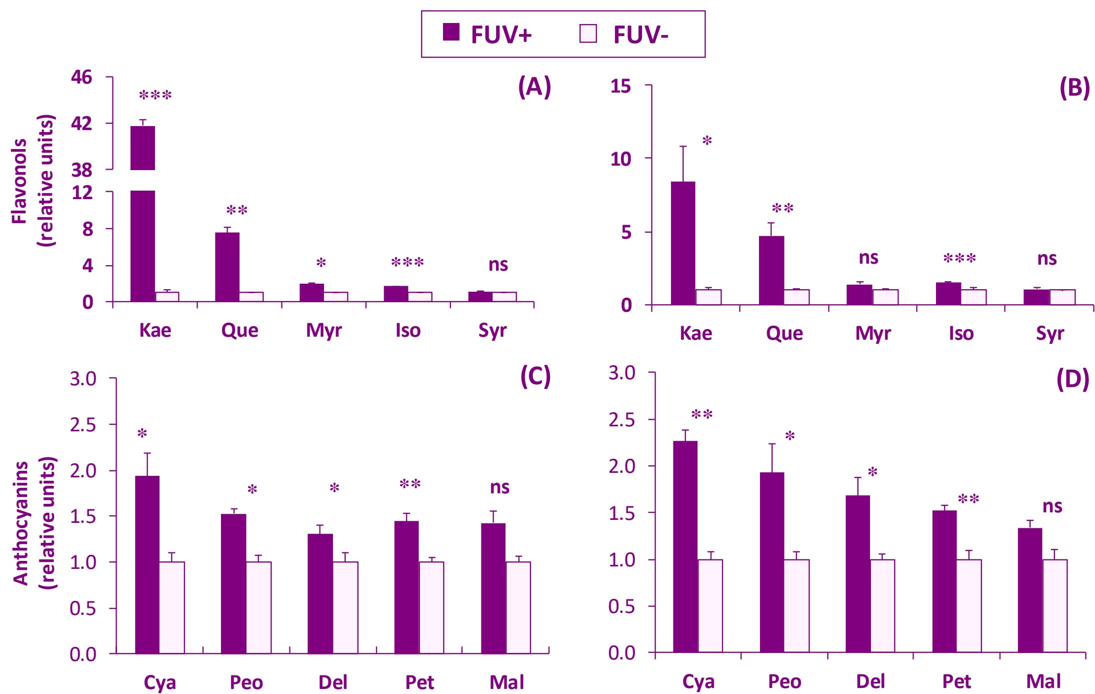


Figure 6.1. Total contents (means ± SE, n=3) of flavonols and anthocyanins in grapevine berry skins (A and C) and the resulting wines (B and D), under the two experimental conditions used in the present study: UV-transmitting filter (FUV+) and UV-blocking filter (FUV-). The diverse types of flavonols and anthocyanins have been differentiated: Kae, kaempferols; Que, quercetins; Myr, myricetins; Iso, isorhamnetins; Syr, syringetins; Cya, cyanidins; Peo, peonidins; Del, delphinidins; Pet, petunidins; Mal, malvidins. Values are expressed in relative units, being the value of each type of compound equivalent to the unit. For each type of compound, significant differences between FUV+ and FUV- samples (Student's t) are shown: ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant.

Chapter 7. Synthesizing the effects of UV radiation on grapes and the resulting wines

Synthesizing the effects of UV radiation on grapes and the resulting wines

ABSTRACT

The effects of UV radiation on *Vitis vinifera* cv. Tempranillo grapes and the resulting wines were studied under field conditions considering integratively the following factors of variation: UV wavelength (differentiating the effects of ambient levels of UV-A and UV-B wavelengths, and also studying the effects of a realistic UV-B enhancement compatible with the predictions of global climate change); phenological stages of the grape (pea size, veraison and harvest); grape components (skin, flesh and seeds); and cell locations of the potentially protecting phenolic UV-absorbing compounds (UVACs).
The variables measured to evaluate UV effects were related to the quality of both grapes and wines: total phenols, total flavonoids, bulk levels of UVACs, antioxidant capacity, and individual phenolic compounds and volatile organic compounds (VOCs), together with grape morphology and enological parameters as wine color. These variables covered the whole way from grape characteristics to wine elaboration. In addition, we incorporated a specific study on the molecular mechanisms (gene expression) underlying the metabolic effects observed. UV effects were wavelength-specific, with ambient UV-B causing stronger effects than ambient UV-A. These effects included increases in the fresh weight, diameter and flesh proportion of grapes, contents of flavonols, and expression of genes related to the synthesis of phenolic compounds (*VvFLS4* and *VvCHS1*). Some synergic effects between UV-B and UV-A were observed. The responses of other phenolic compounds (anthocyanins, flavanols, stilbenes and acids) and volatile organic compounds (VOCs) to UV were more diffuse. Enhanced UV-B led to rather subtle changes in comparison with ambient UV-B, but changes were globally significant as shown by multivariate analysis. Some of these responses of grapes to UV were conserved in the resulting wines, thus affecting their sensorial characteristics, whereas other effects could be modified during the process of winemaking. Responses to UV strongly depended on both phenological stage and grape component, with veraison and skin as the most UV-responsive stage and component, respectively. Nevertheless, the overall natural evolution of phenolic compounds from pea size to harvest was not modified by UV under the experimental conditions used in our study. The cell location of the UVACs was an additional factor influencing UV effects, with the mainly vacuolar methanol-soluble fraction of phenolic compounds more UV-responsive than the methanol-insoluble cell wall-bound fraction, which was UV-insensitive. Some technical constraints influencing UV research under field conditions are discussed.

The overall conclusion was that ambient solar UV-B is essential for the synthesis of a number of phenolic compounds contributing to grape and wine quality, and with potential use as nutraceuticals. This was demonstrated from genes to metabolites. To our knowledge, this is the most integrative study carried out on the effects of UV radiation on grapes and the resulting wines, and it can have implications for an adequate management of UV radiation for a better quality of grapes and wines.

INTRODUCTION

As described in previous chapters and the general Introduction, many studies have been carried out about the effects of UV radiation on grapevine. These studies have considered different organs of the plant (mainly leaves and fruits), different UV managements (filters, lamps, natural UV gradients), different variables to be measured (from gene expression to physiological parameters and secondary metabolites), different grapevine varieties, different campaigns, different development stages of the plant, and even interactions of UV with other environmental factors. Thus, a notable background of knowledge is already available for the scientific community, growers and technicians. However, most studies have reflected partial perspectives of the topic due to the complexity of conducting more global experiments combining several simultaneous experimental scales. In addition, the UV effects on the whole commercial way from grapes to wine has only rarely been studied, which limits the applicability of the knowledge generated.

In the context described, our aim was to synthesize in some way the different experimental scales mentioned above, mainly focusing on the interests of the growers and winemakers. For this aim, we applied different radiation regimes to the plants to differentiate the effects of UV-A and UV-B wavelengths, and also the effects of the enhanced UV-B levels which will probably reach the Biosphere as a consequence of climate change (Bais *et al*. 2019). This gave our study a more global perspective through the inclusion of both agricultural and ecological aspects. In addition, as in previous chapters of this Thesis, we measured variables directly related to the quality of grapes and wines (such as the phenolic composition and the volatile organic compounds, VOCs), covering the whole way from grape characteristics to wine elaboration, but we incorporated here a specific study on the molecular mechanisms (gene expression) underlying the metabolic effects observed. For this specific aim, we started from the knowledge generated by one of the pioneer studies on this matter (Carbonell-Bejerano *et al*. 2014). Finally, given that the development of the grape and its different components strongly influence its

secondary metabolism, we analyzed a great diversity of compounds in three phenological stages (pea size, veraison and harvest) and three grape components (skin, flesh and seeds), also considering the different cell locations of the potentially protecting phenolic UV-absorbing compounds. For a better understanding, the details of this overall approach have been schematized in Figure 7.1.

The present study is only a part (the 2017 campaign) of a more ambitious experiment which took place during several years. However, time limitations have prevented us to show all the results, some of which are still under treatment.

MATERIALS AND METHODS

Plant material, culture conditions and experimental design

The present field experiment was conducted in the 2017 season in an experimental vineyard located in the University of La Rioja (Logroño, La Rioja, northern Spain, 42º 27' N, 2º 25' W, 373 m elevation). The experiment was performed on *Vitis vinifera* L. cv. Tempranillo (clone 43) plants grafted onto 110R rootstock and planted in 50 L pots in 2013. An automatic drip irrigation system maintained the plants at water field capacity, and soil water content was continuously measured with a tensiometer (Watermark, Irrometer Company, Riverside, CA, USA). The soil in the plot was slightly alkaline (pH 8.0) with a medium texture (55% sand, 29% silt, 16% clay) and 1.4% content in organic matter. Plants were kept in a good phytosanitary status along the experiment. A completely randomized block design was set-up. Each block consisted of a frame built with metal profiles that allowed the positioning of different radiation filters (Figure 7.2). Dimensions of each block were: 1.7 m high, 1.5 m wide and 1 m in depth. Block orientation was N-S, and the N side was covered with a shading mesh (PE/RF 70, Rombull Ronets, Alicante, Spain) preventing sunlight to affect the plants but allowing aeration. S, E and W sides of the blocks, together with the top part, were covered with specific cut-off filters. Lateral filters (1.5 m wide and 1.1 m high) were placed at 45° from the vertical axis of the blocks, while top filters were placed at 15º inclination to facilitate rainwater evacuation. Blocks were separated 1.5 m and shading mesh was placed covering the top and S side of the gaps between the blocks. A total of 18 blocks were set-up and were divided into five experimental conditions (radiation regimes), with 3-4 replicates for each treatment:

- P (photosynthetically active radiation, PAR, alone), using XT Vitroflex 395 Solarium Incoloro (Polimertecnic, Girona, Spain), which cut off all UV radiation.
- PA (PAR + UV-A), using acetate Folex 320 (Folex GmbH, Dreieich, Germany), which cut off UV-B and UV-C radiation. As this filter was flexible, it was complemented with an additional

polymetacrylate rigid filter (PMMA XT Vitroflex 295, Polimertecnic, Girona, Spain) preventing the tear of the acetate film by adverse meteorological conditions.

- PAB (PAR + UV-A + UV-B), using PMMA XT Vitroflex 295 (Polimertecnic, Girona, Spain), which cut off UV-C radiation.
- PB (PAR + UV-B), using a Vitroflex 395 filter and UV-B lamps (TL 40W/12 UVB, Philips Lighting, Madrid, Spain). Lamps were switched on during 10-min periods in the central hours of the day to provide the plants with the same UV-B that would receive if exposed to ambient sunlight.
- PAB↑ (PAR + UV-A + enhanced UV-B), using the same filter as in PAB and the same lamps as in PB, but adjusting the time of functioning of the lamps to provide the plants with 10% higher UV-B than that received in the PAB treatment.

Two plants were placed in each block. To prevent the excess of shading on the plants in the treatments that required lamps with respect to the treatments that did not, lamps were placed in all the blocks, although they were switched on only in the PB and PAB↑ treatments. Filters were placed from 6 April (before bud break) to 5 September (harvest). Spectral irradiances under the filters were measured regularly, including daily variations, from the beginning of the experiment (Macam SR9910 spectroradiometer, Macam Photometrics Ltd, Livingstone, Scotland). These meticulous measurements allowed to confirm the stability of the filtering properties of the filters and to precisely establish the radiation received by the plants under each radiation regime. In addition, ambient PAR, UV-A, and UV-B irradiances were continuously recorded close to the experimental plot by broad-band sensors (Skye Quantum SKP 215, SKU 420 and SKU 430, respectively, Skye Instruments Ltd., Powys, UK). The biologically effective UV irradiance (UV_{BE}) was estimated using the action spectra of Caldwell (1971) and Flint and Caldwell (2003). Temperature outside and inside the blocks was measured using a digital thermometer.

Grape sampling and analysis (Figure 7.1)

For each treatment and replicate, grapes were collected from the two plants around noon on sunny days in three different phenological stages: pea size (16 June), veraison (14 July) and harvest (5 September). Grapes used for analysis were immediately frozen in liquid nitrogen, transported to the laboratory and kept at -80ºC until further procedure. The remaining collected grapes were used for vinification.

Phenolic compounds were analyzed in entire grapes (skin and flesh together, discarding seeds) in three phenological stages (pea size, veraison and harvest), from only the methanol-soluble fraction. In addition, at harvest, phenolic compounds were separately analyzed in each of the three grape components (skin, flesh and seeds) and differentiating the methanol-soluble and methanol-insoluble fractions, which are mainly located in the vacuoles and bound to the cell walls, respectively. Methodological details of extraction and analysis can be found in Del-Castillo-Alonso *et al*. (2015). In brief, for extraction of phenolic compounds from the ensemble of skin and flesh, seeds were retired from the frozen grapes and the remaining material was ground together in liquid nitrogen in a mortar to obtain a homogeneous powder, which was stored at -80ºC. For each subsequent extraction, 200 mg FW of this material and 2 mL of extractant was used. For extraction from each of the three grape components separately, frozen berries were allowed to partially thaw, components were separated and material of each component was immediately submerged in liquid nitrogen, lyophilized and ground (UltraTurrax® T25 Basic homogenizer, IKA Labortechnik, Staufen, Germany). For subsequent extraction, 50 mg DW of skins (and 4 ml of extractant), 200 of flesh and 80 of seeds (and 2 mL of extractant in these two last cases), were used. Methanol:water:7M HCl (70:29:1 v:v:v) was used for extraction (24 h at 4 °C in the dark). To differentially extract the methanol-soluble and methanol-insoluble phenolic compounds (MSPC and MIPC, respectively), the extract was centrifuged at 6000 × g for 15 min and the supernatant and pellet were considered the source of MSPC and MIPC, respectively. The pellet was then hydrolyzed with 1 mL of 1 M NaOH for 3 h in a water bath at

80 ºC. Afterwards, 1 mL of HCl (5.6 N) was added and the sample was rinsed three times with ethyl acetate. The supernatant obtained from the rinsing process was then allowed to evaporate (Büchi R-200, Büchi Labortechnik, Flawil, Switzerland) at 40 ºC and the remaining material was resuspended in absolute methanol up to a final volume of 1 mL (for flesh samples) and 2 mL (for the remaining samples).

In both soluble and insoluble fractions of the respective grape components, bulk levels of UV-absorbing compounds (UVAC) were measured as the area under the absorbance curve (AUC) in the wavelength intervals 280-315 nm ($AUC_{280-315}$) and 280-400 nm ($AUC_{280-400}$), respectively covering the UV-B and the ensemble of UV-B plus UV-A ranges (Perkin-Elmer λ 35 spectrophotometer, Perkin-Elmer, Wilton, CT, USA). Soluble and insoluble individual phenolic compounds were analyzed in the respective grape components by UPLC/LC-MS (Waters Acquity Ultra Performance LC system, Waters Corporation, Milford, MA, USA) following Del-Castillo-Alonso *et al*. (2016b). The UPLC system was coupled to a micrOTOF II high-resolution mass spectrometer (Bruker Daltonik, Bremen, Germany) equipped with an Apollo II ESI/APCI multimode source and controlled by the Bruker Daltonics Data Analysis software. A UV detector module was used at 520 nm for the anthocyanins and at 324 nm for the rest of compounds. The electrospray source was operated in negative mode, except for the anthocyanins which operated in positive mode. The different phenolic compounds analyzed were identified and quantified using commercial pure compounds. In absence of the commercial standard, compounds with the same chromophore were used: stilbenes using *t*-resveratrol (Sigma-Adrich, St. Louis, USA); flavanols using catechin, epigallocatechin (Sigma-Adrich, St. Louis, USA) and procyanidin B1 (Fluka, Buchs, Germany); flavonols using kaempferol-glucoside, quercetin-3-*O*-glucuronide (Fluka, Buchs, Germany), myricetin, quercetin, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-glucopyranoside, quercetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside and syringetin-3-*O*-glucoside (Sigma-Adrich, St. Louis, USA); hydroxycinnamic acids and its derivatives using caffeic, *p*-coumaric and ferulic acids (Sigma-Adrich, St. Louis, USA);

hydroxybenzoic acids using gallic acid, syringic acid (Sigma-Adrich, St. Louis, USA) and protocatechuic acid (Fluka, Buchs, Germany); and anthocyanins using malvidin-3-*O*-glucoside (Extrasynthese, Genay, France).

Total phenols were determined using the Folin–Ciocalteu reagent and data were expressed as gallic acid equivalents (GAE) or catequin equivalent (CE) (Farhadi *et al*. 2016). Total flavonoids were determined as in Farhadi *et al*. (2016).

The antioxidant capacity of the different grape components was measured by generating the radical cation 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) as previously described (Del-Castillo-Alonso *et al*. 2016b) and was expressed in terms of Trolox equivalent (TE) antioxidant capacity.

VOCs were analyzed only at harvest in skin and flesh together, following González-Mas *et al*. (2011). For extraction, 0.5 g samples of frozen grape powder were used. Samples were heated at 30ºC for 10 minutes and 1.5 mL of a saturated CaCl2 solution and 300 µL of EDTA 500 mM (pH 7.5) were added. After gentle mixing, 1.5 mL of the resulting mixture were transferred to a 10 mL headspace screw cap vial and subjected to headspace solid-phase microextraction (HS-SPME). A 65 µM PDMS/DVB fiber (Supelco, Bellefonte, PA, USA) was used for the analysis. Pre-incubation and extraction were performed at 50 ºC for 10 and 20 min, respectively. Desorption was performed for 1 min at 250 ºC in splitless mode. VOCs trapped on the fiber were analyzed by GC-MS using an autosampler COMBI PAL CTC Analytics (Zwingen, Switzerland), a 6890N GC Agilent Technologies (Santa Clara, CA, USA) and a 5975B Inert XL MSD Agilent, equipped with an Agilent J&W Scientific DB-5 fused silica capillary column (5%-phenyl-95%-dimethylpolysiloxane as stationary phase, 60 m length, 0.25 mm i.d., and 1 µm thickness film). Oven temperature conditions were 40 ºC for 2 min, 5 ºC min^{-1} ramp until 250 ºC and then held isothermally at 250 ºC for 5 min. Helium was used as carrier gas at 1.4 mL min^{-1} constant flow. Mass/z detection was obtained by an Agilent mass spectrometer operating in the EI mode (ionization energy, 70 eV; source temperature 230 ºC). Data acquisition was performed in scanning mode (mass range *m/z*

35–220). Chromatograms and spectra were recorded and processed using the Enhanced ChemStation software for GC-MS (Agilent). Compound identification was based on both the comparison between the MS for each putative compound with those of the NIST 2005 Mass Spectral library and the match to a GC retention time and Mass Spectra custom library generated using commercially available compounds.

The analysis of gene expression was carried out in grapes of the three phenological stages: pea size, veraison and harvest. Frozen grapes were crushed in porcelain mortar with liquid nitrogen, having previously removed the seeds. The resulting powder was stored at -80 °C until use. RNA was extracted according to Zeng and Yang (2002) and RNA from each sample was treated with DNase (RNasefree) according to the manufacturer instructions (TURBO ADN-free™ Kit, Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) to eliminate contamination with genomic DNA. The concentration of RNA was quantified by a Nanodrop™ 2000c spectrophotometer (ThermoScientific™). Final RNA purification was carried out using the Spektrum™ Plant Total RNA kit (Sigma-Aldrich) according to standard protocols. cDNA was synthesized from 2-4 µg of the treated RNA samples using the NZY First-Strand cDNA Synthesis kit (NZYTech, Lisboa, Portugal). Oligonucleotide primers used in real-time (RT) qPCR analysis were taken from previous studies or designed by the Primer3 program (http://biotools.umassmed.edu/bioapps/primer3_www.cgi), and synthesized from Invitrogen (Table 7.1). Transcript levels were measured by quantitative RT-PCR using an ICycler Bio-Rad instrument. Triplicates of PCR reactions of each sample were done, and relative gene expression levels were calculated according to the $2^{-\Delta\Delta CT}$ methods using ACTIN as control gene to normalize individual gene expression.

At harvest, several morphometric variables of the grapes were measured: fresh weight, diameter, and the proportions between the fresh weights of skin and grape, flesh and grape, and seeds and grape.

Vinification and wine analysis (Figure 7.1)

For each treatment and replicate, grapes were destemmed and crushed and the alcoholic fermentation was carried out (Sampaio *et al*. 2007). Around 3 kg of pomace (must, seed, and skin) were introduced into 2.5 L glass bottles. Potassium metabisulfite (0.09 g kg^{-1}) was added to the samples to give a final total SO_2 concentration of 50 mg L^{-1} and then musts were inoculated with 0.2 g kg^{-1} of commercial *Saccharomyces cerevisiae* r.f. *bayanus* (Enartis, Trecate, Italy). The must was fermented at a controlled temperature of 25 ºC. The alcoholic fermentation finished when reducing sugars were below 2.5 g L^{-1} (two weeks after yeast inoculation). Then, wine was separated from seeds and skins by pressing, and wine analysis was performed. The bulk levels of UV-absorbing compounds (UVAC), individual phenolic compounds, and total antioxidant capacity were analyzed following the same procedures as in berry skins. Color intensity, hue and total polyphenol index (TPI) were analyzed according to official methods (EEC, 1990). Total phenols were determined using the Folin–Ciocalteu reagent and data were expressed as gallic acid equivalents (GAE) (Villaño *et al*. 2004). Total flavonoids were determined as in Farhadi *et al*. (2016). For wine VOCs extraction, 1 mL of wine per sample was transferred to a 10 mL headspace screw cap vial and subjected to HS-SPME following the same procedure as in grapes.

Statistical analysis

The global effects of the radiation regime and the phenological stage on phenolic composition, antioxidant activity and gene expression were tested using a two-way analysis of variance (ANOVA), once proved that the data met the assumptions of normality (Shapiro–Wilks's test) and homoscedasticity (Levene's test). In addition, one-way ANOVA was applied 1) to test the global effect of the radiation regime for each phenological stage; and 2) to test the global effect of radiation regime on the variables measured only at harvest: grape morphology, phenolic and VOCs composition of grapes (for skin, flesh and seeds separately) and wine, and wine color). In

the case of significant differences, means were then compared by the Tukey's test. Non-parametric tests (Kruskal-Wallis) were used if the data did not meet the assumptions. In this case and, when significant differences occurred, means were compared by the Mann-Whitney's test. When only two sets of data had to be compared (for example, PAB *vs.* PAB↑ regimes), differences were assessed using the Student's *t* test. Pearson correlation coefficient (r) was used to examine the relationships between selected variables. The samples were ordinated by Principal Components Analysis (PCA), taking into account the variables that were common to the grapes and wine. All the statistical procedures were performed with SPSS 24.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Radiation and temperature conditions

Radiation conditions in the different regimes are shown in Figure 7.3 and Table 7.2. PAR dose along the experiment was similar in all the regimes. UV-A dose was relatively high and similar in PA, PAB and PAB↑ regimes, whereas in P and PB was around 9%. UV-B dose was relatively high and similar in PB and PAB regimes, and around 10% higher in PAB↑, whereas plants in P and PA only received around 2% of that in PB and PAB (thus, P and PA were not totally deprived of UV-B). UV_{BE} dose (and also UV-B_{BE} dose) were different between PB and PAB regimes, due to the facts that: 1) the UV-B source was different in PB (lamps) and PAB (sunlight), and different sources implied different spectral irradiances; and 2) the action spectra applied to calculate UV_{BE} and UV-B_{BE} doses were different (Flint and Caldwell (2003) and Caldwell (1971), respectively), and in the first case the action spectrum took importantly into account UV-A wavelengths whereas in the second case did not. Consequently, although UV-B dose was similar between PB and PAB regimes, UV_{BE} dose was around 50% lower in PB than in PAB, whereas UV-B_{BE} dose was around 2-fold in PB than in PAB.

Temperature inside the blocks was 4.0±0.6 ^{o}C higher than outside. In addition, in the blocks of the treatments using lamps (PB and PAB↑), temperature of the grape surface was 1.0±0.1 ^{o}C higher in comparison to the temperature found in the blocks not using lamps.

Morphological parameters of grapes (Table 7.3)

UV-A radiation did not seem to modify the morphological parameters of grapes. Conversely, UV-B increased the FW, diameter and flesh proportion of the grapes. The simultaneous presence of UV-A and UV-B (PAB regime) showed a similar trend, but not significantly. UV-B enhancement (PAB↑ regime) had no effect on grape morphology.

Effects of radiation on the phenolic composition of grapes: influence of phenology (Table 7.4, Figure 7.4)

Global variables (total phenols, total flavonoids, bulk levels of UVAC and antioxidant capacity) did not respond to the radiation regime, whereas the phenolic families and individual compounds showed diverse responses. The clearest responses were shown by flavonols, acids and resveratrol. The most consistent responses were those of flavonols, given that they responded in the same manner in every phenological stage: UV-A did not cause any effect (and thus no difference was found between P and PA regimes), whereas UV-B led to an increase in these compounds. Apparently, there was a synergic effect between UV-A and UV-B, since flavonols contents were higher in PAB samples than in the remaining regimes in all the phenological stages. Among flavonols, the strongest response was found in quercetins. Two acids increased only in PB in the pea size stage, whereas stilbenes showed the highest values in P samples.

All the global variables and phenolic compounds showed significant differences between phenological stages. The highest diversity and content of phenolic compounds were found at harvest, with 37 compounds (15 anthocyanins, 13 flavonols, five flavanols, three hydroxycinnamic acids, and one stilbene). In pea size, no anthocyanin was detected, and resveratrol was not found in veraison. Kaempferols and myricetins began to be found in veraison, although in small amounts, and significantly increased at harvest. Flavanols and hydroxycinnamic acids decreased along the season, whereas flavonols and anthocyanins increased.

Effects of radiation on the gene expression of grapes: influence of phenology (Figures 7.5-7.6)

The most consistent response to radiation was that of *VvFLS4*, which was upregulated by UV-B in all the phenological stages, especially in combination with UV-A (higher expressions were

found in PAB). *VvCHS1* also showed a higher expression in PAB in comparison with P, except in the pea size stage, in which expression was similar among all the treatments. HY5 and the remaining transcription factors did not seem to be regulated by UV in any phenological stage.

Regarding the effect of phenology, *VvFLS4* and the bHLH transcription factors showed the highest levels of expression at harvest, whereas HY5 showed rather the contrary, with the lowest levels of expression at harvest. *VvCHS1* showed the lowest expression at veraison.

There was a significant correlation between the content of flavonols and the expression of *VvFLS4* and *VvCHS1* (Figure 7.6). In addition, the content of anthocyanins was significantly correlated with *VvCHS1*.

Effects of radiation on phenolic composition and gene expression of grapes: a polygonal summary (Figure 7.7)

Figure 7.7 represents a synthetic summary of the intensity of response of selected variables (the most UV-responsive genes and phenolic compounds) to UV radiation. This was analyzed by comparing the responses in PAB and P regimes in the three phenological stages studied (pea size, veraison and harvest). Quercetins, isorhamnetins, catechin and two acids (caffeoyl- and coumaroyl-tartaric acids), together with the *VvFLS4* and *VvCHS1* genes, were the variables selected. UV radiation caused important effects in the variables selected in the three phenological stages, but particularly at veraison, when expression of *VvFLS4* and the concomitant synthesis of quercetins and isorhamnetins were highest. In the pea size stage, responses were similar but milder. At harvest, both *VvFLS4* expression and quercetin synthesis were attenuated, but *VvCHS1* expression strongly increased. Responses of catechins and acids was more limited, and similar in the three phenological stages.

Effects of radiation on the phenolic composition of grapes: influence of grape components and cell fractions (Tables 7.5-7.6, Figure 7.8)

The skin was the grape component which showed the strongest response to radiation, given that stilbenes, flavanols and flavonols showed a significant effect of radiation. Flavanols decreased in the presence of both UV-A and UV-B, whereas kaempferols and quercetins increased under UV-B radiation.

The methanol-insoluble fraction was almost completely insensitive to UV radiation. Regarding the methanol-soluble fraction, the seeds were also notably insensitive, with changes only in procyanidins, quercetins and acids that were caused by UV-B and not UV-A. The flesh showed only a few responses (increase in flavonoids and antioxidant capacity in PAB in comparison with P samples).

A higher number of phenolic compounds was identified when the three grape components (skin, flesh and seeds) were separately analyzed: 44 compounds in skins, 16 in flesh and 15 in seeds. The highest contents of total phenols, total flavonoids, together with the highest antioxidant capacities, were found in skins, followed by seeds, both in the methanol-soluble and –insoluble fractions. The insoluble fraction contained the totality of cinnamic and benzoic acids (the former mainly in the skins and the latter equally distributed between skins and seeds. The remaining compounds were only found in the soluble fraction, most of them mainly in the skins, except catechins and procyanidins, that were predominantly found in the seeds. In the flesh, the most abundant compounds were acid derivatives (20%).

Effects of radiation on the VOCs of grapes (Table 7.7, Figure 7.9)

VOCs were analyzed in the ensemble of skin and flesh, where a total of 57 compounds were identified: 14 aldehydes, 13 alcohols, 10 terpenes, six fatty acids, five hydrocarbons, four ketones, three C13-norisoprenoids and two furans. VOCs response to UV radiation was slight. Only UV-B radiation (PB regime) led to a significant increase in fatty acids, menthol and

apocarotenoid. UV-A radiation (PA regime) caused even slighter effects, since only increased the content of phenylacetaldehyde.

Effects of the radiation received by grapes on the phenolic composition of the resulting wines (Table 7.8, Figure 7.10)

The radiation received by grapes did not affect the color of the resulting wines. The presence of only UV-B decreased the antioxidant capacity, total phenols and some flavanols. Acids and flavonols were influenced by UV radiation. In flavonols, the synergy between UV-A and UV-B increased their contents. Among flavonols, the most consistent responses were again found in quercetins and kaempferols, which significantly increased their contents in PAB samples.

Effects of the radiation received by grapes on the VOCs of the resulting wines (Table 7.9, Figure 7.11)

A total of 30 VOCs were identified in wines: 18 esters, four alcohols, four fatty acids, two hyrocarbons, one lactone and one nonaldehyde. Only five of these compounds were also found in grapes. UV radiation received by grapes caused only a weak effect on wine VOCs, although some scattered significant effects of UV-B alone were found, decreasing the percentage of one alcohol and increasing the percentages of most fatty acids.

Effects of enhanced UV-B on the phenolic composition of grapes: influence of phenology (Table 7.10, Figures 7.12-7.13)

Enhanced UV-B did not cause any change in the global variables in any phenological stage, except in the antioxidant capacity, which increased in the pea size stage. Effects of enhanced UV-B on phenolic families were found in this phenological stage, with increases in stilbenes, flavanols and hydroxycinnamic acids. Flavonols showed a similar trend, but differences were not significant. Regarding individual phenolic compounds, most of flavanols and acids increased, as

well as resveratrol, but only in the pea sized stage. In addition, a surprising response was found in kaempferols and quercetins, which decreased under enhanced UV-B.

Effects of enhanced UV-B on gene expression in grapes

In line with the results found in the phenolic composition of grapes, we did not find any clear effect of enhanced UV-B on grapes gene expression, probably due to a high variability of the data.

Effects of enhanced UV-B on the phenolic composition of grapes: influence of grape components and cell fractions (Tables 7.11-7.12, Figures 7.14-7.15)

In general, enhanced UV-B did not cause significant changes in the phenolic composition in any cell fraction (methanol-soluble and -insoluble). Nevertheless, the soluble compounds of seeds were the most affected by UV-B enhancements, with a decrease in all the global variables, as well as the contents of flavonols, flavanols and cinnamic acids. Phenolic compounds in the remaining grape components did not respond significantly to enhanced UV-B.

Effects of enhanced UV-B radiation on the VOCs of grapes (Table 7.12)

Enhanced UV-B increased alcohols, hydrocarbons (due to an increase in the major hydrocarbon) and fatty acids, whereas furans decreased. Fatty acids showed the strongest response, since all of them increased (although two of them not significantly). No effect was found in the remaining VOCs (considered as groups), although some significant changes in individual aldehydes (benzaldehyde increased whereas heptadienal decreased) and terpenes (alcanfor increased and limonene decreased) were found.

Effects of the enhanced UV-B radiation received by grapes on the phenolic composition and VOCs of the resulting wines (Tables 7.13-7.14, Figures 7.16-7.17)

Enhanced UV-B received by grapes had only weak effects on the phenolic and VOCs composition of the resulting wines. Only resveratrol and two alcohols and a decrease in n-nonaldehyde.

Summarizing the effects of UV radiation on grapes and wines (Figures 7.18 and 7.19)

Figure 7.18 compares, on one hand, the response intensity of the phenolic families to UV radiation in both grapes and the resulting wines (P *vs.* PAB regimes), taking into account five phenolic families: anthocyanins, stilbenes, flavanols, flavonols, and acids. On the other hand, the same comparison was obtained between grape skins and the resulting wines. Combining both comparisons, there was a more complete match in the second case. Thus, the influence of the UV radiation received by grapes on the phenolic composition of the resulting wines was better represented (and better predicted) by the composition of skins, rather than by the composition of entire grapes.

Finally, we performed a PCA using the variables that were common to grapes (at harvest) and wines, and particularly the phenolic compounds and VOCs. The accumulated variance by the first three axes was 81% (40% for axis I, 27% for axis II, and 14% for axis III). The plot using the first two axes is shown in Figure 7.19. Loading factors for the positive part of axis I were the global variables (total phenols, total flavonoids, bulk levels of UVAC) and some phenolic groups, such as stilbenes and flavanols. Loading factors for the positive part of axis II were flavonols, anthocyanins and acids, and for the negative part aldehydes (the only VOCs somewhat represented in the PCA). Except for the PA regime, grapes and the resulting wine of the same radiation regime were close in the plot. Thus, there was a clear separation between regimes. PB regime was clearly different to the other regimes, with relatively low values in most of the variables. The remaining regimes were placed on a diagonal crossing axes I and II. On one extreme, grapes and wines of P regime showed high values of global variables and aldehydes, and low values of flavonols, anthocyanins and acids. On the opposite extreme, grapes and wines of PAB↑ regime were placed, showing opposite characteristics. Overall, although an individualized analysis of variables did not show a high effect of enhanced UV-B, the more global perspective supplied by PCA allowed a clear differentiation of PAB↑ grapes and wines.

DISCUSSION

In the present Chapter, the effects of UV radiation were integratively studied incorporating different scales of study including different experimental approaches (the combined use of filters and lamps to differentiate the effects of ambient UV-A, ambient UV-B, and enhanced UV-B levels compatible with climate change predictions), different variables from gene expression to morphology and phenolic and VOCs composition, different phenological stages (pea size, veraison and harvest), different grape components (skin, flesh and seeds), and different cell locations of the potentially protecting UVACs. In addition, the whole way from grape characteristics to wine elaboration was studied, by measuring wine variables (color, antioxidant capacity, phenolic compounds and VOCs) determining key sensorial characteristics. To our knowledge, there is no previous such integrative study in the literature on grapevine and UV.

Effects of ambient UV depended on wavelength

UV-B radiation caused important changes on morphology (increasing grape size, fresh weight and flesh proportion), phenolic composition (particularly increasing flavonols) and gene expression (increasing those genes related to the phenolic changes found). Conversely, the effects of UV-A were much more diffuse. It is already known that UV-A and UV-B can have different effects on plants in general (Verdaguer *et al*. 2017) and, specifically, in grapevine (Jordan, 2017), but these differences have not been well-characterized yet. Our study is one of the few that tried to evaluate these differences under field conditions, because most of the previous studies applied leaf removal or general shading, thus producing changes not only attributable to UV radiation but also to PAR (Feng *et al*. 2015, Verzera *et al*. 2016, Reshef *et al*. 2018), or excluded the whole UV wavelengths (Carbonell-Bejerano *et al*. 2014, Song *et al*. 2015, Del-Castillo-Alonso *et al*. 2015, 2016a). Although in our study UV-B effects were stronger than those of UV-A, a synergic effect between both wavelength bands was sometimes observed, for example regarding the higher increase in flavonols in PAB grapes in comparison with PB samples.

This could be related to the fact that flavonols (as most phenolic compounds) absorb UV-A and thus may act as UV-A screens (Verdaguer *et al*. 2017). Consequently, these compounds can decrease in the absence of UV-A. This apparently synergy between UV-B and UV-A could also be due to the fact that the ratios UV-A/UV-B were different in PAB (where the UV-B source was sunlight) than in PB (where the UV-B sources were lamps). Thus, although the UV-B doses were similar in both regimes, UV_{BE} was much higher in PAB than in PB, and this fact could lead to stronger effects in the former regime in comparison with the latter. Additional research is needed to progress in this topic, and the design of specific action spectra for the induction of phenolic compounds in grapevine should be considered.

Effects of ambient UV-B on grape morphology, phenolic composition, gene expression and VOCs

Ambient UV-B increased grape size, fresh weight and flesh proportion, but did not influence the skin proportion. Conversely, Berli *et al*. (2011) found larger and heavier grapes in Malbec variety when ambient UV-B was excluded, although their results were coincident with ours regarding the skin proportion (no change). Martínez-Lüscher *et al*. (2014a, 2014b) and Carbonell-Bejerano *et al*. (2014) did not find significant changes in grape diameter or weight under different UV treatments applied on Tempranillo. Thus, the effects of UV in general and UV-B in particular may depend on the variety used and the experimental and environmental conditions experienced by grapes (Downey *et al*. 2003). Other wavelengths than UV could also influence grape morphology, since sun-exposed grapes were heavier than shaded grapes (Reshef *et al*. 2018), and interannual variations could be another source of variability (Downey *et al*. 2003).

Ambient UV-B increased the contents of a number of phenolic compounds in grapes, particularly flavonols and especially quercetins and kaempferols. This was expected and has repeatedly been found in several varieties including Tempranillo (Kolb *et al*. 2003, Downey *et al*. 2006, Berli *et al*. 2011, Jug and Rusjan 2012, Del-Castillo-Alonso *et al*. 2016a, Martínez-Lüscher *et al*. 2016, Jordan

2017; see Chapters 6 and 8 and references therein). These compounds contribute to grape and wine quality, and are potentially healthy as nutraceuticals (see Chapters 6 and 8). It is remarkable that the flavonol increase could have been even more consistent if contents had been expressed per berry instead of per DW (as in Berli *et al*. 2015), because UV-B-treated berries in our study were larger and thus the contents of compounds would have been higher. The responses of the remaining phenolic compounds (in general, anthocyanins, flavanols, stilbenes and acids) were more diffuse. Anthocyanins did not show significant differences under any radiation regime, probably because they are more reactive to the interaction of PAR and temperature than to UV (Cortell and Kennedy 2006, Mori *et al*. 2007). Flavanols and cinnamic acid derivatives showed diverse little consistent responses to UV, which also depended on the grape part and phenological stage considered. This may be due to the fact that these compounds show clearer responses to global radiation and temperature (Downey *et al*. 2004, Koyama *et al*. 2012, Cohen *et al*. 2012; see Chapter 6). Similar diffuse responses were found in cinnamic acid derivatives, in line with other studies which did not find responses to ambient UV-B or global radiation (Berli *et al*. 2011, Diago *et al*. 2012, see Chapter 6). The content of stilbenes decreased under regimes containing UV, and more strongly under UV-B. These compounds rarely respond to UV-B and seem to be more dependent on the phenological stage of the berry, the pathogen attacks and artificial UV-C (Downey *et al*. 2006, Pan *et al*. 2009, Petit *et al*. 2009, Del-Castillo-Alonso *et al*. 2016a).

The most UVB-responsive gene was *VvFLS4*, and responded at every phenological stage. In addition, *VvFLS4* was highly correlated with the contents of flavonols, which gave a coherent picture of concomitant increase of both the metabolites and the genes involved in their synthesis. These results were expected concordant with other previous results (Downey *et al*. 2004, Fujita *et al*. 2006, Pastore *et al*. 2013, Loyola *et al*. 2016, Martínez-Lüscher *et al*. 2016). In addition, *VvCHS1* also responded to UV-B at veraison and harvest, but it was better correlated with the content on anthocyanins. Other genes and transcription factors did not seem to be

regulated by UV-B radiation. This was strange for basic helix-loop-helix (bHLH) transcription factors, which are involved in many abiotic stress responses, as well as flavonol and anthocyanin biosynthesis (Jordan 2017). However, when examined in grapes, they did not respond to UV (Wang *et al*. 2018). Among other diverse functions, HY5 is involved in the regulation of flavonol and anthocyanin synthesis in response to light and UV radiation (Loyola *et al*. 2016), thus it was surprising that did not show any significant response to UV in our study (although an increasing trend was found under regimes with UV-B). Early light induced proteins (ELIP) may mainly be induced in *Arabidopsis* by low UV in a UVR8- and HY5-dependent manner (Brown and Jenkins 2008), increasing also with increasing white light (Heddad *et al*. 2006). A slight induction by UV-A in our study could be related with its involvement in the regulation of chlorophyll synthesis and photosynthesis, given that UV-A may increase photosynthesis through an induced better protection of the photosynthetic apparatus (Verdaguer *et al*. 2017). Probably, the lack of clearer responses of gene expression in our study was due to a high variability of the samples.

VOCs showed much less clear responses to solar UV-B (or UV-A) than phenolic compounds and related genes. However, samples receiving UV-B from lamps (PB and PAB↑ regimes) showed some effects, maybe due to the different UV_{BE} or the slight (although measurable) temperature increase under the lamps. Only 12 (out of 57) VOCs responded to UV-B, mostly terpenes and fatty acids, showing higher contents in PB samples. This was in line with the increase of terpenes (together with alcohols and aldehydes) found in Malbec grapes under UV-B (Gil *et al*. 2013). Nevertheless, VOCs increase in response to UV-B depended on the year and the variety, since in Pinot noir only slight changes were found, and mostly in alcohols (Verzera *et al*. 2016). In grapes after leaf removal, VOCs also increased (Feng *et al*. 2015), suggesting that VOCs are more responsive to global radiation than to UV-B (Joubert *et al*. 2016), but this response was not found in another study (Feng *et al*. 2017). Thus, the responses of VOCs to UV-B are not well defined and may depend on additional factors such as the type of compound and the agricultural practices conducted on the plant (Alem *et al*. 2019). Given the importance of VOCs in wine

aroma, more studies are needed to disentangle the effects of UV on their contents and how UV could be managed to influence of grape and wine VOCs.

Influence of the phenological stage

Antioxidant capacity, total phenols and flavonoids, and UVACs showed the highest contents in pea size stage, mainly due to the high levels of flavanols and hydroxycinnamic acids. All these variables decreased at veraison and slightly increased at harvest (probably due to the important presence of anthocyanins). Contrarily, flavonols and anthocyanins showed their highest values at harvest, while stilbenes did not vary along the berry development. This is a common pattern in grapevine, also in Tempranillo (Del-Castillo-Alonso *et al*. 2016a). Starting from this pattern, UV-B effects (increase of flavonols, especially quercetins, and of *VvFLS4* expression) were similar in the three phenological stages studied, but the strongest responses took place at veraison, thus being the most UV-responsive phenological stage of the berry. At harvest, the highest contents of flavonols were found in regimes with UV-B, probably because most flavonols were synthesized between veraison and harvest (Sternad-Lemut *et al*. 2013), among them the most UV-responsive (quercetins and kaempferols: Alonso *et al*. 2016, Liu *et al*. 2018). Similar results were obtained after leaf removal (Lemut *et al*. 2013, Pastore *et al*. 2013), probably due to the UV-B increase derived from this practice. Nevertheless, in our study, UV did not modify the natural evolution of phenolic compounds in grapes from pea size to harvest (neither UV-A, UV-B, nor enhanced UV-B), and no significant interaction was found between radiation regime and phenology (except in stilbenes). Overall, the UV-induced modifications were not so strong to modify the programmed changes of the berry along its development, at least under the experimental conditions used in our study. These programmed changes are so solid that may mask the changes induced by UV treatments (Martínez-Lüscher *et al*. 2014a, 2014b). Identifying the influence of phenology on the UV effects is important to design an adequate temporal UV management to increase specific compounds at a determinate phenological stage.

Regarding the effect of phenology on gene expression, *VvFLS4* and the bHLH transcription factors showed the highest levels of expression at harvest, whereas *VvHY5* showed rather the contrary, with higher expression levels in pea size and veraison than at harvest. *VvCHS1* showed the lowest expression at veraison, when *VvELIP1* showed its highest expression at this stage. Overall, gene expression was highly influenced by the phenological stage, and the changes found in our study were concordant with those found in other studies (Pastore *et al*. 2013, Matus *et al*. 2017, Wang *et al*. 2018). On the other hand, the responses of genes to UV-B depended on the phenological stage, with *VvFLS4* showing response to UV in every stage while *VvCHS1* only responded at veraison and harvest. Also, activation of *VvHY5* at pre-veraison favored flavonol accumulation. *VvHY5* was UV-responsive in white-skinned berries of Sauvignon blanc (Liu *et al*. 2014).

Influence of the grape component

Among the three berry components (skin, flesh and seeds), skin was the most UV-responsive, probably because it receives the highest UV impact. Skin showed the highest levels of total phenols and UVAC under regimes with UV-B, and antioxidant capacity and total flavonoids showed the same (although not-significant) trend. Flesh and seeds showed maximal flavonoids contents in PAB samples. Overall, there was a trend to increase phenols in regimes with UV-B, as UVAC in Martínez-Lüscher *et al*. (2013) or total phenols in Berli *et al*. (2015). Flavanols and cinnamic acids responded differently in each grape component, showing the highest levels in skin and flesh in regimes without UV-B. These results suggest that the responses of global variables to UV were influenced by the grape component, since each component possesses a different phenolic profile and, thus, different UV reactivity.

Influence of cell location of phenolic compounds

The methanol-soluble fraction of phenolic compounds was more UV-responsive than the –insoluble fraction, which was insensitive to UV. The higher responsiveness of the soluble fraction

was probably due to its higher diversity and amount of phenolic compounds. Only a few studies show comparative results of the influence of UV radiation on phenolic compounds from different cell location, and our results were concordant with those shown by Del-Castillo-Alonso *et al*. (2015, 2016a).

Effects of the UV received by grapes on the resulting wines

As in Chapters 6 and 8, UV effects were assessed from grapes to the resulting wines. This allowed to show that some UV effects on grapes, especially those affecting to the skin, were conserved in the resulting wines. This occurred, for example, in flavonols, that were UV-induced in grapes (particularly in skins) and these high levels were conserved in the resulting wines. This was a solid response because it was also found in other studies (Price *et al*. 1995, see Chapters 6 and 8). Conversely, other UV effects on the grapes did not persist in the resulting wines because they could be modified during winemaking. This occurred with total phenols, UVACs and the antioxidant capacity, whose trend in wine was rather the contrary to that found in the grape (UV-induced increase in grape and decrease in wine). This was opposite to the findings in Chapter 6, where the wine derived from UV-exposed grapes had a higher antioxidant capacity. This discrepancy was probably due to different experimental and/or environmental conditions in different years (Teixeira *et al*. 2013, Blancquaert *et al*. 2018, see Chapter 8). Another explanation could be that global variables (such as total phenols or antioxidant capacity) group many different compounds that can show different responses to UV, and grouping these compounds can mask their individual responses. In some cases, interesting compounds (such as the acids present in the methanol-insoluble fraction of skin and seeds) were lost during winemaking. Given their importance in the sensorial traits of wines, this finding may deserve more research to design some viniculture practice that could prevent their disappearance. The manner in which acids are transmitted from grapes to wines is far from clear (Blancquaert *et al*. 2018). Regarding VOCs, only11 compounds out of 30 showed a similar response to UV in grapes and wines, although in general the responses were subtle. The most UV-reactive compounds

were fatty acids and esters. Similar results were found in Tempranillo and other varieties using leaf removal (Vilanova *et al*. 2012, Moreno *et al*. 2017, Bubola *et al*. 2019). In these studies, the variability due to interannual variations and moment of leaf removal were remarked, showing the complexity of these processes. Overall, studying the whole way from grapes to wine is important because the persistence or disappearance in wine of compounds that were UV-induced in grapes can modify the sensorial characteristics of wines.

Effects of a realistic UV-B enhancement

The effects of a realistic 10% UV-B enhancement (as predicted from global change models: Bais *et al*. 2019) caused more subtle changes than ambient UV levels. However, changes due to enhanced UV-B were significantly manifested in a global multivariate analysis using variables measured both in grapes at harvest and in wines. These changes included increases in flavonols, anthocyanins and cinnamic acids, although they could partially be masked by the natural variability of grapes and bunches. The effects of enhanced UV-B were influenced by the phenological stage. For example, flavanols, hydroxycinnamic acids and antioxidant capacity strongly increased in the first development stages of the berry (pea size). Sternad-Lemut *et al*. (2013) found similar results in Pinot noir (stronger increase of hydroxycinnamic acids at pea size than at harvest), but using leaf removal. In our study, we did not find important changes in UVAC due to enhanced UV-B at veraison and harvest, as in Martínez-Lüscher *et al*. (2015). The effects of enhanced UV-B were also influenced by the grape component (Bogs *et al*. 2006), with skin as the most UV-B-reactive part and the seed the least. Thus, each component may have a different regulation. Another aspect to explain the subtle changes caused by enhanced UV-B could be that ambient UV-B doses would be enough to saturate the responses, and thus increasing UV-B beyond the saturation limits would be irrelevant. This was demonstrated by Liu *et al*. (2015) in the gene responses of Sauvignon Blanc. In this line, in our study, enhanced UV-B did not change the expression of most genes analyzed. For example, ELIP1 was subexpressed at veraison under enhanced UV-B, as bHLH-like at harvest. The fact that *VvFLS* was not up-regulated by enhanced

UV-B, despite it was a very UV-responsive gene, also points to UV-B saturation (Liu *et al*. 2015). In wine, the only response to enhanced UV-B received by grapes was the increase of stilbenes. Regarding VOCs, both alcohols and fatty acids in grapes increased under enhanced UV-B, as occurred in PB. This could be due, at least partially, to the increase in both UV-B and temperature (as a consequence of the lamp activity in both regimes). In this line, Gil *et al*. (2013) demonstrated that both radiation and temperature determine VOCs levels. The changes found in grapes were not transmitted to the resulting wines. High UV-B irradiance peaks (see Chapter 8) caused much more important changes than increasing the total UV-B dose in the long-term (as used in the present experiment). This would suggest that, at least under some conditions, increasing peak irradiance can trigger UV-B responses more efficiently than increasing UV-B dose. This opens new possibilities of UV management.

Technical limitations of field studies

As described above, UV research under field conditions shows some technical constraints leading to a relatively high variability, probably because 1) low UV amounts (particularly of UV-B) can trigger a cascade of responses, such is the case of mechanisms regulated by UVR8 (Robson *et al*. 2019), and this fact would lead to homogenize the responses of the different treatments; 2) the interaction of UV and temperature, as pointed out above; and 3) the intrinsic variability of grape characteristics. In this last sense, grapes and bunches were notably variable within each radiation regime, particularly at veraison and harvest, due to differences in ripeness. This may mask the differences due to the radiation regime, which in many cases were not significant although were manifested by multivariate analysis and polygonal figures. For example, malvidins in the ensemble of skin and flesh increased under UV, especially in PB samples, but the increase was not significant. However, malvidins content was a significant loading factor in the PCA.

On the other hand, the optical characteristics of commercially available filters, and the spectral characteristics of lamps, are not always optimal for experimentation. For example, UV filters cut off at 395 nm, and thus some UV-A is transmitted to the plants. In addition, covering plants with

filters leads to temperature increases, although weak. Another interference may be due to UV reaching the plants by reflection in surfaces. As it has been pointed out, even low UV can trigger UV responses that may confound the specific responses due to each radiation regime imposed. Also, the spectral characteristics of the UV lamps do not coincide with the UV solar spectrum (although the supplied doses are the same), which makes some radiation regimes not perfectly comparable. Moreover, the available action spectra are useful but notably general, and specific action spectra for the induction of phenolic compounds (or other UV responses) in grapevine should be developed to prevent ambiguities. Finally, safety measurements for researchers are another critical point that should be taken into account in UV research, given the health problems that UV can cause.

TABLES AND FIGURES

Table 7.1. Oligonucleotide primers used in real-time qPCR analysis.

Primer code	Sequence (5'→ 3')	Gen annotation	Reference
VvActin F	CTTGCATCCCTCAGCACCTT	Actin	(Reid *et al* ., 2006)
VvActin R	TCCTGTGGACAATGGATGGA		
VvFLS4 F	CAGGGCTTGCAGGTTTTTAG	Flavonol synthase	(Downey *et al.* , 2003)
VvFLS4 R	GGGTCTTCTCCTTGTTCACG		
VvCHS1 F	AGCCAGTGAAGCAGGTAGCC	Chalcone synthase	(Goto-Yamamoto *et al* ., 2002)
VvCHS1 R	GTGATCCGGAAGTAGTAAT		
VvHY5 F	CCAGGAGTTACGAGCAGGAG	Basic leucine zipper transcription factor HY5	(Wang *et al* ., 2018)
VvHY5 R	ACGTCGGGAACTCTGAACAA		
VvELIP1 F	GGTCGGTGCTACGTCTTCAA	Chloroplast ELIP early light-induced protein	this study
VvELIP1 R	CGGTTGACGTGACTGTGAGA		
VvbHLH75 F	CCAGTCACTGCAGAACCAGA	Transcription factor bHLH75	this study
VvbHLH75 R	AAAAAGGCCATGTTGAGTGG		
VvbHLH-like F	GCGGCCTTGAAGAATCATAG	Uncharacterized protein	this study
VvbHLH-like R	AGCGTGGAGAGATGACCATT		
VvTPS07 F	ATGGTGCAAGCAACATACCA	Terpene synthase	this study
VvTPS07 R	CCAAAGGAAATTCTCCACCA		

Table 7.2. Doses (in MJ m^{-2}) of photosynthetically active radiation (PAR), UV-A, UV-B, biologically effective ultraviolet radiation (UV_{BE}) and biologically effective UV-B radiation (UV-BBE) received by plants of *Vitis vinifera* cv. Tempranillo under the different radiation regimes (P, PA, PAB, PB and PAB↑) imposed in the experiment (see Materials and Methods for a description of each regime) during the period of study (6 April – 5 September 2017). UV_{BE} and UV-B_{BE} were calculated on the basis of the action spectra of Flint and Caldwell (2003) and Caldwell (1971), respectively.

Radiation	PAR	UV-A	UV-B	UV_{BE}	UV-B_{BE}
P	840	6.2	0.04	0.03	0.01
PA	802	73	0.1	0.1	0.00
PAB	834	77	1.90	1.4	0.37
PB	832	7.0	1.91	0.76	0.76
PAB↑	833	78	2.12	1.5	0.44

Table 7.3. Morphometric parameters of the grapes in the five radiation regimes (P, PA, PAB, PB and PAB↑) imposed in the experiment. FW, fresh weight. For each variable, the statistical significance of a one-way ANOVA test is shown, and different letters mean significant differences between radiation regimes (Tukey's test). Means ± SE are shown. **, $p < 0.01$; ns, not significant.

Morphometric parameters	P	PA	PB	PAB	PAB↑	statistical significance
FW_{berry} (mg)	1404 ± 52 **a**	1465 ± 44 **a**	1759 ± 32 **b**	1578 ± 81 **ab**	1668 ± 69 **ab**	**
Diameter (cm)	1.0 ± 0.0 **a**	1.0 ± 0.0 **a**	1.4 ± 0.1 **b**	1.2 ± 0.1 **ab**	1.4 ± 0.1 **b**	**
FW_{skin} / berry (mg)	218 ± 9	233 ± 10	273 ± 21	241 ± 16	267 ± 9	**ns**
FW_{flesh} / berry (mg)	1085 ± 41 **a**	1129 ± 38 **a**	1377 ± 16 **b**	1237 ± 60 **ab**	1294 ± 57 **ab**	**
FW_{seed} / berry (mg)	101 ± 64	103 ± 1	109 ± 2	100 ± 4	106 ± 2	**ns**

	Pea size				Veraison				Harvest				statistical significance		
Phenolic compounds and antioxidant capacity	P	PA	PB	PAB	P	PA	PB	PAB	P	PA	PB	PAB	time	treatment	interaction
Total phenols (mg g^{-1} FW)	5.7 ± 0.1	8.3 ± 1.0	9.3 ± 0.3	8.9 ± 0.9	5.1 ± 0.2	4.7 ± 0.7	4.5 ± 0.2	5.3 ± 0.5	6.1 ± 0.1	5.2 ± 0.3	5.1 ± 0.3	5.5 ± 0.3	***	ns	**
Total flavonoids (mg g^{-1} FW)	2.3 ± 0.4	3.0 ± 0.4	3.6 ± 0.2	3.1 ± 0.3	1.7 ± 0.1	1.5 ± 0.2	1.4 ± 0.1	1.6 ± 0.2	1.5 ± 0.1 b	1.0 ± 0.1 a	1.1 ± 0.1 ab	1.2 ± 0.1 ab	***	ns	ns
Antioxidant capacity (μmol TE g^{-1} FW)	472 ± 64	471 ± 38	514 ± 10	468 ± 20	153 ± 5	147 ± 10	147 ± 11	141 ± 19	149 ± 8 b	106 ± 7 a	116 ± 8 ab	127 ± 12 ab	***	ns	ns
UVAC ($AUC_{280\text{-}315}$ mg^{-1} FW)	1.4 ± 0.0	1.4 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	0.81 ± 0.05	0.63 ± 0.06	0.69 ± 0.03	0.78 ± 0.1	1.6 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.5 ± 0.1	***	ns	ns
UVAC ($AUC_{280\text{-}400}$ mg^{-1} FW)	2.3 ± 0.0	2.3 ± 0.1	2.6 ± 0.2	2.6 ± 0.2	1.3 ± 0.1	1.0 ± 0.1	1.1 ± 0.0	1.3 ± 0.1	2.6 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	2.5 ± 0.1	***	ns	ns
Stilbenes (μg g^{-1} FW)															
Resveratrol	6.1 ± 0.2 c	2.3 ± 0.0 b	1.2 ± 0.0 a	1.2 ± 0.2 a					3.2 ± 0.8 ab	4.4 ± 0.0 b	1.8 ± 0.1 a	4.1 ± 0.4 b	*	***	***
Flavanols (μg g^{-1} FW)															
Catechin	218 ± 34 a	264 ± 8 a	374 ± 17 b	250 ± 20 a	225 ± 0 b	114 ± 22 a	162 ± 24 ab	184 ± 17 ab	41 ± 4	43 ± 4	47 ± 9	52 ± 12	***	**	***
Epicatechin	5.9 ± 0.4	7.2 ± 0.6	9.2 ± 1.4	7.9 ± 0.5	18 ± 3	8.0 ± 2.1	17 ± 8	13 ± 2	24 ± 2 b	11 ± 3 a	11 ± 1 a	14 ± 2 a	**	ns	ns
Catechin gallate	20 ± 2	26 ± 1	28 ± 3	31 ± 7	26 ± 9	5 ± 1	16 ± 7	9 ± 1	15 ± 1 b	6.5 ± 1.5 a	8.0 ± 1.2 ab	10 ± 3 ab	***	ns	ns
Epigallocatechin gallate	3.7 ± 0.7	4.9 ± 0.6	6.8 ± 0.5	6.1 ± 1.4	2.0 ± 0.4	1.6 ± 0.2	1.9 ± 0.5	2.9 ± 0.7	1.6 ± 0.7	1.3 ± 0.1	1.3 ± 0.1	1.9 ± 0.5	***	ns	ns
Procyanidin B1	114 ± 16 a	131 ± 4 a	195 ± 1 b	127 ± 14 a	60 ± 9 b	39 ± 19 a	42 ± 12 a	48 ± 9 a	60 ± 19	39 ± 3	42 ± 6	48 ± 11	***	ns	**
Flavonols (μg g^{-1} FW)															
Kaempferol-3-*O*-glucoside					0.30 ± 0.07	1.4 ± 0.8	0.65 ± 0.17	2.5 ± 0.9	4.7 ± 0.5 a	4.1 ± 0.7 a	6.1 ± 1.9 ab	10 ± 1 b	***	**	ns
Kaempferol-3-*O*-galactoside					2.1 ± 0.5	1.8 ± 0.4	2.1 ± 0.8	2.6 ± 1.3	5.6 ± 0.4	5.4 ± 0.6	3.4 ± 0.3	5.6 ± 0.6	***	ns	ns
Myricetin									2.6 ± 0.3	2.8 ± 0.6	3.0 ± 0.5	2.3 ± 0.5	-	ns	-
Myricetin-3-*O*-glucoside					2.7 ± 0.3	3.0 ± 0.9	3.5 ± 1.3	3.5 ± 1.5	46 ± 4	47 ± 8	52 ± 8	53 ± 6	***	ns	ns
Myricetin-3-*O*-glucuronide									3.6 ± 0.4	4.6 ± 1.0	4.3 ± 0.4	4.2 ± 0.7	-	ns	-
Quercetin-3-*O*-galactoside	1.5 ± 0.4 a	1.7 ± 0.5 a	3.4 ± 0.8 ab	4.8 ± 0.6 b	0.82 ± 0.09 a	1.1 ± 0.2 a	1.9 ± 0.1 a	5.5 ± 1.4 b	8.2 ± 0.5 a	6.7 ± 1.6 a	7.8 ± 1.8 a	14 ± 1 b	***	***	ns
Quercetin-3-*O*-glucoside	6.4 ± 1.8 a	7.5 ± 1.2 a	10 ± 2 ab	17 ± 1 b	6.9 ± 1.3 a	8.1 ± 0.5 a	10 ± 0 a	35 ± 9 b	57 ± 2 a	47 ± 8 a	60 ± 14 a	81 ± 3 b	***	**	ns
Quercetin-3-*O*-glucuronide	42 ± 11 a	49 ± 3 a	62 ± 0 b	80 ± 5 c	26 ± 4 a	25 ± 2 a	45 ± 11 b	79 ± 16 b	19 ± 2 a	20 ± 3 a	21 ± 3 a	31 ± 4 b	***	***	ns
Quercetin-3-*O*-rutinoside	3.7 ± 1.0 a	5.2 ± 1.0 a	8.9 ± 1.5 ab	11.2 ± 1.2 b	1.8 ± 0.4 a	2.5 ± 0.2 a	5.3 ± 0.5 b	8.7 ± 1.5 b	1.3 ± 0.1 a	1.6 ± 0.3 a	2.7 ± 0.5 ab	3.5 ± 0.4 b	***	***	*
Isorhamnetin-3-*O*-glucoside									6.9 ± 0.4 ab	6.1 ± 0.7 a	5.1 ± 1.2 a	10 ± 1 b	-	*	-
Isorhamnetin-3-*O*-galactoside									23 ± 2	25 ± 2	27 ± 1	25 ± 3	-	ns	-
Isorhamnetin-3-*O*-glucuronide	7.6 ± 2.2 a	8.5 ± 0.4 a	10 ± 1 a	14 ± 1 b	5.0 ± 0.6 a	5.6 ± 0.9 a	6.3 ± 0.4 a	18 ± 2 b	4.1 ± 0.8	4.3 ± 0.5	4.0 ± 0.4	6.0 ± 0.7	***	***	**
Syringetin-3-*O*-glucoside									11 ± 1 ab	11 ± 1 ab	10 ± 1 a	14 ± 1 b	-	*	-
Hydroxycinnamic acid derivatives (μg g^{-1} FW)															
Caffeoyl tartaric acid	121 ± 15 a	124 ± 15 a	218 ± 0 b	94 ± 1 a	43 ± 7	30 ± 8	42 ± 5	37 ± 2	17 ± 1	14 ± 0	17 ± 1	16 ± 2	***	***	***
Coumaroyl tartaric acid	729 ± 90 a	771 ± 20 a	1125 ± 25 b	708 ± 51 a	274 ± 41	207 ± 83	249 ± 27	227 ± 34	94 ± 11	72 ± 2	86 ± 6	87 ± 12	***	**	***
Feruloyl tartaric acid	52 ± 8	59 ± 4	63 ± 0	56 ± 1	52 ± 3	37 ± 9	37 ± 5	44 ± 2	13 ± 5	8.7 ± 0.9	10 ± 2	11 ± 3	***	ns	ns
Anthocyanins (mg g^{-1} FW)															
Cyanidin-3-*O*-glucoside					14 ± 3	11 ± 3	12 ± 5	16 ± 8	40 ± 2	39 ± 6	24 ± 2	37 ± 5	***	ns	ns
Delphinidin-3-*O*-glucoside					20 ± 3	20 ± 5	27 ± 9	26 ± 12	87 ± 9	96 ± 15	91 ± 6	98 ± 16	***	ns	ns
malvidin-3-*O*-glucoside					36 ± 5	36 ± 8	58 ± 22	42 ± 19	240 ± 16	271 ± 30	294 ± 19	261 ± 45	***	ns	ns
Peonidin-3-*O*-glucoside					26 ± 5	21 ± 2	25 ± 10	29 ± 15	104 ± 6	99 ± 12	68 ± 6	93 ± 12	***	ns	ns
Petunidin-3-*O*-glucoside					16 ± 2	17 ± 4	23 ± 9	20 ± 10	81 ± 8	89 ± 12	88 ± 5	89 ± 14	***	ns	ns
Cyanidin-3-*O*-(6'-acetyl)glucoside									1.7 ± 0.0	1.7 ± 0.2	1.2 ± 0.1	1.7 ± 0.3	-	ns	-
Delphinidin-3-*O*-(6'-acetyl)glucoside									3.4 ± 0.1	3.6 ± 0.4	3.4 ± 0.3	3.5 ± 0.6	-	ns	-
Malvidin-3-*O*-(6'-acetyl)glucoside									20 ± 0	25 ± 2	26 ± 1	24 ± 5	-	ns	-
Peonidin-3-*O*-(6'-acetyl)glucoside									5.3 ± 0.2	5.6 ± 0.6	4.4 ± 0.4	5.6 ± 0.9	-	ns	-
Petunidin-3-*O*-(6'-acetyl)glucoside									4.7 ± 0.4	5.7 ± 0.7	5.5 ± 0.6	5.6 ± 0.9	-	ns	-
Cyanidin-3-*O*-(6'-p-coumaroyl)glucoside					4.1 ± 0.4	4.4 ± 1.0	5.5 ± 2.2	5.6 ± 2.5	13 ± 1	13 ± 2	13 ± 1	15 ± 2	***	ns	ns
Delphinidin-3-*O*-(6'-p-coumaroyl)glucoside					3.4 ± 0.3	4.0 ± 1.1	5.6 ± 2.2	4.3 ± 2.0	21 ± 1	26 ± 4	28 ± 1	26 ± 5	***	ns	ns
Malvidin-3-*O*-(6'-p-coumaroyl)glucoside					9.3 ± 0.5	11 ± 3	18 ± 8	12 ± 5	114 ± 7	138 ± 13	166 ± 4	140 ± 30	***	ns	ns
Peonidin-3-*O*-(6'-p-coumaroyl)glucoside					6.9 ± 0.7	7.2 ± 1.4	9.1 ± 4.0	8.9 ± 4.0	36 ± 3	36 ± 3	32 ± 2	37 ± 6	***	ns	ns
Petunidin-3-*O*-(6'-p-coumaroyl)glucoside					3.6 ± 0.3	4.2 ± 1.0	5.8 ± 2.4	4.6 ± 2.0	24 ± 1	32 ± 5	36 ± 1	33 ± 7	***	ns	ns

Table 7.4. Global variables (total phenols, total flavonoids, bulk levels of UV-absorbing compounds (UVAC) and antioxidant capacity) and individual phenolic compounds of grapes in three phenological stages (pea size, veraison and harvest) and four radiation regimes (P, PA, PB and PAB). Phenolic variables were measured in the soluble fraction of the ensemble of skin and flesh. For each variable, the statistical significance of a two-way ANOVA test (using time (phenological stage) and treatment (radiation regime) as main factors) is shown, together with the interaction between these two factors, and different letters mean significant differences between radiation regimes for each phenological stage (Tukey's test). Means ± SE are shown. FW, fresh weight. TE, Trolox equivalent. $AUC_{280\text{–}315}$ and $AUC_{280\text{–}400}$, area under the absorbance curve in the intervals 280–315 and 280-400 nm, respectively. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant.

SKIN

Compound	Soluble fraction P	PA	PB	PAB	statistical significance	Insoluble fraction P	PA	PB	PAB	statistical significance
Stilbenes (μg g⁻¹ DW)										
Resveratrol	5.9 ± 0.8	5.4 ± 0.6	2.6 ± 0.8	5.9 ± 1.3	ns					
Resveratrol-3-*O*-glucoside	134 ± 19 bc	129 ± 1 bc	43 ± 6 a	82 ± 6 ab	**					
Total stilbenes	140 ± 4 c	134 ± 0 c	45.9 ± 7.1 a	87.5 ± 6.8 b	**					
Flavanols (μg g⁻¹ DW)										
Catechin	115 ± 3 c	83.4 ± 6.4 ab	71.2 ± 4.7 a	98.3 ± 4.2 bc	**					
Epicatechin	18 ± 2 b	14 ± 1 ab	11 ± 0 a	13 ± 0 ab	*					
Catechin gallate	12 ± 1	10 ± 2	7 ± 1	11 ± 0	ns					
Epigallocatechin	3.0 ± 0.2	3.3 ± 0.2	3.2 ± 0.4	3.0 ± 0.7	ns					
Gallocatechin	30 ± 3	31 ± 4	34 ± 3	31 ± 4	ns					
Epicatechin gallate										
Procyanidin B1	130 ± 8 b	117 ± 2 ab	87.0 ± 6.2 a	114 ± 4 ab	*					
Procyanidin B2	21 ± 1 b	16 ± 1 ab	13 ± 1 a	17 ± 1 ab	*					
Procyanidin B3										
Total flavanols	329 ± 7 c	274 ± 1 ab	226 ± 13 a	288 ± 3 bc	**					
Flavonols (μg g⁻¹ DW)										
Kaempferol	0.25 ± 0.05 a	0.23 ± 0.06 a	0.17 ± 0.02 a	0.56 ± 0.06 b	**					
Kaempferol-3-*O*-glucoside	61 ± 13	39 ± 18	44 ± 4	107 ± 5	ns					
Myricetin	48 ± 7	55 ± 18	48 ± 3	53 ± 3	ns					
Myricetin-3-*O*-glucoside	1429 ± 128	1456 ± 130	1273 ± 71	1607 ± 44	ns					
Myricetin-3-*O*-glucuronide	152 ± 18	149 ± 14	115 ± 11	148 ± 12	ns					
Laricitrin-3-*O*-glucoside	132 ± 4 ab	169 ± 12 c	111 ± 5 a	144 ± 4 bc	**					
Quercetin	2.5 ± 0.1 ab	2.5 ± 0.1 ab	2.3 ± 0.3 a	3.8 ± 0.5 b	*					
Quercetin-3-*O*-glucoside	411 ± 93 ab	455 ± 41 ab	203 ± 9 a	703 ± 21 b	*					
Quercetin-3-*O*-galactoside	87 ± 26	47 ± 5	90 ± 6	87 ± 5	ns					
Quercetin-3-*O*-glucuronide	502 ± 84 a	604 ± 84 ab	636 ± 59 ab	871 ± 25 b	*					
Quercetin-3-*O*-rutinoside	27 ± 8 a	33 ± 2 a	50 ± 4 a	77 ± 4 b	**					
Isorhamnetin-3-*O*-glucoside	152 ± 8 b	116 ± 13 b	67 ± 3 a	142 ± 3 b	***					
Isorhamnetin-3-*O*-glucuronide	10.0 ± 1.8	8.1 ± 0.7	7.8 ± 1.5	13.8 ± 0.2	ns					
Syringetin	3.2 ± 0.3	2.9 ± 0.2	2.8 ± 0.4	3.4 ± 0.2	ns					
Syringetin-3-*O*-glucoside	88 ± 11	62 ± 0	48 ± 6	72 ± 7	ns					
Total flavonols	3104 ± 377 ab	3199 ± 178 ab	2699 ± 179 a	4031 ± 120 b	*					
Hydroxybenzoic acids (μg g⁻¹ DW)										
Protocatechuic acid						16 ± 4	11 ± 1	13 ± 1	15 ± 1	ns
Gallic acid						55 ± 10	34 ± 4	70 ± 6	51 ± 3	ns
Total hydroxybenzoic acids						70 ± 18	45 ± 17	83 ± 8	66 ± 2	ns
Hydroxycinnamic acid (μg g⁻¹ DW)										
Caffeic acid						5.1 ± 1.6	3.8 ± 0.5	3.8 ± 0.6	4.0 ± 0.6	ns
Coumaric acid						305 ± 42	288 ± 11	272 ± 20	294 ± 21	ns
Ferulic acid						147 ± 11 b	147 ± 32 b	87 ± 6 ab	59 ± 2 a	*
Total hydroxycinnamic acids						457 ± 60	438 ± 15	363 ± 36	357 ± 31	ns
Hydroxycinnamic acid derivatives (μg g⁻¹ DW)										
Caffeoyl tartaric acid	243 ± 15 ab	183 ± 25 a	283 ± 28 b	288 ± 20 b	*					
Coumaroyl tartaric acid	122 ± 11	98 ± 13	133 ± 11	97 ± 1	ns					
Feruloyl tartaric acid	55 ± 4	55 ± 3	49 ± 1	55 ± 4	ns					
Total hydroxycinnamic acids derivatives	420 ± 22	336 ± 9	465 ± 33	441 ± 19	ns					
Anthocyanins (mg g-1 DW)										
Cyanidin-3-*O*-glucoside	2.7 ± 0.6	2.8 ± 0.3	1.6 ± 0.3	2.0 ± 0.1	ns					
Delphinidin-3-*O*-glucoside	14 ± 2	16 ± 2	16 ± 2	15 ± 2	ns					
Malvidin-3-*O*-glucoside	42 ± 5	50 ± 6	53 ± 8	44 ± 10	ns					
Peonidin-3-*O*-glucoside	8.1 ± 1.5	8.1 ± 0.8	5.6 ± 1.0	5.9 ± 0.5	ns					
Petunidin-3-*O*-glucoside	15 ± 2	17 ± 2	17 ± 2	15 ± 3	ns					
Cyanidin-3-*O*-(6'-acetyl)glucoside	0.10 ± 0.01	0.09 ± 0.01	0.06 ± 0.00	0.08 ± 0.01	ns					
Delphinidin-3-*O*-(6'-acetyl)glucoside	0.30 ± 0.13	0.17 ± 0.02	0.15 ± 0.01	0.18 ± 0.01	ns					
Malvidin-3-*O*-(6'-acetyl)glucoside	10 ± 1	12 ± 2	8.7 ± 3.9	10 ± 3	ns					
Peonidin-3-*O*-(6'-acetyl)glucoside	0.24 ± 0.01 b	0.24 ± 0.02 b	0.16 ± 0.01 a	0.22 ± 0.01 ab	*					
Petunidin-3-*O*-(6'-acetyl)glucoside	0.30 ± 0.04	0.25 ± 0.10	0.31 ± 0.03	0.34 ± 0.03	ns					
Cyanidin-3-*O*-(6'-p-coumaroyl)glucoside	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	ns					
Delphinidin-3-*O*-(6'-p-coumaroyl)glucoside	0.72 ± 0.09	0.86 ± 0.14	0.83 ± 0.04	0.90 ± 0.04	ns					
Malvidin-3-*O*-(6'-p-coumaroyl)glucoside	28 ± 4	35 ± 4	43 ± 6	30 ± 7	ns					
Peonidin-3-*O*-(6'-p-coumaroyl)glucoside	5.6 ± 0.4	5.6 ± 0.7	5.4 ± 0.7	4.7 ± 0.7	ns					
Petunidin-3-*O*-(6'-p-coumaroyl)glucoside	3.9 ± 0.5	4.5 ± 0.6	4.9 ± 0.4	5.0 ± 0.5	ns					
Total anthocyanins	131 ± 14	152 ± 4	157 ± 17	134 ± 22	ns					

FLESH

Compound	Soluble fraction P	PA	PB	PAB	statistical significance	Insoluble fraction P	PA	PB	PAB	statistical significance
Stilbenes (μg g⁻¹ DW)										
Resveratrol										
Resveratrol-3-*O*-glucoside										
Total stilbenes										
Flavanols (μg g⁻¹ DW)										
Catechin	4.8 ± 0.2 b	4.2 ± 0.3 ab	4.3 ± 0.3 ab	3.5 ± 0.1 a	*					
Epicatechin	2.8 ± 0.2 b	2.0 ± 0.1 b	2.1 ± 0.1 ab	2.0 ± 0.1 a	*					
Catechin gallate										
Epigallocatechin										
Gallocatechin										
Epicatechin gallate	2.0 ± 0.4	1.3 ± 0.1	2.0 ± 0.3	1.3 ± 0.2	ns					
Procyanidin B1	5.7 ± 0.1	5.8 ± 0.3	6.1 ± 0.4	4.9 ± 0.2	ns					
Procyanidin B2	0.69 ± 0.04 b	0.60 ± 0.01 ab	0.64 ± 0.02 a	0.56 ± 0.02 a	*					
Procyanidin B3										
Total flavanols	16.0 ± 0.3 b	14.0 ± 1.0 ab	15.1 ± 1.0 ab	12.3 ± 0.2 a	*					
Flavonols (μg g⁻¹ DW)										
Kaempferol										
Kaempferol-3-*O*-glucoside										
Myricetin										
Myricetin-3-*O*-glucoside										
Myricetin-3-*O*-glucuronide										
Laricitrin-3-*O*-glucoside										
Quercetin										
Quercetin-3-*O*-glucoside	3.1 ± 0.4 ab	2.4 ± 0.3 a	4.2 ± 0.3 ab	4.4 ± 0.3 b	*					
Quercetin-3-*O*-galactoside										
Quercetin-3-*O*-glucuronide	0.56 ± 0.05	0.67 ± 0.17	0.78 ± 0.05	0.66 ± 0.15	ns					
Quercetin-3-*O*-rutinoside	0.23 ± 0.05	0.21 ± 0.03	0.38 ± 0.06	0.30 ± 0.07	ns					
Isorhamnetin-3-*O*-glucoside										
Isorhamnetin-3-*O*-glucuronide										
Syringetin										
Syringetin-3-*O*-glucoside										
Total flavonols	3.9 ± 0.3	3.3 ± 0.7	5.4 ± 0.4	5.3 ± 0.6	ns					
Hydroxybenzoic acids (μg g⁻¹ DW)										
Protocatechuic acid						2.4 ± 0.7	2.8 ± 0.8	0.93 ± 0.12	1.5 ± 0.1	ns
Gallic acid						5.6 ± 0.9	3.2 ± 0.6	4.6 ± 0.1	4.9 ± 0.6	ns
Total hydroxybenzoic acids						8.0 ± 0.3	6.0 ± 1.3	5.5 ± 0.1	6.4 ± 0.7	ns
Hydroxycinnamic acid (μg g⁻¹ DW)										
Caffeic acid						1.1 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	1.1 ± 0.0	ns
Coumaric acid						1.9 ± 0.2	1.6 ± 0.0	1.4 ± 0.1	1.3 ± 0.0	ns
Ferulic acid						0.52 ± 0.11	0.49 ± 0.09	0.52 ± 0.06	0.48 ± 0.09	ns
Total hydroxycinnamic acids						3.5 ± 0.4	3.4 ± 0.1	3.0 ± 0.2	2.9 ± 0.1	ns
Hydroxycinnamic acid derivatives (μg g⁻¹ DW)										
Caffeoyl tartaric acid	97 ± 6	81 ± 4	92 ± 7	76 ± 2	ns					
Coumaroyl tartaric acid	30 ± 1 ab	26 ± 1 bc	31 ± 1 a	23 ± 1 c	**					
Feruloyl tartaric acid	19 ± 1	17 ± 1	17 ± 1	16 ± 0	ns					
Total hydroxycinnamic acids derivatives	146 ± 8	124 ± 6	140 ± 9	115 ± 3	*					
Anthocyanins (mg g-1 DW)										
Cyanidin-3-*O*-glucoside										
Delphinidin-3-*O*-glucoside										
Malvidin-3-*O*-glucoside										
Peonidin-3-*O*-glucoside										
Petunidin-3-*O*-glucoside										
Cyanidin-3-*O*-(6'-acetyl)glucoside										
Delphinidin-3-*O*-(6'-acetyl)glucoside										
Malvidin-3-*O*-(6'-acetyl)glucoside										
Peonidin-3-*O*-(6'-acetyl)glucoside										
Petunidin-3-*O*-(6'-acetyl)glucoside										
Cyanidin-3-*O*-(6'-p-coumaroyl)glucoside										
Delphinidin-3-*O*-(6'-p-coumaroyl)glucoside										
Malvidin-3-*O*-(6'-p-coumaroyl)glucoside										
Peonidin-3-*O*-(6'-p-coumaroyl)glucoside										
Petunidin-3-*O*-(6'-p-coumaroyl)glucoside										
Total anthocyanins										

SEED

Compound	Soluble fraction P	PA	PB	PAB	statistical significance	Insoluble fraction P	PA	PB	PAB	statistical significance
Stilbenes (μg g⁻¹ DW)										
Resveratrol										
Resveratrol-3-*O*-glucoside										
Total stilbenes										
Flavanols (μg g⁻¹ DW)										
Catechin	152 ± 16	141 ± 7	156 ± 6	173 ± 6	ns	7.5 ± 2.1 a	11 ± 0 ab	15 ± 2 b	9.4 ± 0.1 ab	ns
Epicatechin	90 ± 5 ab	83 ± 9 a	89 ± 4 ab	113 ± 6 b	*					
Catechin gallate										
Epigallocatechin										
Gallocatechin										
Epicatechin gallate	307 ± 7 ab	315 ± 12 ab	269 ± 23 a	342 ± 14 b	*					
Procyanidin B1	145 ± 8	143 ± 10	157 ± 2	165 ± 13	ns					
Procyanidin B2	59 ± 2 a	57 ± 3 a	63 ± 3 ab	72 ± 2 b	*					
Procyanidin B3	6.5 ± 0.5 a	6.7 ± 0.5 a	7.4 ± 0.3 ab	9.4 ± 0.5 b	**					
Total flavanols	760 ± 39	746 ± 30	742 ± 27	874 ± 79	ns	7.5 ± 2.1 a	11 ± 0 ab	15 ± 2 b	9.4 ± 0.1 ab	ns
Flavonols (μg g⁻¹ DW)										
Kaempferol										
Kaempferol-3-*O*-glucoside										
Myricetin										
Myricetin-3-*O*-glucoside										
Myricetin-3-*O*-glucuronide										
Laricitrin-3-*O*-glucoside										
Quercetin	0.37 ± 0.04 ab	0.37 ± 0.04 ab	0.27 ± 0.04 a	0.55 ± 0.07 b	*					
Quercetin-3-*O*-glucoside	3.8 ± 0.1	3.5 ± 0.3	4.0 ± 0.4	3.7 ± 0.3	ns					
Quercetin-3-*O*-galactoside	33 ± 2 b	33 ± 1 b	23 ± 2 a	34 ± 1 b	**					
Quercetin-3-*O*-glucuronide	0.63 ± 0.05 ab	0.49 ± 0.14 a	0.72 ± 0.14 ab	0.94 ± 0.06 b	*					
Quercetin-3-*O*-rutinoside										
Isorhamnetin-3-*O*-glucoside										
Isorhamnetin-3-*O*-glucuronide										
Syringetin										
Syringetin-3-*O*-glucoside										
Total flavonols	37 ± 2	37 ± 1	28 ± 3	39 ± 1	ns					
Hydroxybenzoic acids (μg g⁻¹ DW)										
Protocatechuic acid						5.8 ± 0.1	5.0 ± 0.6	5.0 ± 0.5	5.0 ± 0.2	ns
Gallic acid						79 ± 5 b	54 ± 3 a	49 ± 3 a	62 ± 5 ab	*
Total hydroxybenzoic acids						85 ± 10	59 ± 4	54 ± 4	67 ± 0	ns
Hydroxycinnamic acid (μg g⁻¹ DW)										
Caffeic acid										
Coumaric acid						16 ± 2	16 ± 2	17 ± 1	15 ± 1	ns
Ferulic acid										
Total hydroxycinnamic acids						16 ± 2	16 ± 2	17 ± 1	15 ± 1	ns
Hydroxycinnamic acid derivatives (μg g⁻¹ DW)										
Caffeoyl tartaric acid	2.3 ± 0.1	2.0 ± 0.1	2.3 ± 0.0	2.2 ± 0.0	ns					
Coumaroyl tartaric acid	129 ± 10 b	93 ± 3 ab	83 ± 7 a	118 ± 2 ab	*					
Feruloyl tartaric acid										
Total hydroxycinnamic acids derivatives	131 ± 10 b	95 ± 3 ab	85 ± 7 a	120 ± 10 ab	*					
Anthocyanins (mg g-1 DW)										
Cyanidin-3-*O*-glucoside										
Delphinidin-3-*O*-glucoside										
Malvidin-3-*O*-glucoside										
Peonidin-3-*O*-glucoside										
Petunidin-3-*O*-glucoside										
Cyanidin-3-*O*-(6'-acetyl)glucoside										
Delphinidin-3-*O*-(6'-acetyl)glucoside										
Malvidin-3-*O*-(6'-acetyl)glucoside										
Peonidin-3-*O*-(6'-acetyl)glucoside										
Petunidin-3-*O*-(6'-acetyl)glucoside										
Cyanidin-3-*O*-(6'-p-coumaroyl)glucoside										
Delphinidin-3-*O*-(6'-p-coumaroyl)glucoside										
Malvidin-3-*O*-(6'-p-coumaroyl)glucoside										
Peonidin-3-*O*-(6'-p-coumaroyl)glucoside										
Petunidin-3-*O*-(6'-p-coumaroyl)glucoside										
Total anthocyanins										

Table 7.5. Individual phenolic compounds from the methanol-soluble and methanol-insoluble fractions of the three components of grapes (skin, flesh and seeds) in four radiation regimes (P, PA, PB and PAB) at harvest. For each fraction of each component, the statistical significance of a one-way ANOVA test using radiation regime as main factor is shown, and different letters mean significant differences between radiation regimes (Tukey's test). Means ± SE are shown. DW, dry weight. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant.

Table 7.6. Relative abundance (percentages) of methanol-soluble and methanol-insoluble phenolic compounds (main groups) in the three grape components (skin, flesh and seeds).

	Soluble compounds			Insoluble compounds		
	Skin	Flesh	Seed	Skin	Flesh	Seed
Resveratrols	100					
Catechins	21	1.1	77			1.5
PAs	36	1.7	62			
Myricetins	100					
Quercetins	97	0.4	2.8			
Kaempferols	100					
Isorhamnetins	100					
Syringetins	100					
Benzoic acids				48	4.8	48
Cinnamic acids				95	0.8	3.8
Hydroxycinnamic acid derivatives	64	20	17			
Anthocyanins	100					

Table 7.7. Relative abundance (percentages) of individual volatile organic compounds (VOCs) in the ensemble of skin and flesh in four radiation regimes (P, PA, PB and PAB) at harvest. For each compound, the statistical significance of a one-way ANOVA test using radiation regime as main factor is shown, and different letters mean significant differences between radiation regimes (Tukey's test). Means ± SE are shown. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant.

Volatile organic compounds (VOCs)	P	PA	PB	PAB	statistical significance
Alcohols					
1-butanol	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	ns
3-buten-2-ol-2methyl	0.04 ± 0.00	0.03 ± 0.01	0.05 ± 0.01	0.04 ± 0.00	ns
1-decanol	7.97 ± 0.98	10 ± 2	12 ± 7	15 ± 8	ns
Ethylhexanol	0.07 ± 0.01	0.07 ± 0.01	1.05 ± 0.96	0.06 ± 0.01	ns
2 heptanol n	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	ns
1-hexanol	4.2 ± 0.3	3.5 ± 0.2	4.0 ± 0.8	3.1 ± 0.4	ns
(E)-2-hexen-1-ol	3.2 ± 0.7	3.7 ± 0.3	3.4 ± 0.2	3.1 ± 0.4	ns
2-methyl-2-propanol	0.03 ± 0.00 **ab**	0.03 ± 0.00 **ab**	0.04 ± 0.00 **b**	0.02 ± 0.00 **a**	*
1-octanol	0.14 ± 0.00	0.14 ± 0.01	0.15 ± 0.01	0.09 ± 0.05	ns
1-pentanol	0.06 ± 0.00	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	ns
1-penten-3-ol	0.09 ± 0.00	0.12 ± 0.02	0.10 ± 0.00	0.09 ± 0.02	ns
(Z)-2-penten-1-ol n	0.06 ± 0.00	0.08 ± 0.02	0.06 ± 0.00	0.07 ± 0.01	ns
2-phenylethanol	0.04 ± 0.01	0.06 ± 0.00	0.06 ± 0.00	0.05 ± 0.00 **s**	ns
Ketones					
3-hexen-2-one	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.0 ± 0.2	ns
4-methyl-2-heptanone	0.06 ± 0.00	0.06 ± 0.00	0.03 ± 0.00	0.06 ± 0.01	ns
6-methyl-5-hepten-2-one	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	ns
2-pentanone	0.08 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.06 ± 0.01	ns
Hydrocarbons					
Dodecane n	0.08 ± 0.01	0.10 ± 0.01	0.07 ± 0.00	0.10 ± 0.02	ns
Heptane, 2, 2, 4, 6, 6-pentamethyl	0.02 ± 0.00	0.02 ± 0.00	0.28 ± 0.27	0.02 ± 0.00	ns
Hexane	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	ns
Propane, 2-methoxy-2-methyl	0.04 ± 0.03 **ab**	0.13 ± 0.02 **b**	0.14 ± 0.03 **b**	0.03 ± 0.01 **a**	*
Tridecane n	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.05 ± 0.01	ns
Aldehydes					
Benzaldehyde	0.03 ± 0.00	0.02 ± 0.00	0.09 ± 0.07	0.03 ± 0.00	ns
Decanal	0.07 ± 0.01	0.08 ± 0.01	0.09 ± 0.02	0.10 ± 0.02	ns
(E,E)-2,4-heptadienal	0.12 ± 0.01	0.20 ± 0.05	0.08 ± 0.01	0.16 ± 0.02	ns
Heptanal	0.06 ± 0.01	0.05 ± 0.00	0.06 ± 0.00	0.05 ± 0.01	ns
(E)-2-heptenal	1.4 ± 0.1	1.4 ± 0.1	1.1 ± 0.4	1.3 ± 0.2	ns
(E,E)-2,4-hexadienal	0.07 ± 0.00	0.09 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	ns
Hexanal	22 ± 2	20 ± 1	17 ± 2	19 ± 3	ns
(Z)-3-hexenal	4.0 ± 0.1	3.8 ± 0.2	4.4 ± 0.3	3.7 ± 0.8	ns
(E)-2-hexenal	45 ± 1	44 ± 2	36 ± 2	43 ± 5	ns
n-nonaldehyde	0.41 ± 0.06	0.43 ± 0.01	0.32 ± 0.03	0.39 ± 0.05	ns
Octanal	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.00	0.09 ± 0.01	ns
(E)-2-octenal	0.08 ± 0.01 **ab**	0.06 ± 0.00 **a**	0.08 ± 0.00 **ab**	0.09 ± 0.01 **b**	*
Pentanal	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	ns
Phenylacetaldehyde	0.06 ± 0.01 **a**	0.14 ± 0.01 **b**	0.05 ± 0.01 **a**	0.07 ± 0.01 **a**	**
Furans					
2-ethylfuran	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.03 ± 0.01	ns
2-pentylfuran	0.95 ± 0.10	1.55 ± 0.31	0.41 ± 0.21	1.29 ± 0.17	ns
Terpenes					
Alcanfor	1.7 ± 0.1 **ab**	1.6 ± 0.1 **ab**	2.5 ± 0.3 **b**	1.5 ± 0.2 **a**	*
Alpha, alpha-Dichloroacetone	0.21 ± 0.01	0.20 ± 0.03	0.33 ± 0.05	0.18 ± 0.03	ns
Alpha-pinene n	0.15 ± 0.02	0.27 ± 0.07	0.14 ± 0.02	0.22 ± 0.03	ns
3-carene	1.7 ± 0.1	1.7 ± 0.1	1.6 ± 0.8	1.5 ± 0.2	ns
β-cyclocitral	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.01	ns
Limonene	0.40 ± 0.04	0.50 ± 0.17	0.62 ± 0.03	0.37 ± 0.03	ns
Linalool	0.24 ± 0.02	0.31 ± 0.09	0.48 ± 0.12	0.35 ± 0.06	ns
(Z)-linalool oxide	0.04 ± 0.00	0.05 ± 0.00	0.07 ± 0.00	0.05 ± 0.00	ns
Menthol	0.03 ± 0.00 **a**	0.03 ± 0.00 **a**	0.05 ± 0.01 **b**	0.03 ± 0.00 **a**	**
Terpineol	0.08 ± 0.00	0.09 ± 0.01	0.13 ± 0.02	0.10 ± 0.01	ns

Volatile organic compounds (VOCs)	P	PA	PB	PAB	statistical significance
C13-norsioprenoids					
Apocarotenoid	0.06 ± 0.01 **a**	0.06 ± 0.01 **a**	0.12 ± 0.00 **b**	0.06 ± 0.01 **a**	**
β-damascenone	0.36 ± 0.02	0.31 ± 0.05	0.32 ± 0.02	0.40 ± 0.05	ns
β-ionone	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	ns
Acids					
2-ethylhexanoic acid	0.12 ± 0.01 **a**	0.12 ± 0.02 **a**	0.88 ± 0.10 **b**	0.23 ± 0.07 **a**	***
Heptanoic acid	0.16 ± 0.02 **a**	0.19 ± 0.04 **a**	0.51 ± 0.04 **b**	0.31 ± 0.06 **a**	**
Hexanoic acid	1.2 ± 0.1	1.0 ± 0.2	0.9 ± 0.1	1.1 ± 0.2	ns
Nonanoic acid	0.55 ± 0.06 **a**	1.35 ± 0.05 **a**	5.48 ± 0.08 **b**	1.33 ± 0.63 **a**	***
Octanoic acid	0.11 ± 0.01 **a**	0.12 ± 0.01 **a**	1.27 ± 0.04 **b**	0.37 ± 0.15 **a**	***
Propanoic acid, 2, 2-dimethyl	0.29 ± 0.02	0.27 ± 0.05	0.30 ± 0.04	0.26 ± 0.04	ns

Table 7.8. Global variables (color, total polyphenol index, total phenols, bulk levels of UV-absorbing compounds (UVAC) and antioxidant capacity) and individual phenolic compounds in wines elaborated from grapes exposed to four radiation regimes (P, PA, PB and PAB). For each variable, the statistical significance of a one-way ANOVA test using radiation regime as main factor is shown, and different letters mean significant differences between radiation regimes (Tukey's test). Means ± SE are shown. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant.

	P	PA	PB	PAB	statistical significance
Color intensity (CI)	14 ± 1	14 ± 1	13 ± 0	14 ± 0	ns
Hue	0.76 ± 0.06	0.73 ± 0.02	0.76 ± 0.76	0.76 ± 0.76	ns
Antioxidant capacity and phenolic compounds					
Total polyphenol index (TPI)	60 ± 4 b	55 ± 3 ab	46 ± 2 a	54 ± 0 ab	*
Total phenols (GAE, g L^{-1})	2.6 ± 0.1	2.7 ± 0.2	2.2 ± 0.1	2.6 ± 0.1	ns
Antioxidant capacity (mM TE)	24 ± 1 b	23 ± 0 ab	19 ± 0 a	23 ± 1 ab	**
UVAC ($AUC_{280-315}$)	1523 ± 106	1425 ± 70	1231 ± 34	1394 ± 5	ns
UVAC ($AUC_{280-400}$)	2616 ± 154	2626 ± 150	2336 ± 69	2655 ± 16	ns
Stilbenes (mg L^{-1})					
Resveratrol	0.09 ± 0.02	0.12 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	ns
Resveratrol-3-*O*-glucoside	1.7 ± 0.2 ab	2.1 ± 0.2 b	1.2 ± 0.1 a	1.5 ± 0.1 ab	*
Flavanols (mg L^{-1})					
Catechin	15 ± 2 b	12 ± 1 ab	8 ± 0 a	11 ± 1 ab	*
Epicatechin	6.7 ± 0.6 b	5.3 ± 0.1 ab	3.6 ± 0.2 a	5.6 ± 0.9 ab	*
Gallocatechin	0.90 ± 0.15	0.99 ± 0.12	1.3 ± 0.2	1.2 ± 0.2	ns
Epigallocatechin	6.7 ± 1.8 a	13.0 ± 0.0 b	10.3 ± 1.1 ab	12.9 ± 1.3 ab	*
Catechin gallate	0.13 ± 0.03 ab	0.18 ± 0.03 b	0.08 ± 0.01 ab	0.05 ± 0.02 a	*
Procyanidin B1	8.0 ± 0.5 ab	8.7 ± 0.9 b	5.6 ± 0.8 a	7.5 ± 0.4 ab	*
Procyanidin B2	3.0 ± 0.3 b	2.5 ± 0.0 b	1.4 ± 0.1 a	2.3 ± 0.3 ab	**
Flavonols (mg L^{-1})					
Kaempferol	1.2 ± 0.7	1.9 ± 0.1	0.77 ± 0.13	0.72 ± 0.18	ns
Kaempferol-3-*O*-glucoside	0.02 ± 0.00 a	0.04 ± 0.01 a	0.06 ± 0.00 a	0.14 ± 0.03 b	**
Kaempferol-3-*O*-glucuronide	0.02 ± 0.00 b	0.02 ± 0.00 ab	0.01 ± 0.00 a	0.03 ± 0.00 c	***
Myricetin	3.3 ± 0.2 a	5.4 ± 0.7 b	3.8 ± 0.1 a	6.0 ± 0.7 b	*
Myricetin-3-*O*-glucoside	3.0 ± 0.4	4.0 ± 0.4	3.9 ± 0.1	4.4 ± 0.4	ns
Myricetin-3-*O*-glucuronide	1.4 ± 0.1 a	1.5 ± 0.1 ab	1.4 ± 0.0 a	1.8 ± 0.1 b	*
Laricitrin	0.57 ± 0.06 ab	0.58 ± 0.02 ab	0.42 ± 0.05 a	0.66 ± 0.05 b	*
Laricitrin-3-*O*-glucoside	0.76 ± 0.13	0.91 ± 0.15	0.63 ± 0.02	0.84 ± 0.03	ns
Quercetin	4.7 ± 1.6 a	2.1 ± 0.3 a	1.4 ± 0.2 a	4.2 ± 0.8 a	*
Quercetin-3-*O*-galactoside	5.3 ± 0.1 a	8.6 ± 0.8 ab	8.6 ± 0.5 ab	13 ± 2 b	*
Quercetin-3-*O*-glucoside	5.6 ± 0.4 a	4.9 ± 0.7 a	3.5 ± 0.6 a	13 ± 2 b	**
Quercetin-3-*O*-glucuronide	3.0 ± 0.2 a	2.7 ± 0.3 a	1.9 ± 0.2 a	4.5 ± 0.3 b	***
Isorhamnetin-3-*O*-glucoside	0.08 ± 0.01	0.09 ± 0.01	0.09 ± 0.02	0.11 ± 0.01	ns
Isorhamnetin-3-*O*-glucuronide	0.87 ± 0.16 a	1.07 ± 0.07 ab	0.86 ± 0.06 a	1.71 ± 0.29 b	*
Syringetin	0.31 ± 0.06			0.16 ± 0.03	ns
Syringetin-3-*O*-glucoside	2.2 ± 0.2	2.0 ± 0.1	1.6 ± 0.1	2.5 ± 0.3	ns
Hydroxybenzoic acids (mg L^{-1})					
Protocatechuic acid	0.63 ± 0.06 ab	0.59 ± 0.03 ab	0.33 ± 0.02 a	0.73 ± 0.14 b	*
Gallic acid	9.7 ± 1.0	10 ± 1	8 ± 0	12 ± 1	ns
Hydroxycinnamic acid derivatives (mg L^{-1})					
Caffeoyl tartaric acid	27 ± 5	35 ± 4	31 ± 3	35 ± 1	ns
Coumaroyl tartaric acid	15 ± 4	11 ± 0	17 ± 2	12 ± 1	ns
Caffeic acid ethyl ester	1.1 ± 0.2	0.61 ± 0.07	0.21 ± 0.00	0.80 ± 0.10	ns
Anthocyanins (mg L^{-1})					
Cyanidin-3-*O*-glucoside	2.2 ± 0.2 b	2.2 ± 0.3 b	0.82 ± 0.01 a	1.8 ± 0.2 ab	*
Delphinidin-3-*O*-glucoside	26 ± 1 a	60 ± 9 b	18 ± 5 a	48 ± 7 ab	*
Malvidin-3-*O*-glucoside	156 ± 5	230 ± 18	262 ± 26	210 ± 33	ns
Peonidin-3-*O*-glucoside	14 ± 2 b	14 ± 2 b	3.4 ± 0.9 a	12 ± 1 b	**
Petunidin-3-*O*-glucoside	41 ± 5	66 ± 4	60 ± 11	66 ± 7	ns
Cyanidin-3-*O*-(6'-acetyl)glucoside	1.7 ± 0.3	1.8 ± 0.1	1.0 ± 0.2	1.8 ± 0.0	ns
Delphinidin-3-*O*-(6'-acetyl)glucoside	0.91 ± 0.18	1.5 ± 0.1	1.5 ± 0.2	1.4 ± 0.1	ns
Malvidin-3-*O*-(6'-acetyl)glucoside	20 ± 2	33 ± 4	37 ± 4	28 ± 5	ns
Peonidin-3-*O*-(6'-acetyl)glucoside	1.3 ± 0.1	1.4 ± 0.2	0.94 ± 0.23	1.2 ± 0.0	ns
Petunidin-3-*O*-(6'-acetyl)glucoside	2.3 ± 0.7	4.2 ± 0.4	2.9 ± 0.5	3.7 ± 0.2	ns
Cyanidin-3-*O*-(6'-p-coumaroyl)glucoside	4.3 ± 0.4	5.5 ± 0.8	2.9 ± 0.7	4.6 ± 0.2	ns
Delphinidin-3-*O*-(6'-p-coumaroyl)glucoside	6.7 ± 1.2	15 ± 2	14 ± 2	14 ± 2	ns
Malvidin-3-*O*-(6'-p-coumaroyl)glucoside	19 ± 3	46 ± 2	34 ± 3	29 ± 7	ns
Peonidin-3-*O*-(6'-p-coumaroyl)glucoside	1.4 ± 0.2	1.9 ± 0.3	1.0 ± 0.3	1.4 ± 0.1	ns
Petunidin-3-*O*-(6'-p-coumaroyl)glucoside	1.7 ± 0.3 a	4.1 ± 0.6 b	3.6 ± 0.3 ab	3.3 ± 0.5 ab	*

Table 7.9. Relative abundance (percentages) of individual volatile organic compounds (VOCs) in wines elaborated from grapes exposed to four radiation regimes (P, PA, PB and PAB). For each compound, the statistical significance of a one-way ANOVA test using radiation regime as main factor is shown, and different letters mean significant differences between radiation regimes (Tukey's test). Means ± SE are shown. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant.

Volatile organic compounds (VOCs)	P	PA	PB	PAB	statistical significance
Alcohols					
2-methyl-1-butanol	19 ± 2	19 ± 2	25 ± 3	20 ± 2	ns
3-methyl butanol	25 ± 1 b	24 ± 1 b	14 ± 2 a	21 ± 1 b	**
1-hexanol	1.0 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	ns
Phenyl ethyl alcohol	21 ± 2	22 ± 2	18 ± 2	25 ± 2	ns
Hydrocarbons					
Tridecane	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	ns
Tetradecane	0.03 ± 0.00 a	0.04 ± 0.00 ab	0.05 ± 0.00 b	0.05 ± 0.00 b	**
Esters					
Acetic acid, 2-phenylethyl ester	0.09 ± 0.01	0.10 ± 0.00	0.10 ± 0.01	0.12 ± 0.00	ns
Diethyl succinate	0.70 ± 0.13	0.85 ± 0.07	0.72 ± 0.05	0.79 ± 0.14	ns
Ethyl acetate	6.5 ± 1.0	6.5 ± 0.4	6.4 ± 0.7	6.0 ± 0.9	ns
Ethyl butanoate	0.29 ± 0.04	0.43 ± 0.07	0.43 ± 0.05	0.35 ± 0.03	ns
Ethyl decanoate	0.97 ± 0.13 a	1.25 ± 0.18 a	2.14 ± 0.04 b	1.15 ± 0.08 a	***
Ethyl dodecanoate	0.52 ± 0.06 c	0.43 ± 0.02 bc	0.30 ± 0.02 ab	0.26 ± 0.01 a	**
Ethyl heptanoate	0.11 ± 0.01	0.09 ± 0.01	0.06 ± 0.01	0.09 ± 0.01	ns
Ethyl hexanoate	7.1 ± 0.6	6.4 ± 0.5	8.2 ± 0.5	7.2 ± 0.5	ns
Ethyl hexyl salycilate	0.10 ± 0.01	0.10 ± 0.03	0.06 ± 0.00	0.07 ± 0.01	ns
Ethyl nonanoate	1.2 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.3 ± 0.2	ns
Ethyl octanoate	8.4 ± 1.4 a	8.9 ± 1.0 a	16 ± 1 b	10 ± 1 a	**
Hexadecanoic acid, ethyl ester	4.7 ± 0.2	4.8 ± 1.0	3.0 ± 0.1	2.7 ± 0.6	ns
2-methylbutyl acetate	0.23 ± 0.05	0.20 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	ns
3-methylbutyl acetate	0.64 ± 0.10	0.86 ± 0.08	0.88 ± 0.02	0.91 ± 0.06	ns
Methyl hexanoate	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	ns
Methyl octanoate	0.08 ± 0.01	0.09 ± 0.01	0.13 ± 0.02	0.11 ± 0.02	ns
Octanoic acid, 3-methylbutyl ester	0.04 ± 0.00	0.04 ± 0.00	0.07 ± 0.01	0.04 ± 0.00	ns
Tetradecanoic acid ethyl ester	0.50 ± 0.08 b	0.32 ± 0.05 ab	0.12 ± 0.02 a	0.10 ± 0.02 a	**
Acids					
Ethanoic acid	0.95 ± 0.25	0.71 ± 0.21	0.81 ± 0.07	0.83 ± 0.30	ns
Hexanoic acid	0.46 ± 0.04 a	0.42 ± 0.01 a	0.73 ± 0.05 b	0.59 ± 0.05 ab	**
Nonanoic acid	0.07 ± 0.00 a	0.09 ± 0.01 ab	0.21 ± 0.01 c	0.12 ± 0.01 b	***
Octanoic acid	0.22 ± 0.03 a	0.31 ± 0.03 ab	0.70 ± 0.02 c	0.45 ± 0.05 b	***
Other compounds					
Hydroxybutyric acid lactone	0.03 ± 0.01	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	ns
n-nonaldehyde	0.08 ± 0.00 ab	0.07 ± 0.00 ab	0.06 ± 0.00 a	0.09 ± 0.01 b	*

Table 7.10. Effect of enhanced UV-B (comparison between PAB and PAB↑ regimes) on global variables (total phenols, total flavonoids, bulk levels of UV-absorbing compounds (UVAC) and antioxidant capacity) and individual phenolic compounds of grapes in three phenological stages (pea size, veraison and harvest). Phenolic variables were measured in the soluble fraction of the ensemble of skin and flesh. For each variable and phenological stage, the statistical significance of a Student's t test comparing both radiation regimes is shown. Means ± SE are shown. FW, fresh weight. TE, Trolox equivalent. $AUC_{280–315}$ and $AUC_{280–400}$, area under the absorbance curve in the intervals 280–315 and 280-400 nm, respectively. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant.

	Pea Size		statistical significance	Veraison		statistical significance	Harvest		statistical significance
Phenolic compounds and antioxidant capacity	PAB	PAB↑		PAB	PAB↑		PAB	PAB↑	
Total phenols (mg g^{-1} FW)	8.9 ± 0.9	9.9 ± 0.2	ns	5.3 ± 0.5	6.0 ± 0.2	ns	5.5 ± 0.3	5.6 ± 0.4	ns
Total flavonoids (mg g^{-1} FW)	3.1 ± 0.3	4.0 ± 0.2	ns	1.6 ± 0.2	2.0 ± 0.1	ns	1.2 ± 0.1	1.5 ± 0.1	ns
Antioxidant capacity (μmol TE g^{-1} FW)	468 ± 20	547 ± 15	*	141 ± 19	184 ± 12	ns	127 ± 12	128 ± 20	ns
UVAC ($AUC_{280-315}$ mg^{-1} FW)	1.6 ± 0.1	1.8 ± 0.1	ns	0.78 ± 0.07	0.83 ± 0.07	ns	1.5 ± 0.1	1.4 ± 0.1	ns
UVAC ($AUC_{280-400}$ mg^{-1} FW)	2.6 ± 0.2	2.9 ± 0.1	ns	1.3 ± 0.1	1.3 ± 0.1	ns	2.5 ± 0.1	2.3 ± 0.1	ns
Stilbenes (μg g^{-1} FW)									
Resveratrol	1.2 ± 0.2	2.4 ± 0.2	**				4.1 ± 0.4	2.8 ± 0.4	ns
Flavanols (μg g^{-1} FW)									
Catechin	250 ± 20	444 ± 19	**	184 ± 17	229 ± 58	ns	52 ± 12	46 ± 12	ns
Epicatechin	7.9 ± 0.5	12 ± 1	*	13 ± 2	46 ± 34	ns	14 ± 2	16 ± 3	ns
Catechin gallate	31 ± 7	35 ± 1	ns	9.2 ± 0.9	51 ± 38	ns	9.8 ± 3.2	10.0 ± 3.5	ns
Epigallocatechin gallate	6.1 ± 1.4	12 ± 0	*	2.9 ± 0.7	2.4 ± 0.5	ns	1.9 ± 0.5	1.3 ± 0.3	ns
Procyanidin B1	127 ± 14	216 ± 4	**	103 ± 9	107 ± 21	ns	48 ± 11	49 ± 10	ns
Flavonols (μg g^{-1} FW)									
Kaempferol-3-*O*-glucoside				2.5 ± 0.9	1.4 ± 0.2	ns	10 ± 1	7.2 ± 0.6	*
Kaempferol-3-*O*-galactoside				2.6 ± 1.3	2.0 ± 0.5	ns	5.6 ± 0.6	5.7 ± 0.4	ns
Myricetin							2.3 ± 0.5	3.2 ± 0.2	ns
Myricetin-3-*O*-glucoside				3.5 ± 1.5	3.5 ± 1.0	ns	53 ± 6	55 ± 2	ns
Myricetin-3-*O*-glucuronide						-	4.2 ± 0.7	4.2 ± 0.1	ns
Quercetin-3-*O*-galactoside	4.8 ± 0.6	5.2 ± 1.1	ns	5.5 ± 1.4	2.4 ± 0.1	ns	14 ± 1	10 ± 0	*
Quercetin-3-*O*-glucoside	17 ± 1	20 ± 4	ns	35 ± 9	20 ± 3	ns	81 ± 3	75 ± 4	ns
Quercetin-3-*O*-glucuronide	80 ± 5	106 ± 13	ns	79 ± 16	74 ± 9	ns	31 ± 4	26 ± 3	ns
Quercetin-3-*O*-rutinoside	11 ± 1	15 ± 3	ns	8.7 ± 1.5	7.6 ± 1.1	ns	3.5 ± 0.4	2.7 ± 0.5	ns
Isorhamnetin-3-*O*-glucoside							10 ± 1	6.6 ± 0.9	ns
Isorhamnetin-3-*O*-galactoside							25 ± 3	29 ± 2	*
Isorhamnetin-3-*O*-glucuronide	14 ± 1	18 ± 3	ns	18 ± 2	14 ± 1	ns	6.0 ± 0.7	4.5 ± 0.9	ns
Syringetin-3-*O*-glucoside							14 ± 1	12 ± 1	ns
Hydroxycinnamic acid derivatives (μg g^{-1} FW)									
Caffeoyl tartaric acid	94 ± 1	239 ± 23	*	37 ± 2	38 ± 3	ns	16 ± 2	16 ± 1	ns
Coumaroyl tartaric acid	708 ± 51	1276 ± 70	**	227 ± 34	239 ± 31	ns	87 ± 12	96 ± 7	ns
Feruloyl tartaric acid	56 ± 1	74 ± 6	ns	44 ± 2	56 ± 17	ns	11 ± 3	9.4 ± 2.4	ns
Anthocyanins (mg g^{-1} FW)									
Cyanidin-3-*O*-glucoside				16 ± 8	12 ± 3	ns	37 ± 5	41 ± 1	ns
Delphinidin-3-*O*-glucoside				26 ± 12	23 ± 7	ns	98 ± 16	106 ± 3	ns
malvidin-3-*O*-glucoside				42 ± 19	46 ± 16	ns	261 ± 45	298 ± 20	ns
Peonidin-3-*O*-glucoside				29 ± 15	24 ± 6	ns	93 ± 12	101 ± 6	ns
Petunidin-3-*O*-glucoside				20 ± 10	19 ± 6	ns	89 ± 14	103 ± 7	ns
Cyanidin-3-*O*-(6'-acetyl)glucoside							1.7 ± 0.3	1.8 ± 0.2	ns
Delphinidin-3-*O*-(6'-acetyl)glucoside							3.5 ± 0.6	4.3 ± 0.4	ns
Malvidin-3-*O*-(6'-acetyl)glucoside							24 ± 5	29 ± 3	ns
Peonidin-3-*O*-(6'-acetyl)glucoside							5.6 ± 0.9	5.9 ± 0.4	ns
Petunidin-3-*O*-(6'-acetyl)glucoside							5.6 ± 0.9	6.3 ± 0.7	ns
Cyanidin-3-*O*-(6'-p-coumaroyl)glucoside				5.6 ± 2.5	5.1 ± 1.3	ns	15 ± 2	18 ± 1	ns
Delphinidin-3-*O*-(6'-p-coumaroyl)glucoside				4.3 ± 2.0	4.4 ± 1.5	ns	26 ± 5	30 ± 3	ns
Malvidin-3-*O*-(6'-p-coumaroyl)glucoside				12 ± 5	14 ± 5	ns	140 ± 30	165 ± 16	ns
Peonidin-3-*O*-(6'-p-coumaroyl)glucoside				8.9 ± 4.0	7.9 ± 2.2	ns	37 ± 6	42 ± 4	ns
Petunidin-3-*O*-(6'-p-coumaroyl)glucoside				4.6 ± 2.0	4.8 ± 1.7	ns	33 ± 7	39 ± 4	ns

	SKIN						FLESH						SEED					
	Soluble fraction			Insoluble fraction			Soluble fraction			Insoluble fraction			Soluble fraction			Insoluble fraction		
	PAB	PAB↑	statistical significance	PAB	PAB↑	statistical significance	PAB	PAB↑	statistical significance	PAB	PAB↑	statistical significance	PAB	PAB↑	statistical significance	PAB	PAB↑	statistical significance
Stilbenes ($\mu g\ g^{-1}$ DW)																		
Resveratrol	5.9 ± 1.3	8.1 ± 1.0	ns															
Resveratrol-3-*O*- glucoside	82 ± 6	87 ± 24	ns															
Total stilbenes	88 ± 7	95 ± 25	ns															
Flavanols ($\mu g\ g^{-1}$ DW)																		
Catechin	98 ± 4	80 ± 6	ns				3.5 ± 0.1	4.4 ± 0.2	*				173 ± 6	180 ± 17	ns	9.4±0.1	12.1±1.4	ns
Epicatechin	13 ± 0	13 ± 1	ns				2.0 ± 0.1	2.1 ± 0.2	ns				113 ± 6	87.8 ± 5.5	*			
Catechin gallate							1.3 ± 0.2	1.4 ± 0.2	ns									
Epigallocatechin	11 ± 0	8.7 ± 0.5	*										342 ± 14	284 ± 27	ns			
Gallocatechin	3.0 ± 0.7	3.1 ± 0.4	ns															
Epicatechin gallate	31 ± 4	32 ± 1	ns															
Procyanidin B1	114 ± 4	110 ± 4	ns				4.9 ± 0.2	5.7 ± 0.2	ns				165 ± 13	144 ± 8	ns			
Procyanidin B2	17 ± 1	14 ± 1	ns				0.56 ± 0.02	0.58 ± 0.01	ns				72 ± 2	54 ± 5	*			
Procyanidin B3													9.4 ± 0.5	6.7 ± 0.3	**			
Total flavanols	288 ± 3	261 ± 21	ns				12 ± 0	14 ± 0	*				874 ± 79	757 ± 16	ns	9.4±0.1	12.1±1.4	ns
Flavonols ($\mu g\ g^{-1}$ DW)																		
Kaempferol	0.56 ± 0.06	0.51 ± 0.09	ns															
Kaempferol-3-*O* -glucoside	107 ± 5	102 ± 1	ns															
Myricetin	53 ± 3	65 ± 8	ns															
Myricetin-3-*O* -glucoside	1607 ± 44	1608 ± 220	ns															
Myricetin-3-*O* -glucuronide	148 ± 12	161 ± 24	ns															
Laricitrin-3-*O*- glucoside	144 ± 4	155 ± 9	ns															
Quercetin	3.8 ± 0.5	3.0 ± 0.7	ns										0.55 ± 0.07	0.25 ± 0.03	*			
Quercetin-3-*O* -glucoside	703 ± 21	714 ± 53	ns				4.4 ± 0.3	4.2 ± 1.1	ns				3.7 ± 0.3	3.3 ± 0.3	ns			
Quercetin-3-*O* -galactoside	87 ± 5	91 ± 1	ns															
Quercetin-3-*O* -glucuronide	871 ± 25	936 ± 25	ns				0.66 ± 0.15	0.64 ± 0.00	ns				34 ± 1	23 ± 0	*			
Quercetin-3-*O* -rutinoside	76.8 ± 4.1	61.4 ± 1.3	ns				0.30 ± 0.07	0.35 ± 0.10	ns				0.94 ± 0.06	0.71 ± 0.09	ns			
Isorhamnetin-3-*O* -glucoside	142 ± 3	131 ± 5	ns															
Isorhamnetin-3-*O* -glucuronide	14 ± 0	14 ± 1	ns															
Syringetin	3.4 ± 0.2	3.0 ± 0.2	ns															
Syringetin-3-*O* -glucoside	72 ± 7	51 ± 3	ns															
Total flavonols	4031 ± 120	4096 ± 367	ns				5.3 ± 0.6	5.2 ± 1.4	ns				39 ± 1	28 ± 1	**			
Hydroxybenzoic acids ($\mu g\ g^{-1}$ DW)																		
Protocatechuic acid				15 ± 1	10 ± 0	ns				1.5 ± 0.1	1.0 ± 0.4	ns				5.0±0.2	3.5±0.0	*
Gallic acid				51 ± 3	26 ± 2	*				4.9 ± 0.6	4.0 ± 0.3	ns				62±5	33±2	*
Total hydroxybenzoic acids				66 ± 2	37 ± 6	ns				6.4 ± 0.7	5.0 ± 0.4	ns				67±0	36±2	*
Hydroxycinnamic acid ($\mu g\ g^{-1}$ DW)																		
Caffeic acid				4.0 ± 0.6	2.6 ± 0.6	ns				1.1 ± 0.0	1.6 ± 0.2	*						
Coumaric acid				294 ± 21	290 ± 39	ns				1.3 ± 0.0	1.2 ± 0.0	*				15±1	13±1	ns
Ferulic acid				59 ± 2	78 ± 12	ns				0.48 ± 0.09	0.48 ± 0.04	ns						
Total hydroxycinnamic acids				357 ± 31	370 ± 47	ns				2.9 ± 0.1	3.3 ± 0.2	ns				15±1	13±1	ns
Hydroxycinnamic acid derivatives ($\mu g\ g^{-1}$ DW)																		
Caffeoyl tartaric acid	288 ± 20	432 ± 224	ns				76 ± 2	90 ± 5	ns									
Coumaroyl tartaric acid	97 ± 1	101 ± 9	ns				23 ± 1	27 ± 2	ns				2.2 ± 0.0	1.8 ± 0.1	*			
Feruloyl tartaric acid	55 ± 4	46 ± 6	ns				16 ± 0	15 ± 1	ns				118 ± 2	58 ± 9	*			
Total hydroxycinnamic acids derivatives	441 ± 19	579 ± 15	*				115 ± 3	133 ± 7	ns				129 ± 10	60 ± 2	*			
Anthocyanins (mg g-1 DW)																		
Cyanidin-3-*O* -glucoside	2.0 ± 0.1	1.9 ± 0.5	ns															
Delphinidin-3-*O* -glucoside	15 ± 2	18 ± 3	ns															
Malvidin-3-*O* -glucoside	44 ± 10	54 ± 9	ns															
Peonidin-3-*O* -glucoside	5.9 ± 0.5	5.7 ± 1.0	ns															
Petunidin-3-*O* -glucoside	15 ± 3	18 ± 3	ns															
Cyanidin-3-*O* -(6'-acetyl)glucoside	0.08 ± 0.01	0.09 ± 0.00	ns															
Delphinidin-3-*O* -(6'-acetyl)glucoside	0.18 ± 0.01	0.17 ± 0.01	ns															
Malvidin-3-*O* -(6'-acetyl)glucoside	10 ± 3	12 ± 1	ns															
Peonidin-3-*O* -(6'-acetyl)glucoside	0.22 ± 0.01	0.21 ± 0.01	ns															
Petunidin-3-*O* -(6'-acetyl)glucoside	0.34 ± 0.03	0.31 ± 0.02	ns															
Cyanidin-3-*O* -(6'-p-coumaroyl)glucoside	0.04 ± 0.00	0.04 ± 0.00	ns															
Delphinidin-3-*O* -(6'-p-coumaroyl)glucoside	0.90 ± 0.04	0.89 ± 0.04	ns															
Malvidin-3-*O* -(6'-p-coumaroyl)glucoside	30 ± 7	38 ± 6	ns															
Peonidin-3-*O* -(6'-p-coumaroyl)glucoside	4.7 ± 0.7	6.0 ± 0.7	ns															
Petunidin-3-*O* -(6'-p-coumaroyl)glucoside	5.0 ± 0.5	5.1 ± 0.3	ns															
Total anthocyanins	134 ± 22	161 ± 2	ns															

Table 7.11. Effect of enhanced UV-B (comparison between PAB and PAB↑ regimes) on individual phenolic compounds from the methanol-soluble and methanol-insoluble fractions of the three components of grapes (skin, flesh and seeds) at harvest. For each fraction of each component, the statistical significance of a Student's t test comparing both radiation regimes is shown. Means ± SE are shown. DW, dry weight. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant.

Table 7.12. Effect of enhanced UV-B (comparison between PAB and PAB↑ regimes) on the relative abundance (percentages) of individual volatile organic compounds (VOCs) in the ensemble of skin and flesh at harvest. For each compound, the statistical significance of a Student's t test comparing both radiation regimes is shown. Means ± SE are shown. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant.

Volatile organic compounds (VOCs)	PAB	PAB↑	statistical significance
alcohols			
1-butanol	0.03 ± 0.00	0.02 ± 0.00	*
3-buten-2-ol-2methyl	0.04 ± 0.00	0.05 ± 0.00	ns
1-decanol	15 ± 8	13 ± 2	ns
ethylhexanol	0.06 ± 0.01	2.4 ± 0.1	***
2 heptanol n	0.03 ± 0.01	0.03 ± 0.00	ns
1-hexanol	3.1 ± 0.4	4.7 ± 0.4	ns
(E)-2-hexen-1-ol	3.1 ± 0.4	2.5 ± 0.4	ns
2-methyl-2-propanol	0.02 ± 0.00	0.04 ± 0.01	*
1-octanol	0.09 ± 0.05	0.13 ± 0.00	ns
1-pentanol	0.06 ± 0.01	0.06 ± 0.01	ns
1-penten-3-ol	0.09 ± 0.02	0.08 ± 0.00	ns
(Z)-2-penten-1-ol n	0.07 ± 0.01	0.04 ± 0.01	ns
2-phenylethanol	0.05 ± 0.00	0.05 ± 0.00	ns
ketones			
3-hexen-2-one	1.0 ± 0.2	1.2 ± 0.1	ns
4-methyl-2-heptanone	0.06 ± 0.01	0.03 ± 0.00	ns
6-methyl-5-hepten-2-one	0.06 ± 0.01	0.06 ± 0.00	ns
2-pentanone	0.06 ± 0.01	0.09 ± 0.01	ns
hydrocarbons			
dodecane n	0.10 ± 0.02	0.08 ± 0.00	ns
heptane, 2, 2, 4, 6, 6-pentamethyl	0.02 ± 0.00	1.1 ± 0.1	**
hexane	0.03 ± 0.01	0.02 ± 0.00	ns
propane, 2-methoxy-2-methyl	0.03 ± 0.01	0.05 ± 0.01	ns
tridecane n	0.05 ± 0.01	0.05 ± 0.02	ns
aldehydes			
benzaldehyde	0.03 ± 0.00	0.19 ± 0.01	***
decanal	0.10 ± 0.02	0.08 ± 0.01	ns
(E,E)-2,4-heptadienal	0.16 ± 0.02	0.07 ± 0.01	**
heptanal	0.05 ± 0.01	0.06 ± 0.00	ns
(E)-2-heptenal	1.3 ± 0.2	1.2 ± 0.0	ns
(E,E)-2,4-hexadienal	0.06 ± 0.01	0.07 ± 0.00	ns
hexanal	19 ± 3	18 ± 1	ns
(Z)-3-hexenal	3.7 ± 0.8	4.1 ± 0.1	ns
(E)-2-hexenal	43 ± 5	35 ± 1	ns
n-nonaldehyde	0.39 ± 0.05	0.26 ± 0.00	ns
octanal	0.09 ± 0.01	0.08 ± 0.00	ns
(E)-2-octenal	0.09 ± 0.01	0.08 ± 0.00	ns
pentanal	0.03 ± 0.00	0.03 ± 0.00	ns
phenylacetaldehyde	0.07 ± 0.01	0.04 ± 0.01	*

Table 7.13. Global variables (color, total polyphenol index, total phenols, bulk levels of UV-absorbing compounds (UVAC) and antioxidant capacity) and individual phenolic compounds in wines elaborated from grapes exposed to ambient (PAB regime) or enhanced (PAB↑ regime) UV-B. For each variable, the statistical significance of a Student's t test comparing both radiation regimes is shown. Means ± SE are shown. GAE, gallic acid equivalent. TE, Trolox equivalent. $AUC_{280–315}$ and $AUC_{280–400}$, area under the absorbance curve in the intervals 280–315 and 280-400 nm, respectively. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant.

	PAB	PAB↑	statistical significance
Color intensity (CI)	14 ± 0	13 ± 0	ns
Hue	0.76 ± 0.76	0.72 ± 0.03	ns
Antioxidant capacity and phenolic compounds			
Total polyphenol index (TPI)	54 ± 0	50 ± 3	ns
Total phenols (GAE, g L^{-1})	2.6 ± 0.1	2.5 ± 0.1	ns
Antioxidant capacity (mM TE)	23 ± 1	22 ± 1	ns
UVAC ($AUC_{280-315}$)	1394 ± 5	1281 ± 80	ns
UVAC ($AUC_{280-400}$)	2655 ± 16	2390 ± 155	ns
Stilbenes (mg L^{-1})			
Resveratrol	0.08 ± 0.01	0.12 ± 0.00	*
Resveratrol-3-*O*-glucoside	1.5 ± 0.1	1.7 ± 0.2	ns
Flavanols (mg L^{-1})			
Catechin	11 ± 1	11 ± 1	ns
Epicatechin	5.6 ± 0.9	5.0 ± 0.4	ns
Gallocatechin	1.2 ± 0.2	1.3 ± 0.2	ns
Epigallocatechin	13 ± 1	14 ± 2	ns
Catechin gallate	0.05 ± 0.02	0.11 ± 0.02	ns
Procyanidin B1	7.5 ± 0.4	8.2 ± 0.7	ns
Procyanidin B2	2.3 ± 0.3	2.2 ± 0.3	ns
Flavonols (mg L^{-1})			
Kaempferol	0.72 ± 0.18	0.68 ± 0.01	ns
Kaempferol-3-*O*-glucoside	0.14 ± 0.03	0.11 ± 0.04	ns
Kaempferol-3-*O*-glucuronide	0.03 ± 0.00	0.04 ± 0.01	ns
Myricetin	6.0 ± 0.7	3.4 ± 1.0	ns
Myricetin-3-*O*-glucoside	4.4 ± 0.4	4.6 ± 0.4	ns
Myricetin-3-*O*-glucuronide	1.8 ± 0.1	1.9 ± 0.2	ns
Laricitrin	0.66 ± 0.05	0.53 ± 0.04	ns
Laricitrin-3-*O*-glucoside	0.84 ± 0.03	1.2 ± 0.1	ns
Quercetin	4.2 ± 0.8	2.5 ± 0.5	ns
Quercetin-3-*O*-galactoside	13 ± 2	14 ± 1	ns
Quercetin-3-*O*-glucoside	13 ± 2	12 ± 1	ns
Quercetin-3-*O*-glucuronide	4.5 ± 0.3	5.0 ± 0.8	ns
Isorhamnetin-3-*O*-glucoside	0.11 ± 0.01	0.10 ± 0.01	ns
Isorhamnetin-3-*O*-glucuronide	1.7 ± 0.3	1.5 ± 0.2	ns
Syringetin	0.16 ± 0.03	0.17 ± 0.01	ns
Syringetin-3-*O*-glucoside	2.5 ± 0.3	2.3 ± 0.2	ns

	PAB	PAB↑	statistical significance
Hydroxybenzoic acids (mg L^{-1})			
Protocatechuic acid	0.73 ± 0.14	0.63 ± 0.03	ns
Gallic acid	12 ± 1	11 ± 0	ns
Hydroxycinnamic acid derivatives (mg L^{-1})			
Caffeoyl tartaric acid	35 ± 1	36 ± 2	ns
Coumaroyl tartaric acid	12 ± 1	16 ± 2	ns
Caffeic acid ethyl ester	0.80 ± 0.10	1.5 ± 0.4	ns
Anthocyanins (mg L^{-1})			
Cyanidin-3-*O*-glucoside	1.8 ± 0.2	2.1 ± 0.2	ns
Delphinidin-3-*O*-glucoside	48 ± 7	73 ± 10	ns
Malvidin-3-*O*-glucoside	210 ± 33	295 ± 44	ns
Peonidin-3-*O*-glucoside	12 ± 1	15 ± 0	ns
Petunidin-3-*O*-glucoside	66 ± 7	78 ± 11	ns
Cyanidin-3-*O*-(6'-acetyl)glucoside	1.8 ± 0.0	2.0 ± 0.4	ns
Delphinidin-3-*O*-(6'-acetyl)glucoside	1.4 ± 0.1	1.6 ± 0.1	ns
Malvidin-3-*O*-(6'-acetyl)glucoside	28 ± 5	30 ± 3	ns
Peonidin-3-*O*-(6'-acetyl)glucoside	1.2 ± 0.0	1.3 ± 0.1	ns
Petunidin-3-*O*-(6'-acetyl)glucoside	3.7 ± 0.2	4.5 ± 0.6	ns
Cyanidin-3-*O*-(6'-p-coumaroyl)glucoside	4.6 ± 0.2	4.5 ± 0.7	ns
Delphinidin-3-*O*-(6'-p-coumaroyl)glucoside	14 ± 2	15 ± 4	ns
Malvidin-3-*O*-(6'-p-coumaroyl)glucoside	29 ± 7	27 ± 3	ns
Peonidin-3-*O*-(6'-p-coumaroyl)glucoside	1.4 ± 0.1	1.3 ± 0.1	ns
Petunidin-3-*O*-(6'-p-coumaroyl)glucoside	3.3 ± 0.5	3.3 ± 0.8	ns

Table 7.14. Relative abundance (percentages) of individual volatile organic compounds (VOCs) in wines elaborated from grapes exposed to ambient (PAB regime) or enhanced (PAB↑ regime) UV-B. For each compound, the statistical significance of a Student's t test comparing both radiation regimes is shown. Means ± SE are shown. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant.

Volatile organic compounds (VOCs)	PAB	PAB↑	statistical significance
Alcohols			
2-methyl-1-butanol	20 ± 2	34 ± 2	*
3-methyl butanol	21 ± 1	14 ± 0	*
1-hexanol	1.2 ± 0.1	1.0 ± 0.1	ns
Phenyl ethyl alcohol	25 ± 2	22 ± 1	ns
Hydrocarbons			
Tridecane	0.02 ± 0.00	0.02 ± 0.00	ns
Tetradecane	0.05 ± 0.00	0.06 ± 0.01	ns
Esters			
Acetic acid, 2-phenylethyl ester	0.12 ± 0.00	0.11 ± 0.01	ns
Diethyl succinate	0.79 ± 0.14	0.55 ± 0.04	ns
Ethyl acetate	6.0 ± 0.9	4.3 ± 0.5	ns
Ethyl butanoate	0.35 ± 0.03	0.34 ± 0.04	ns
Ethyl decanoate	1.1 ± 0.1	1.7 ± 0.3	ns
Ethyl dodecanoate	0.26 ± 0.01	0.20 ± 0.03	ns
Ethyl heptanoate	0.09 ± 0.01	0.06 ± 0.01	ns
Ethyl hexanoate	7.2 ± 0.5	6.2 ± 0.9	ns
Ethyl hexyl salycilate	0.07 ± 0.01	0.07 ± 0.01	ns
Ethyl nonanoate	1.3 ± 0.2	0.63 ± 0.02	ns
Ethyl octanoate	10 ± 1	13 ± 2	ns
Hexadecanoic acid, ethyl ester	2.7 ± 0.6	2.1 ± 1.8	ns
2-methylbutyl acetate	0.21 ± 0.01	0.16 ± 0.02	ns
3-methylbutyl acetate	0.91 ± 0.06	0.68 ± 0.11	ns
Methyl hexanoate	0.04 ± 0.00	0.02 ± 0.01	ns
Methyl octanoate	0.11 ± 0.02	0.18 ± 0.01	ns
Octanoic acid, 3-methylbutyl ester	0.04 ± 0.00	0.05 ± 0.01	ns
Tetradecanoic acid ethyl ester	0.10 ± 0.02	0.05 ± 0.00	ns
Acids			
Ethanoic acid	0.83 ± 0.30	0.50 ± 0.05	ns
Hexanoic acid	0.59 ± 0.05	0.60 ± 0.08	ns
Nonanoic acid	0.12 ± 0.01	0.31 ± 0.05	ns
Octanoic acid	0.45 ± 0.05	0.57 ± 0.11	ns
Other compounds			
Hydroxybutyric acid lactone	0.03 ± 0.00	0.02 ± 0.00	ns
n-nonaldehyde	0.09 ± 0.01	0.05 ± 0.00	**

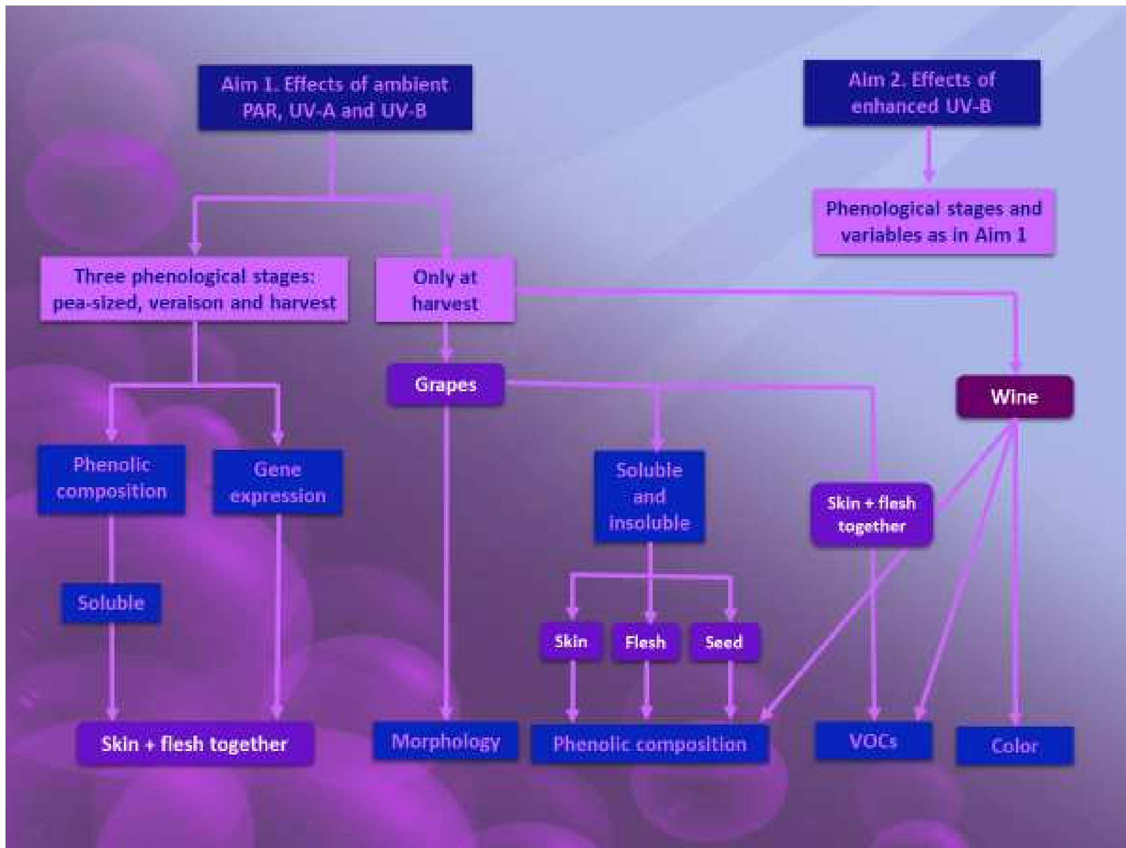


Figure 7.1. Schematic representation of the experiment performed, showing the aims and UV wavelengths used (dark blue background), the variables measured (blue background, differentiating the cell fractions, methanol-soluble and –insoluble, from which phenolic compounds were extracted), the phenological stages (lilac background), and the grape components (purple background) in which measurements were taken. Appropriate variables were also measured in wine (red garnet background).

Figure 7.2. Experimental set-up at Universidad de La Rioja, which consisted of 18 blocks arranged in three parallel rows. Transparent specific cut-off filters covering each block and white shading mesh in the gaps between blocks can be seen. Two grapevine plants were placed in pots in each block.

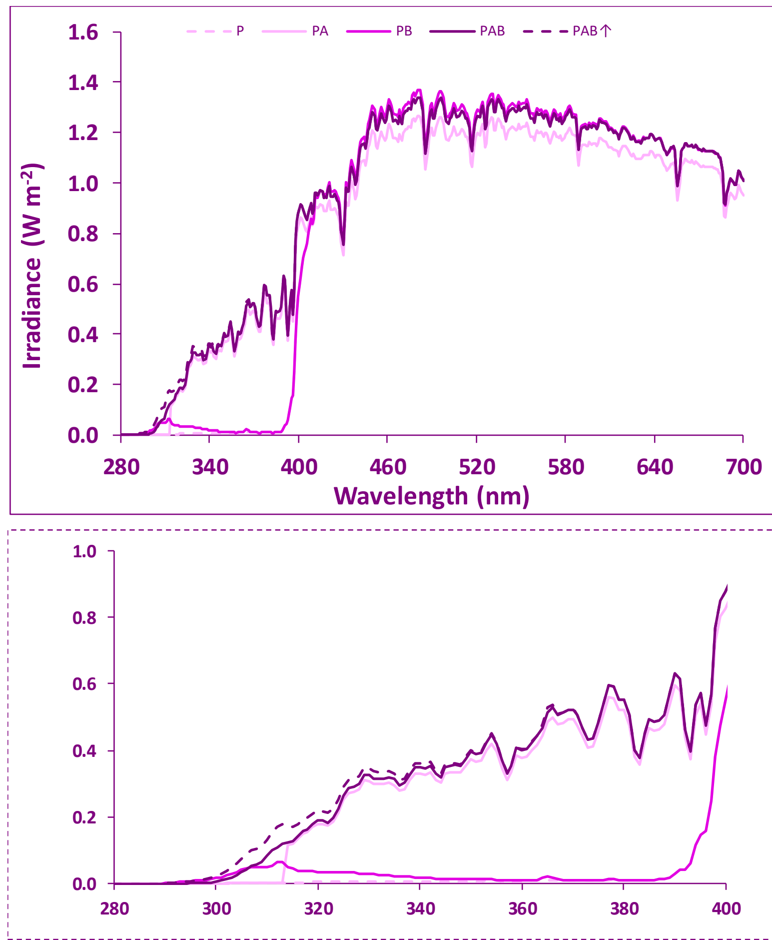


Figure 7.3. Spectral irradiances in the wavelength interval 280-700 nm (top), and an amplified detail in the UV-B plus UV-A interval (280-400 nm, bottom) measured in the different radiation regimes (P, PA, PB, PAB and PAB↑) imposed in the experiment (see Materials and Methods for a description of each regime).

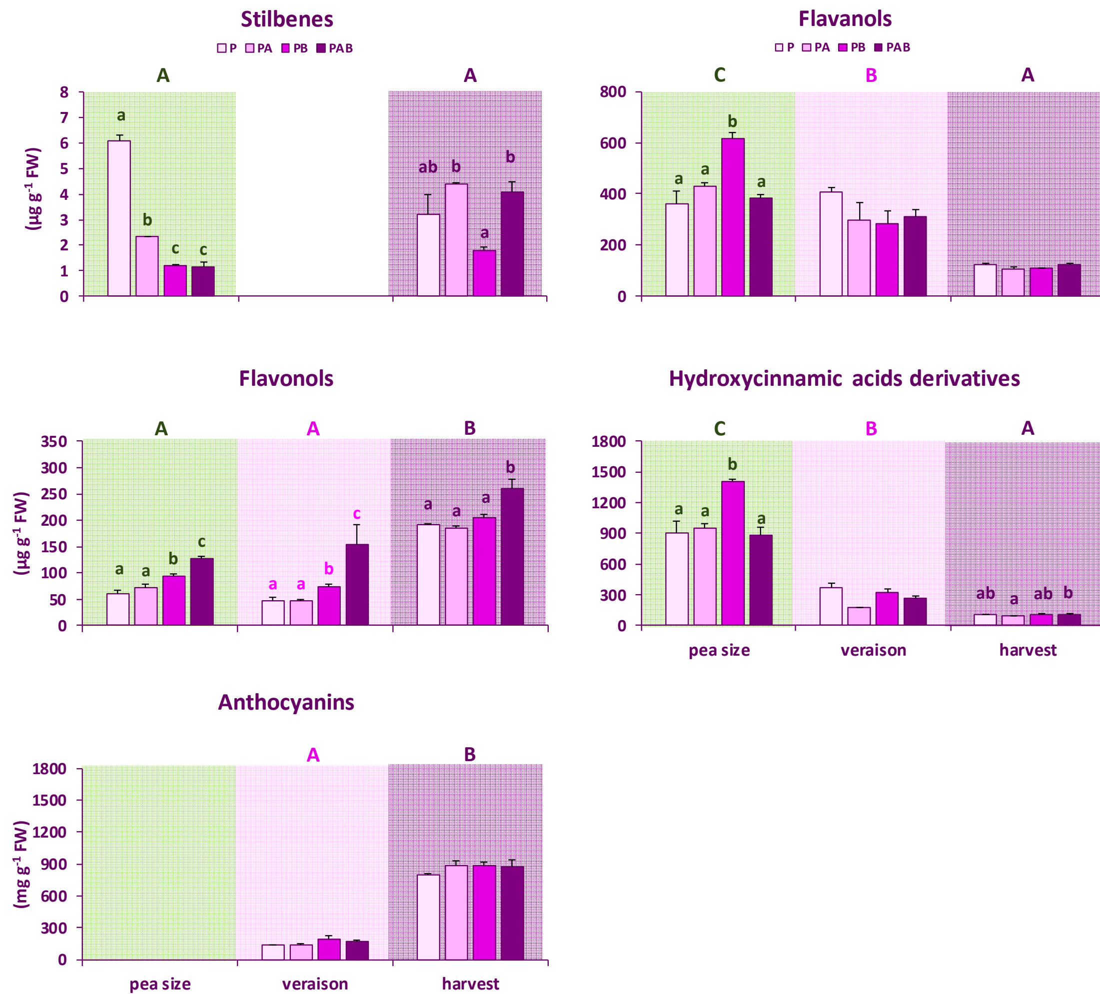


Figure 7.4. Families of phenolic compounds of grapes in three phenological stages (pea size, veraison and harvest) and four radiation regimes (P, PA, PB and PAB), measured in the soluble fraction of the ensemble of skin and flesh. Different capital letters show significant differences between phenological stages, and different lower-case letters between radiation regimes for each phenological stage (results of post-hoc Tukey's tests after a two-way ANOVA test using time (phenological stage) and treatment (radiation regime) as main factors). Means ± SE are shown. FW, fresh weight.

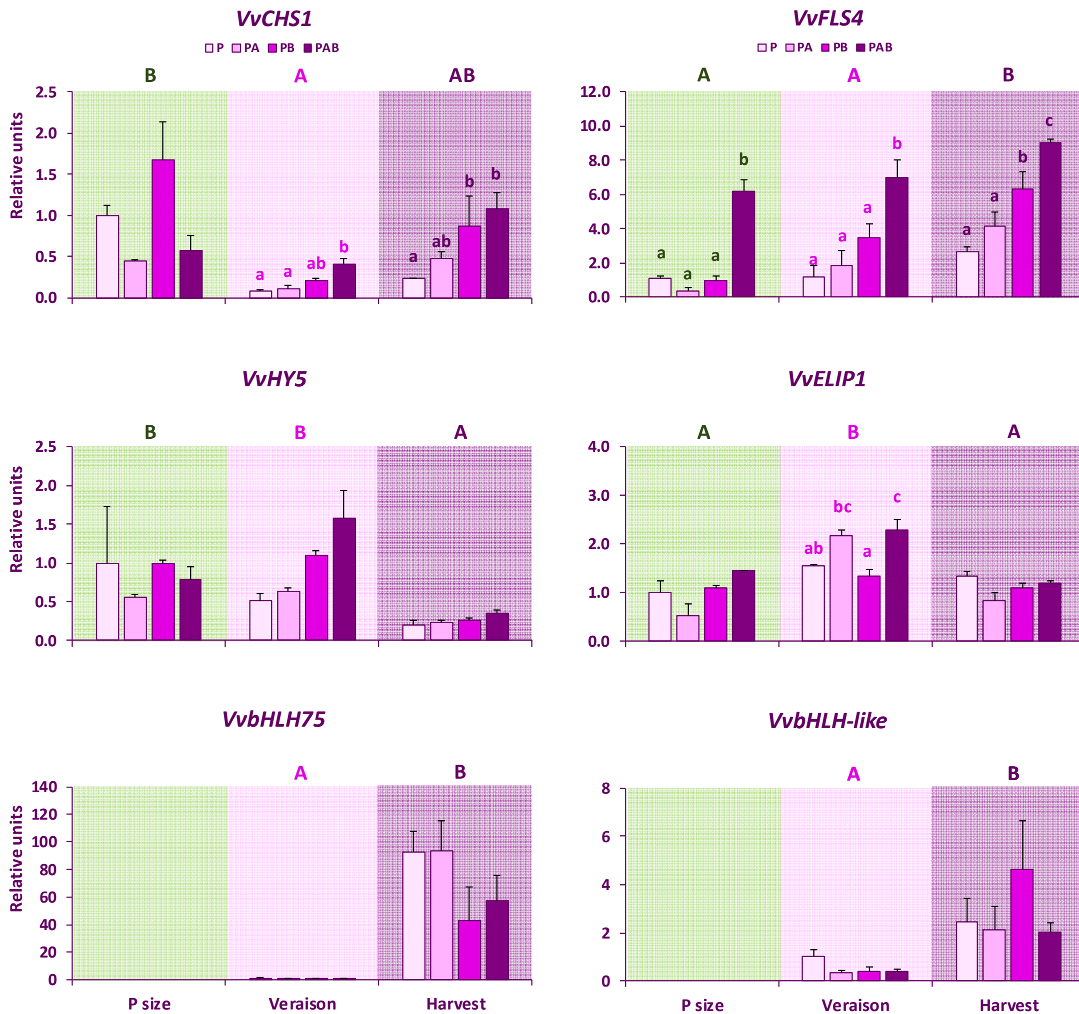


Figure 7.5. Relative expression of the specified genes (see Table 7.1) of grapes in three phenological stages (pea size, veraison and harvest) and four radiation regimes (P, PA, PB and PAB), measured in the ensemble of skin and flesh. Gene expression is shown in relative units, taking the expression in P regime in pea size samples as the unit (in absence of it, the expression in P regime in veraison samples would be the unit). Different capital letters show significant differences between phenological stages, and different lower-case letters between radiation regimes for each phenological stage (results of post-hoc Tukey's tests after a two-way ANOVA test using time (phenological stage) and treatment (radiation regime) as main factors). Means ± SE are shown.

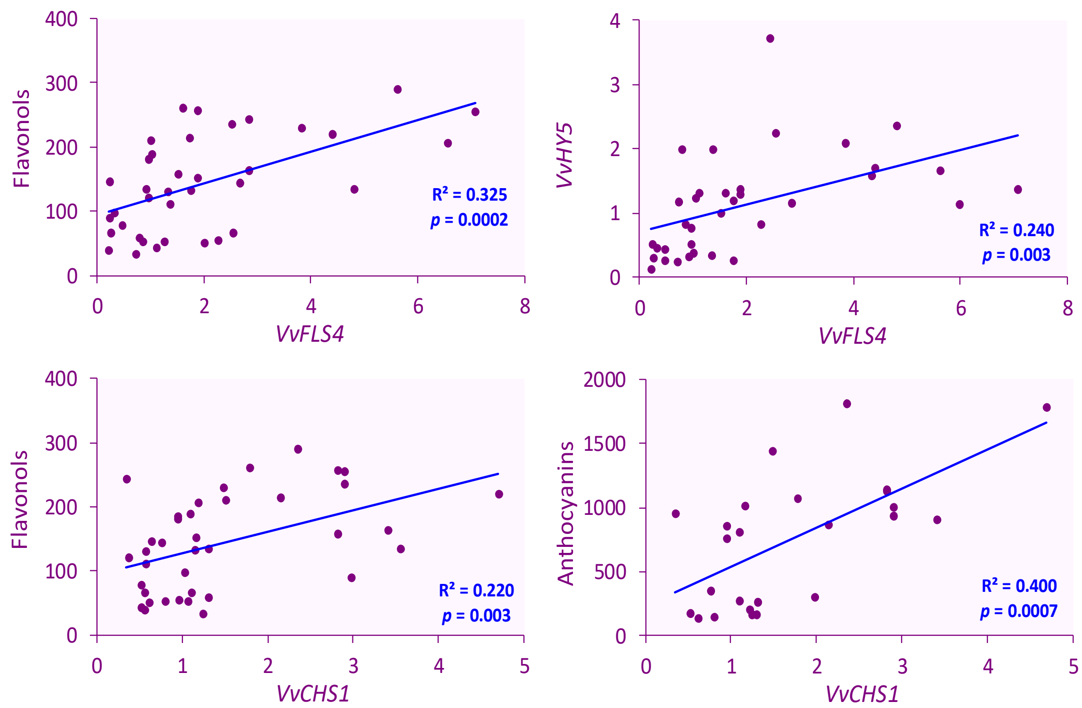


Figure 7.6. Selected regressions between the contents of specific phenolic families and gene expression of particular genes (see Table 7.1). The coefficients of determination (R^2) and probability levels are indicated. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant.

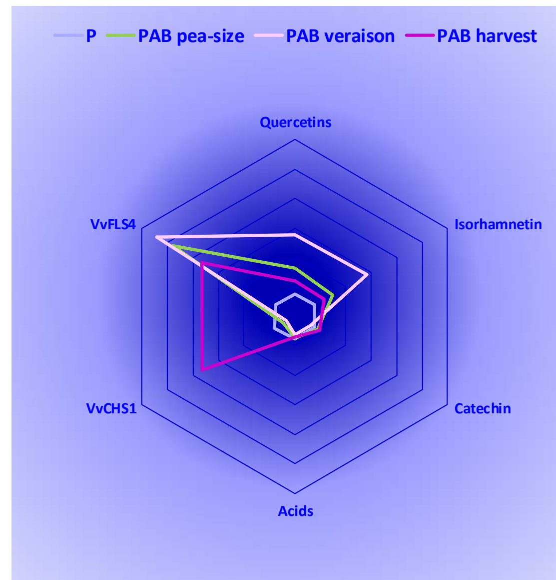


Figure 7.7. Intensity of response to UV radiation (comparison of P and PAB regimes) of the phenolic compounds and genes that were found to be the most UV-responsive, in the three phenological stages studied (pea size, veraison and harvest). Each vertex represent one variable, and the values of the each variable increase from the polygon center to its respective vertex.

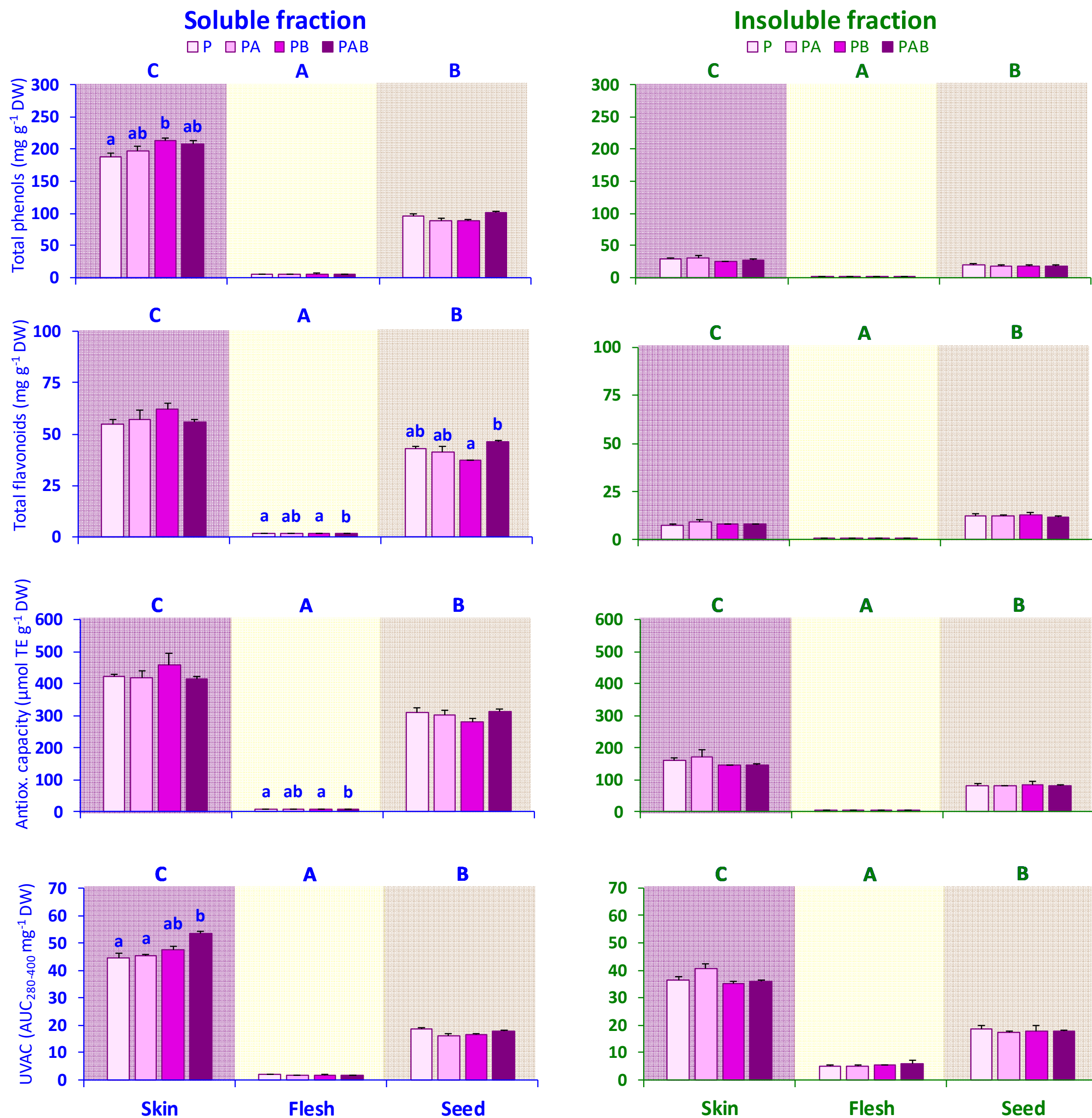


Figure 7.8. Global variables (total phenols, total flavonoids, antioxidant capacity, and bulk levels of UV-absorbing compounds (UVAC)) from the methanol-soluble and methanol-insoluble fractions of the three components of grapes (skin, flesh and seeds) in four radiation regimes (P, PA, PB and PAB) at harvest. Different capital letters show significant differences between grape components, and different lower-case letters between radiation regimes for each component (results of post-hoc Tukey's tests after a two-way ANOVA test using grape components and radiation regimes as main factors). Means ± SE are shown. DW, dry weight. TE, Trolox equivalent. $AUC_{280–400}$, area under the absorbance curve in the interval 280-400 nm.

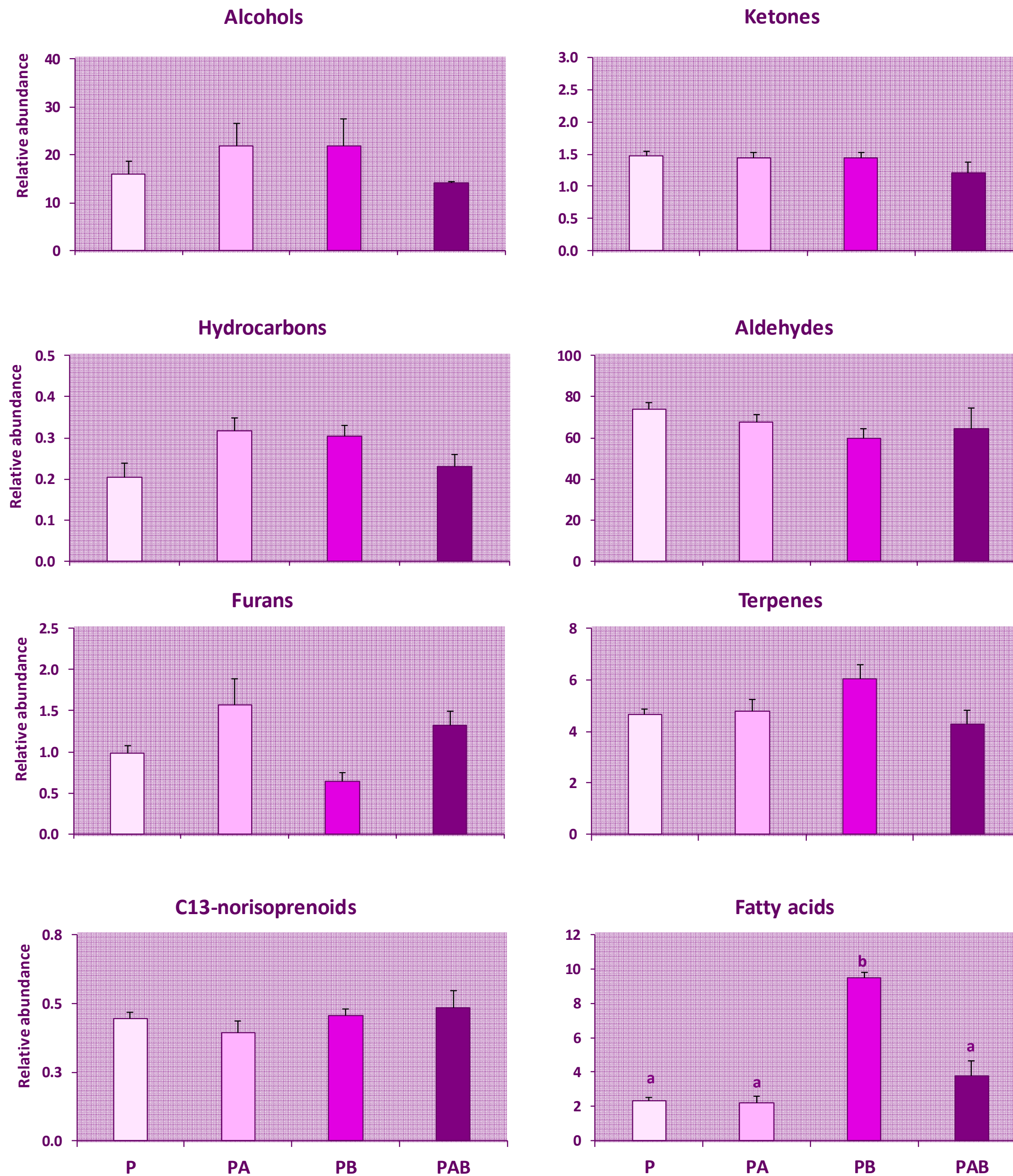


Figure 7.9. Relative abundance (percentages) of families of volatile organic compounds (VOCs) in the ensemble of skin and flesh in four radiation regimes (P, PA, PB and PAB) at harvest. For each variable, different letters mean significant differences between radiation regimes (post-hoc Tukey's test after a one-way ANOVA test using radiation regime as main factor). Means ± SE are shown.

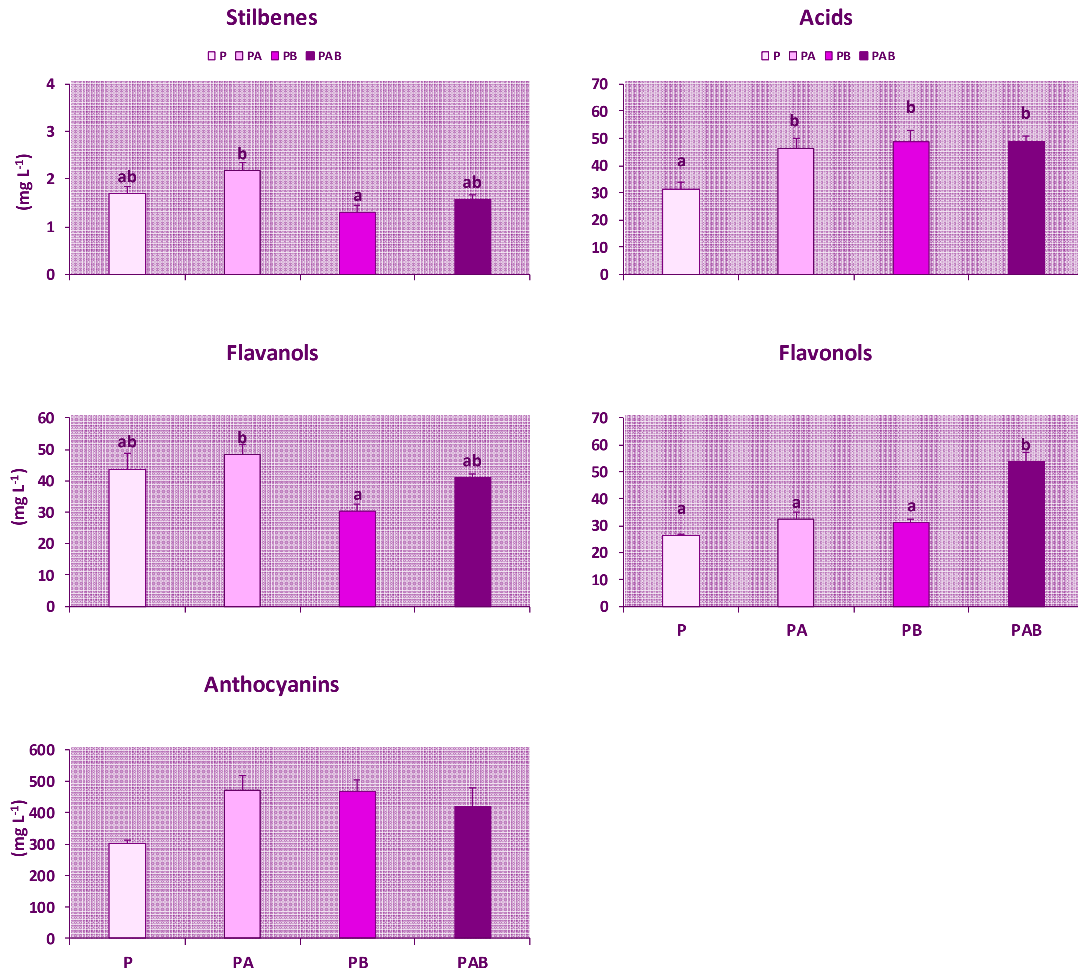


Figure 7.10. Contents of phenolic families in wines elaborated from grapes exposed to four radiation regimes (P, PA, PB and PAB). For each variable, different letters mean significant differences between radiation regimes (post-hoc Tukey's test after a one-way ANOVA test using radiation regime as main factor). Means ± SE are shown.

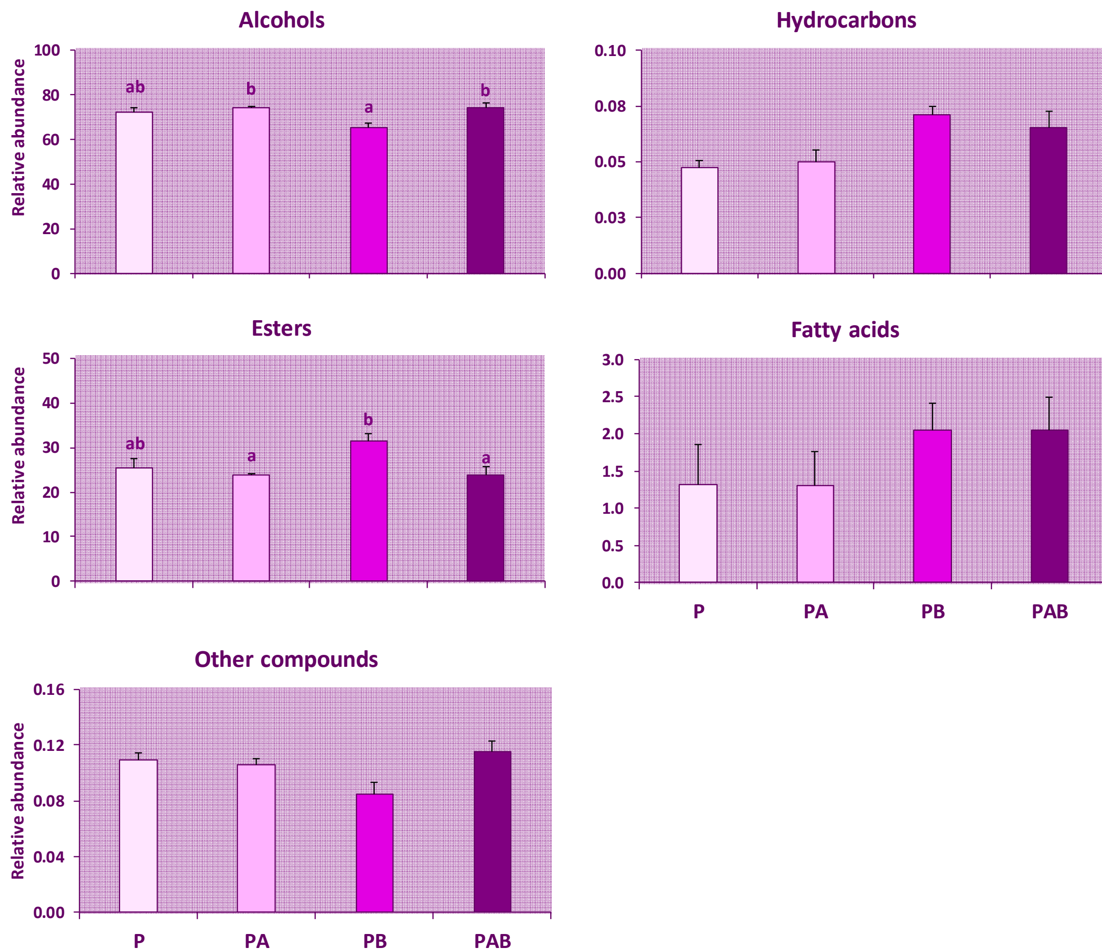


Figure 7.11. Relative abundance (percentages) of families of volatile organic compounds (VOCs) in wines elaborated from grapes exposed to four radiation regimes (P, PA, PB and PAB). For each variable, different letters mean significant differences between radiation regimes (post-hoc Tukey's test after a one-way ANOVA test using radiation regime as main factor). Means ± SE are shown.

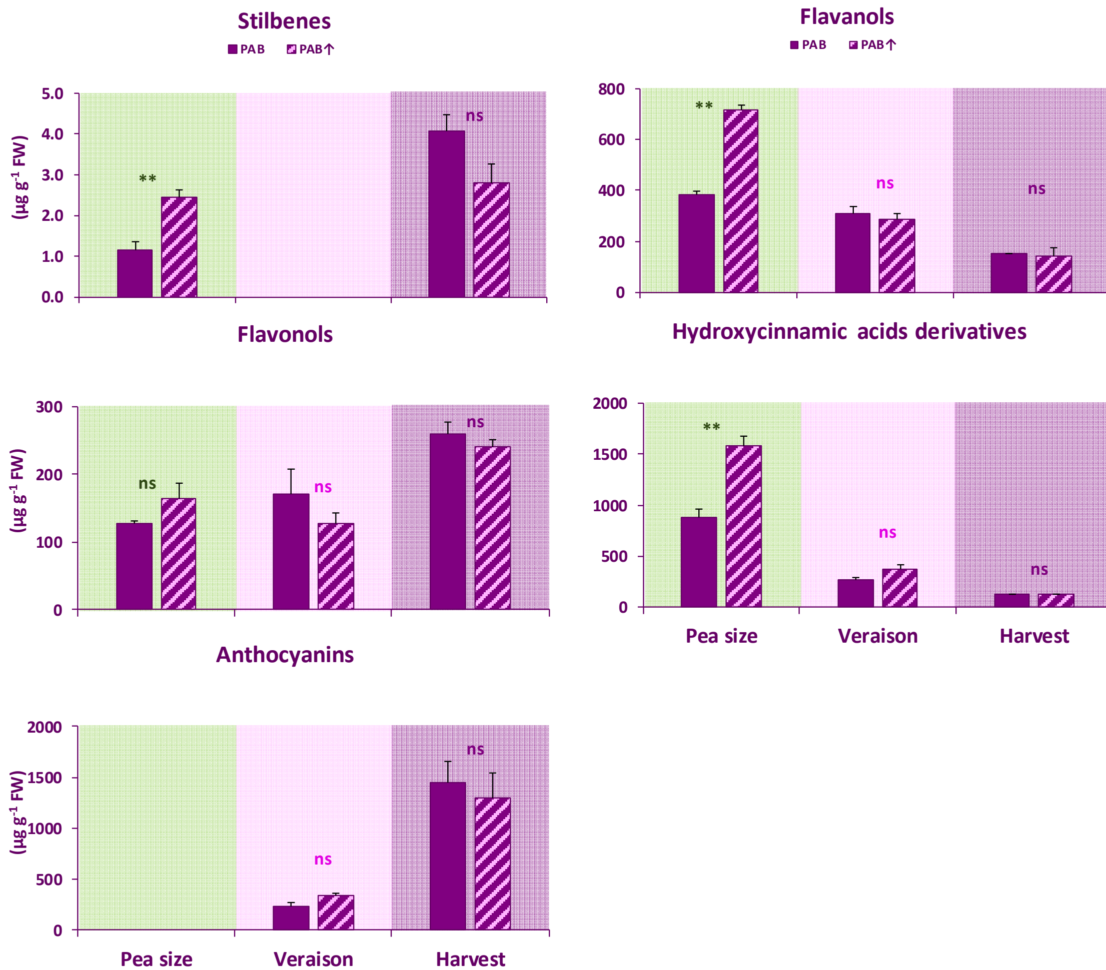


Figure 7.12. Effect of enhanced UV-B (comparison between PAB and PAB↑ regimes) on the contents of phenolic families of grapes in three phenological stages (pea size, veraison and harvest). Variables were measured in the soluble fraction of the ensemble of skin and flesh. For each variable and phenological stage, the statistical significance of a Student's t test comparing both radiation regimes is shown. Means ± SE are shown. FW, fresh weight. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant.

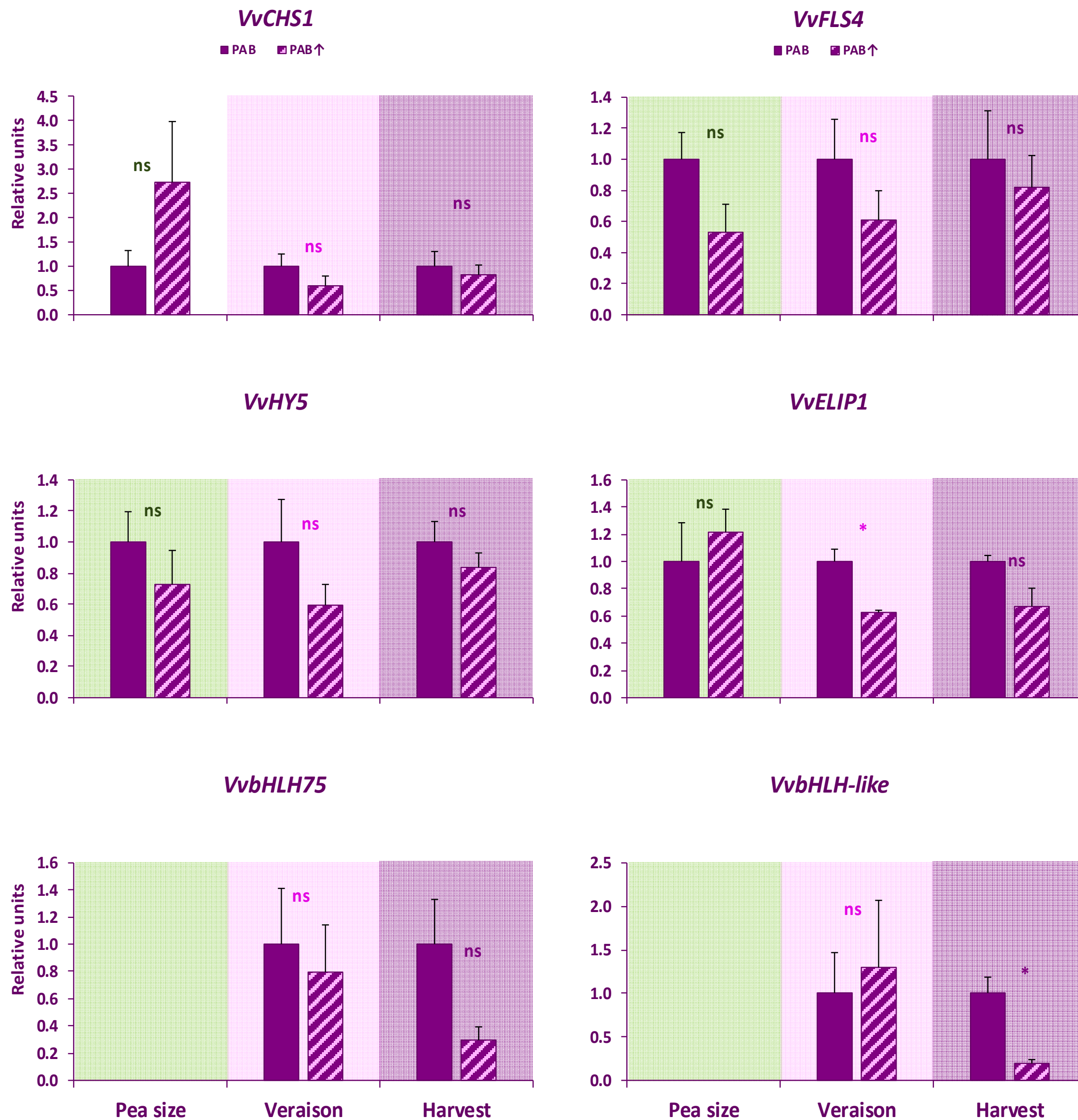


Figure 7.13. Effect of enhanced UV-B (comparison between PAB and PAB↑ regimes) on the relative expression of the specified genes (see Table 7.1) of grapes in three phenological stages (pea size, veraison and harvest), measured in the ensemble of skin and flesh. Gene expression is shown in relative units, taking the expression in PAB regime as the unit. For each variable and phenological stage, the statistical significance of a Student's t test comparing both radiation regimes is shown. Means ± SE are shown. *, $p < 0.05$; ns, not significant.

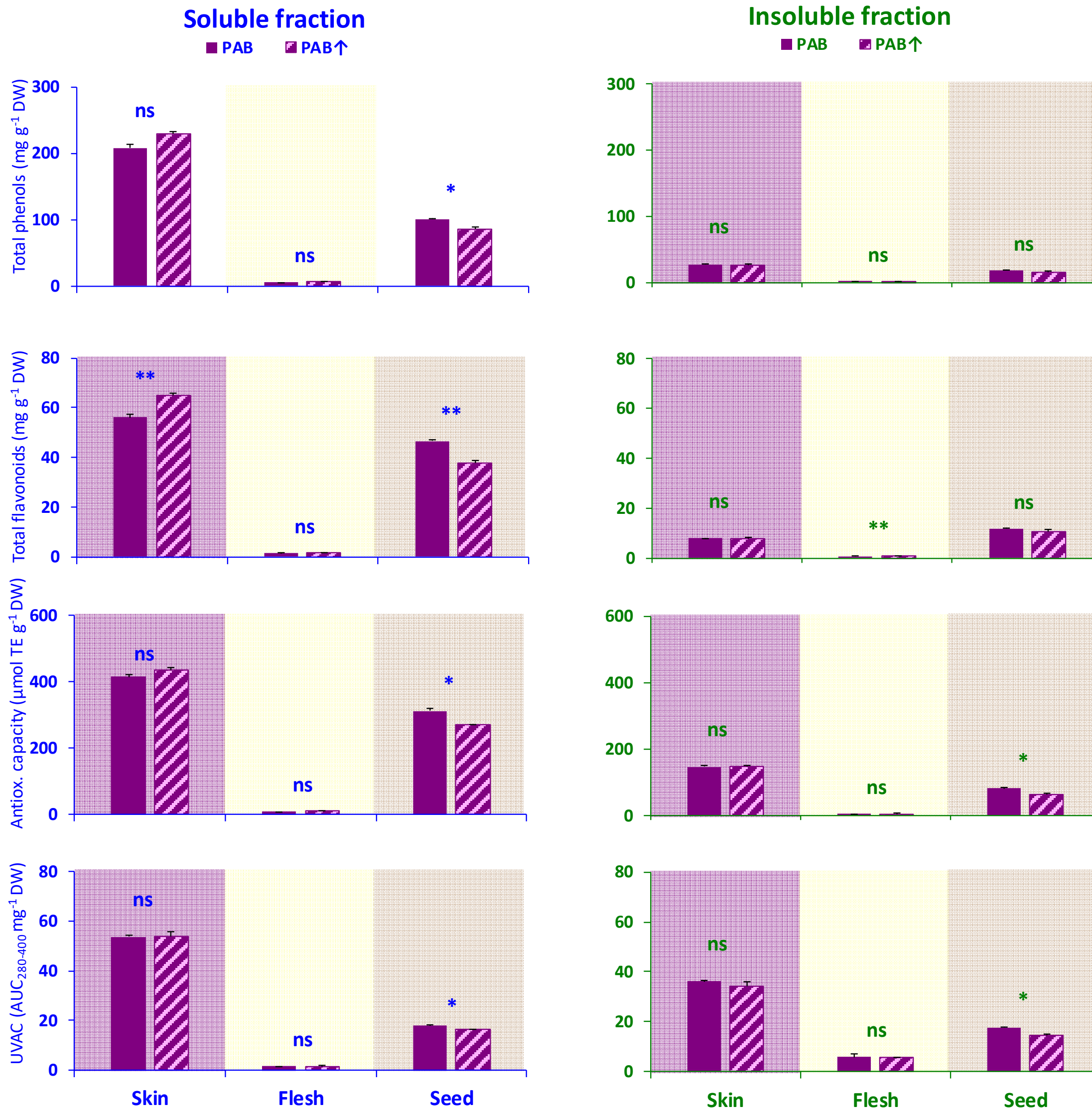


Figure 7.14. Effect of enhanced UV-B (comparison between PAB and PAB↑ regimes) on global variables (total phenols, total flavonoids, antioxidant capacity, and bulk levels of UV-absorbing compounds (UVAC)) from the methanol-soluble and methanol-insoluble fractions of the three components of grapes (skin, flesh and seeds) at harvest. For each fraction of each component, the statistical significance of a Student's t test comparing both radiation regimes is shown. Means ± SE are shown. DW, dry weight. TE, Trolox equivalent. **, $p < 0.01$; *, $p < 0.05$; ns, not significant; n.d., not detected.

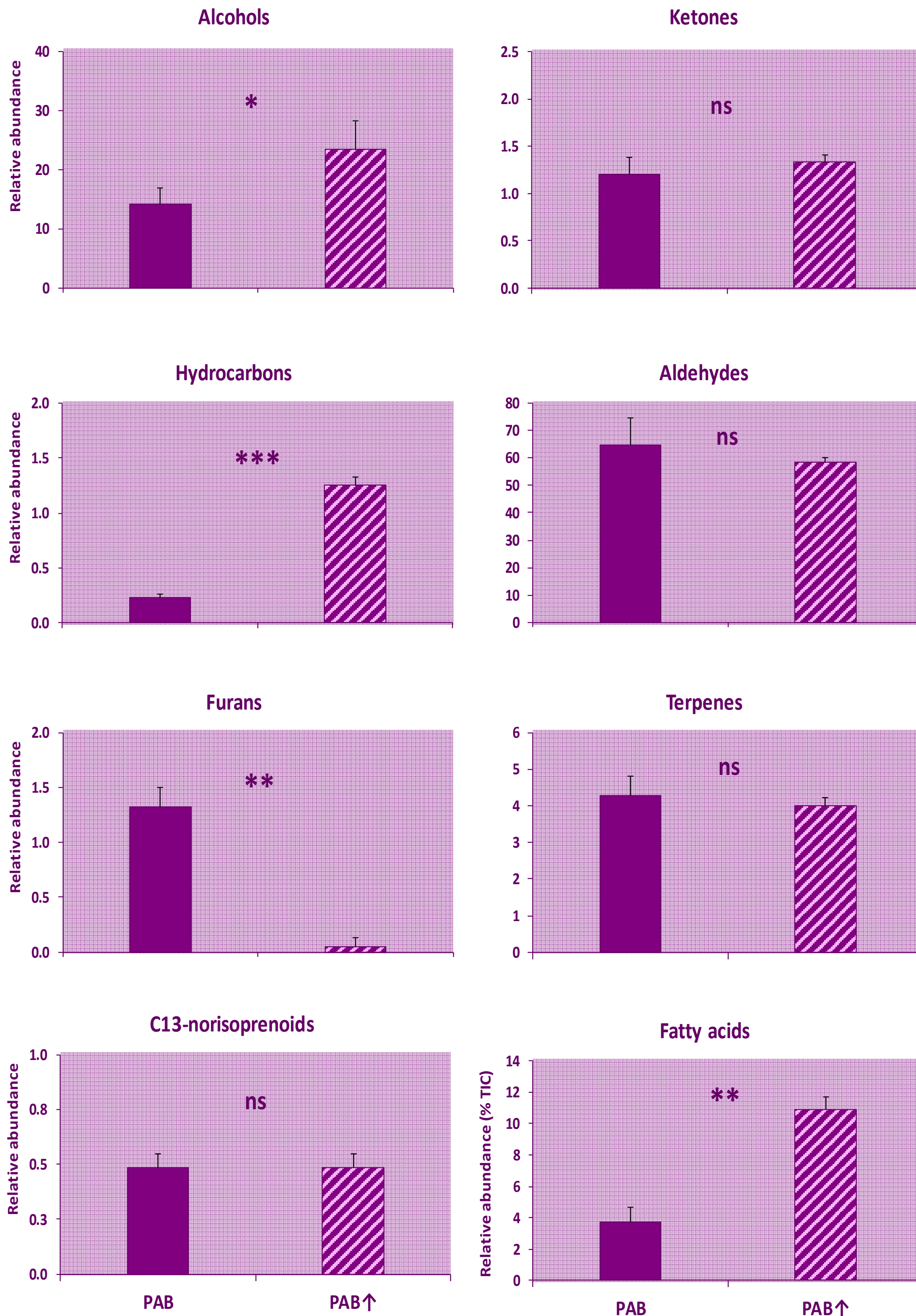


Figure 7.15. Effect of enhanced UV-B (comparison between PAB and PAB↑ regimes) on the relative abundance (percentages) of families of volatile organic compounds (VOCs) in the ensemble of skin and flesh at harvest. For each variable, the statistical significance of a Student's t test comparing both radiation regimes is shown. Means ± SE are shown. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant.

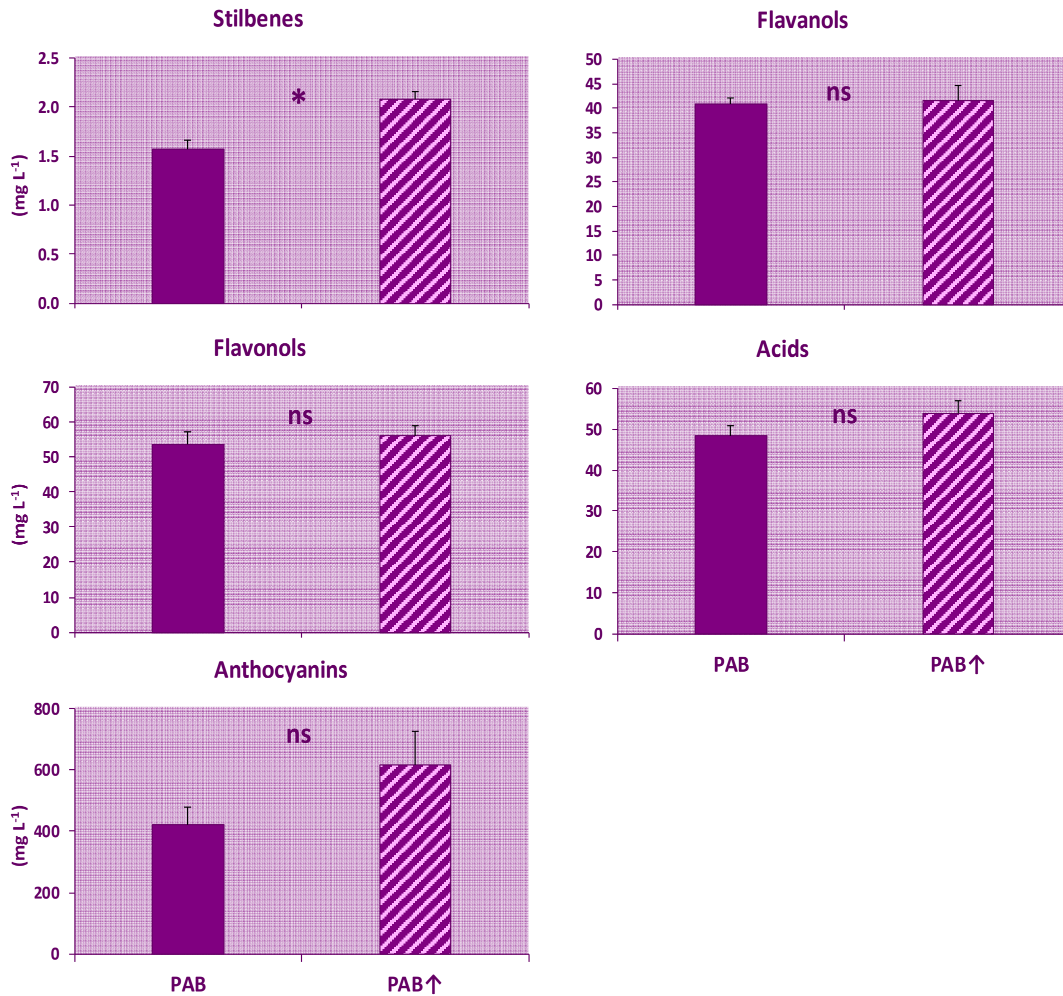


Figure 7.16. Contents of phenolic families in wines elaborated from grapes exposed to ambient (PAB regime) or enhanced (PAB↑ regime) UV-B. For each variable, the statistical significance of a Student's t test comparing both radiation regimes is shown. Means ± SE are shown. *, $p < 0.05$; ns, not significant.

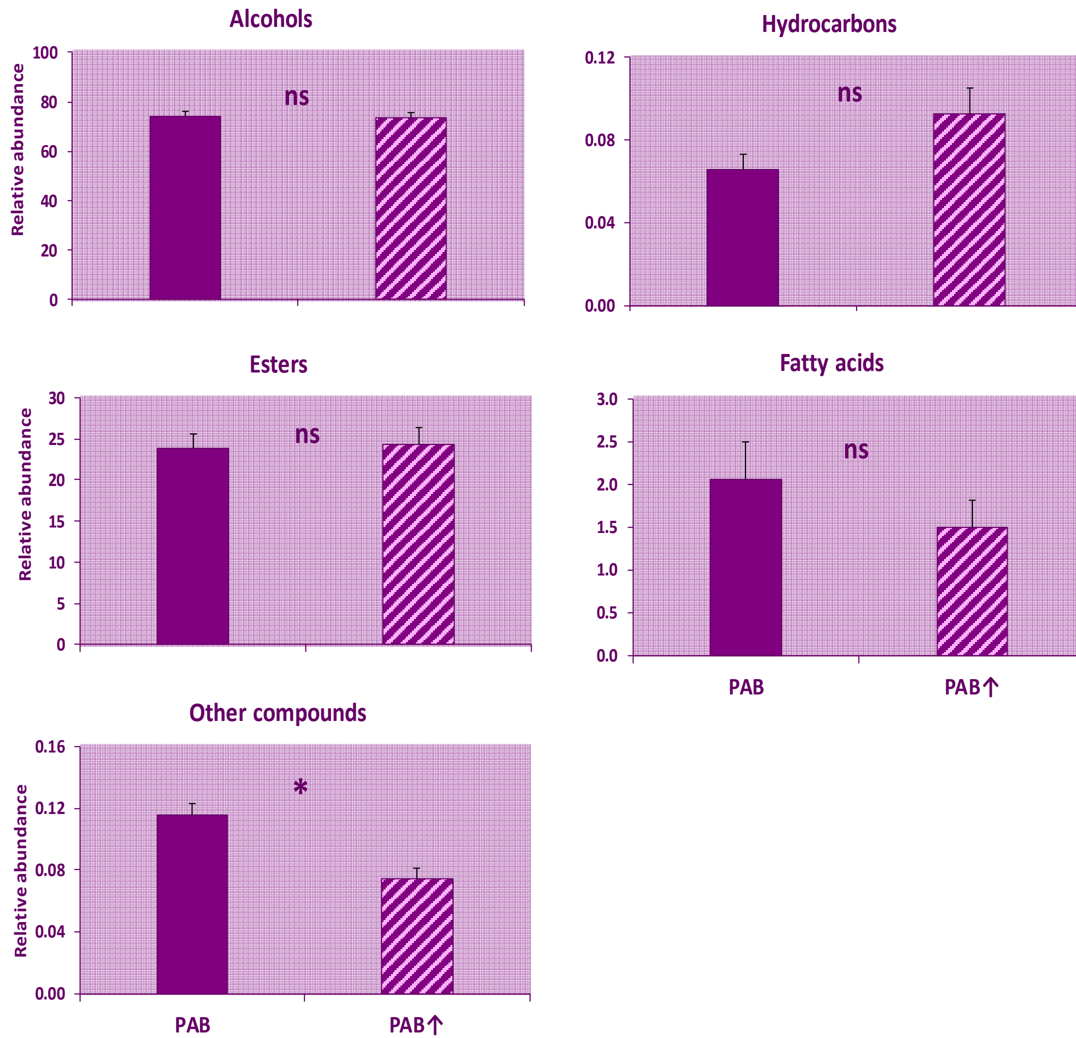


Figure 7.17. Relative abundance (percentages) of families of volatile organic compounds (VOCs) in wines elaborated from grapes exposed to ambient (PAB regime) or enhanced (PAB↑ regime) UV-B. For each variable, the statistical significance of a Student's t test comparing both radiation regimes is shown. Means ± SE are shown. *, $p < 0.05$; ns, not significant.

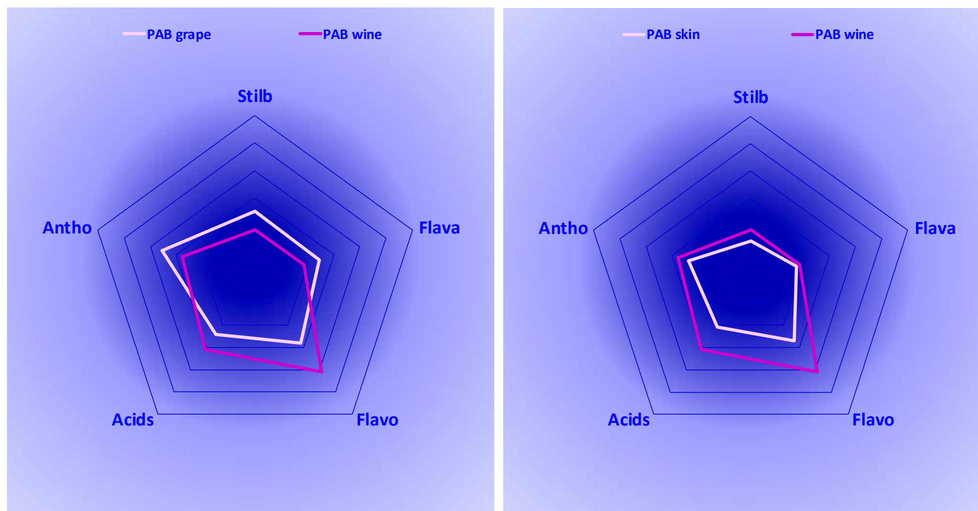


Figure 7.18. Comparison of the response intensity of the phenolic families to UV radiation in grapes and the resulting wines (left) and in the grape skins and the resulting wines (right). Each vertex represent the ratio between the contents of a specific phenolic family in the PAB and P regimes. Values of each variable increase from the polygon center to its respective vertex. Antho, anthocyanins. Stilb, stilbenes. Flava, flavanols. Flavo, flavonols.

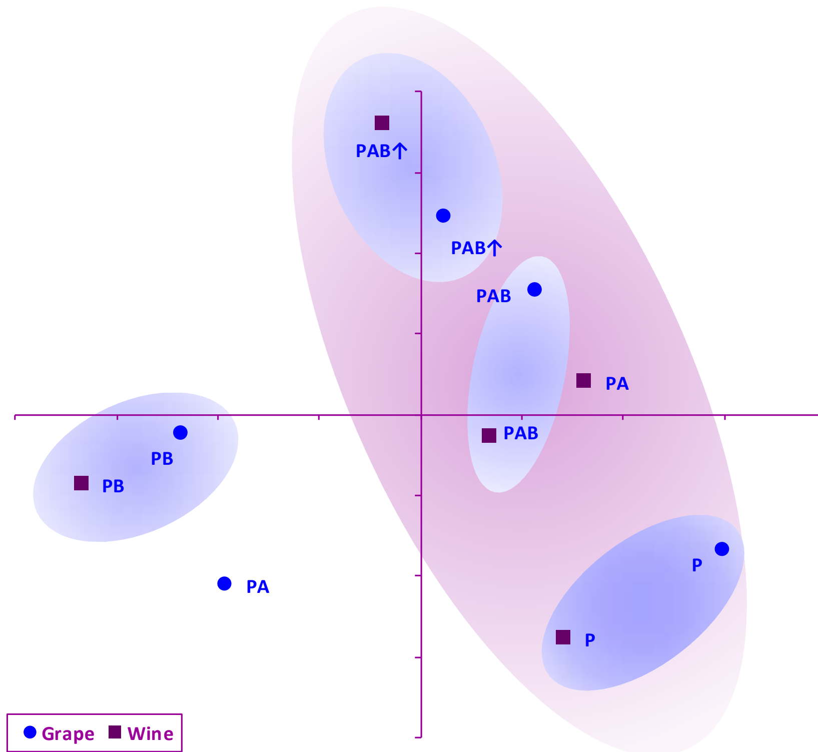


Figure 7.19. Ordination, through Principal Components Analysis (PCA), of the samples of grapes and wines used in the experiment. Grapes grown under the five different radiation regimes imposed in the experiment (P, PA, PAB, PB and PAB↑), and the resulting wines for each regime, are shown. PCA was performed using the variables that were common to grapes (at harvest) and wines. Loading factors for each axis are described in the text. Axis I is the horizontal one, and axis II is the vertical one. Each tick-mark on axes I and II represents 0.5 units.

Chapter 8. UV-B radiation as a cultural practice to control the phenolic ripeness of berry and wine quality

UV-B radiation as a cultural practice to control the phenolic ripeness of berry and wine quality

ABSTRACT

Background and Aims: Many studies on the effects of ambient levels of UV-B radiation on grapevine have been carried out, but no previous study has evaluated the effects of artificially enhanced UV-B on the phenolic composition and volatile organic compounds (VOCs) in both grape skins and the resulting wines at a crop scale.

Methods and Results: Bunches of Tempranillo grapevines were supplemented with UV-B under field commercial conditions, by using a lamp mounted on a tractor. The phenolic profiles (around 45 individual compounds) and antioxidant capacity of both grape skins and the resulting wines were analyzed in two different campaigns (2015 and 2017). Additionally, 27 wine VOCs were analyzed in 2017. The year of experimentation significantly influenced 36 variables of grape skins (out of 53) and 34 of wines (out of 54). This was expected because many factors influencing skin and wine characteristics change from year to year. Enhanced UV-B affected 23 variables of skins and 27 of wine. Particularly, flavonols (mainly quercetins, but also myricetins, isorhamnetins and kaempferols), phenolic acids and the antioxidant capacity increased in UV-B-supplemented skins and the resulting wines. Flavonols increase could contribute to a better wine color stabilization. Moreover, six VOCs affecting aroma characteristics of wine, among which fatty acids, alcohols, esters and aldehydes were represented, increased in the wines elaborated with UV-B-supplemented grapes. Probably, the changes described were due rather to UV-B irradiance peaks than to the supplemental UV-B dose.

Conclusions: Artificially enhanced UV-B improved the phenolic quality and antioxidant capacity of both grape skins and the resulting wines, mainly through the increase in flavonols. These changes were modulated by the specific year of application.
Significance of the Study: Our study opens new possibilities to realistically introduce the mechanical application of supplemental UV-B radiation as an additional agricultural practice under field commercial conditions at a crop scale, in order to improve the quality of grapes and the resulting wine. This could particularly be applied in countries or years with insufficient natural sunlight and, consequently, insufficient UV-B.

INTRODUCTION

Many studies on the effects of UV radiation on grapevine have been carried out, mostly on leaves and berry skins. These studies have mostly assessed the effects of ambient solar UV levels by comparing plants receiving either full solar radiation or this radiation deprived of UV-A and/or UV-B wavelengths, by using specific cut-off filters (Berli *et al*. 2008, Carbonell-Bejerano *et al*. 2014, Del-Castillo-Alonso *et al*. 2015, Song *et al*. 2015, Del-Castillo-Alonso *et al*. 2016a, Liu *et al*. 2018, Marfil *et al*. 2019). Other studies have evaluated the effects of artificially enhanced UV levels, by using lamps providing supplemental UV (Martínez-Lüscher *et al*. 2014, Liu *et al*. 2018). In addition, the effects of natural gradients of UV radiation have occasionally been studied (Del-Castillo-Alonso *et al*. 2016b). Regarding the second type of studies, they have preferentially been performed under more or less controlled conditions, for example growing plants in pots and/or in greenhouses, or using special cuttings. These studies, although valuable, may not totally replicate the typical commercial conditions of culture. Thus, studies evaluating the effects of enhanced UV under commercial conditions are lacking, and this would be the type of knowledge particularly needed to derive applications. In addition, no study has previously tried to follow the effects of supplemental UV application on grapes until wine elaboration, to check if the effects caused on grapes are conserved in the resulting wines.

In the described context, the aim of our study was to develop a method to mechanically apply a supplement of UV-B to grapes in a commercial vineyard at a crop scale, and to evaluate if the effects obtained in grapes were conserved in the resulting wines. If so, this method would represent a new agricultural practice to manipulate UV-B radiation in a realistic way to potentially improve the quality of both grapes and wine in commercial vineyards.

MATERIALS AND METHODS

Plant material and culture conditions

This field experiment was conducted in the 2015 and 2017 seasons on an experimental vineyard located at the University of La Rioja (Logroño, La Rioja, northern Spain, 42º 27' N, 2º 25' W, 373 m elevation). Plants of *Vitis vinifera* L. cv. Tempranillo (clone 261), grafted onto 110R rootstock and planted in 2011 on loamy soil with N-S row orientation, were used. The plant spacing was 2.7 m between rows and 1.0 m between plants within rows. The vines were spur-pruned (12 buds per vine) in a bilateral cordon and trained to a vertical shoot positioning trellis system. The annual precipitation was 398.4 mm in 2015 and 320.5 mm in 2017, and the average annual temperatures were 13.4 °C and 14.4 °C, respectively. Vines were not irrigated during the growing season.

Experimental design

A completely randomized block design was set-up. Six blocks of ten vines each were divided into two experimental conditions (three replicates per experimental condition): Ambient UV-B (non-irradiated plants that received only ambient solar UV-B) and Enhanced UV-B (UV-B↑, plants that received an artificial UV-B supplement over ambient levels). In September, five days before grape harvest, leaf removal was carried out in both Ambient and UV-B↑ plants to let the bunches exposed. On the same day of leaf removal, and during five consecutive days, UV-B↑ plants were irradiated at the height of the bunches using a manufactured lamp that was mounted on the front part of a tractor with the aid of a commercial inter-row weeding boom (Stagric BHV2L5M, Carvoeira, Portugal). Extension and elevation of this tool was controlled from the driver's seat, allowing horizontal and vertical movements of 70 and 50 cm, respectively. Consequently, the position of the lamp could be finely controlled to guarantee that irradiation was homogeneously applied in all the plants, at the height of the bunches and at a distance of 20 cm from the lamp to the bunches. The lamp was autonomous because it was connected to a

current converter and the tractor battery. The lamp was manufactured with four fluorescent tubes (Philips UV-B Narrowband TL 20W/01, Philips Lighting, Madrid, Spain) covered by a metal case. These tubes emitted only UV-B wavelengths between 305 and 315 nm, peaking at 311 nm. Bunches were irradiated at the evening before sunset. The average speed of the tractor was 84 m h^{-1}, allowing an irradiation period of 25 s for the bunches of each plant. The spectral irradiance of the lamp was checked daily using a spectroradiometer (Macam SR9910, Macam Photometrics Ltd., Livingstone, Scotland) to confirm stability. During the five days of irradiation, ambient photosynthetically active radiation (PAR), UV-A, and UV-B irradiances were continuously recorded close to the experimental plot by broad-band sensors (Skye Quantum SKP 215, SKU 420 and SKU 430, respectively, Skye Instruments Ltd., Powys, UK). The biologically effective UV and UV-B irradiances (UV_{BE} and UV-B_{BE}, respectively) received by the plants were calculated using the action spectra of Flint and Caldwell (2003) and Caldwell (1971), respectively. Mean temperature during the irradiation period was 19.6 °C in 2015 and 18.6 °C in 2017. No precipitation occurred in these periods.

Berry sampling and berry skin analysis

The day after the last irradiation, berries were collected around noon. For each treatment and replicate, 30 berries from 10 different plants were immediately frozen in liquid nitrogen, transported to the laboratory and kept at -80 ºC until analysis. The remaining berries were used for vinification. Frozen berries were allowed to partially thaw and skin was carefully removed from the flesh using a scalpel, without rupturing the hypodermal cells. The skins were immediately submerged in liquid nitrogen, lyophilized and ground (UltraTurrax® T25 Basic homogenizer, IKA Labortechnik, Staufen, Germany). For each analytical sample, 50 mg of this material was subsequently ground in a TissueLyser (Qiagen, Hilden, Germany) to obtain a homogeneous powder. Then, 4 mL of methanol:water:7M HCl (70:29:1 v:v:v) was added for extraction (24 h at 4 °C in the dark). The extract was centrifuged at 6000 g for 15 min and the supernatant was considered the source of phenolic compounds.

The analysis of phenolic compounds was carried out as in Del Castillo Alonso *et al*. (2016b). The bulk level of UV-absorbing compounds (UVAC) was measured as the area under the absorbance curve in the interval 280–400 nm ($AUC_{280–400}$) per unit of dry weight (DW), using a Perkin-Elmer λ35 spectrophotometer (Perkin-Elmer, Wilton, CT, USA). Individual phenolic compounds were analyzed by UPLC using a Waters Acquity Ultra Performance LC system (Waters Corporation, Milford, USA). The UPLC system was coupled to a micrOTOF II high-resolution mass spectrometer (Bruker Daltonik, Germany) equipped with an Apollo II ESI/APCI multimode source and controlled by the Bruker Daltonics DataAnalysis software. A UV detector module was used at 520 nm for anthocyanins and at 324 nm for the remaining compounds. The electrospray source was operated in negative mode, except for the anthocyanins which operated in positive mode. The capillary potential was set to 4 kV; the drying gas temperature was 200 °C and its flow 9 L min^{-1}; the nebulizer gas was set to 3.5 bar and 25 °C. Spectra were acquired between *m/z* 120 and 1505 in both modes. The different phenolic compounds were identified and quantified using specific commercial pure compounds or, in their absence, compounds with the same chromophore: *t*-resveratrol, catechin, epigallocatechin, procyanidin B2, kaempferol-glucoside, quercetin-3-*O*-glucuronide, myricetin, quercetin, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-rutinoside, isorhamnetin-3-*O*-glucoside, syringetin-3-*O*-glucoside, caffeic acid, *p*-coumaric acid, gallic acid, protocatechuic acid, and malvidin-3-*O*-glucoside (Sigma-Aldrich, St. Louis, MO, USA; Fluka, Buchs, Germany; Extrasynthese, Genay, France). Total contents of the different phenolic groups (stilbenes, flavanols, flavonols, hydroxybenzoic acids, hydroxycinnamic acids, and anthocyanins) were obtained as the sum of the respective individual compounds. Total phenols were determined using the Folin–Ciocalteu reagent and data were expressed as gallic acid equivalents (GAE) (Villaño *et al*. 2004).

The antioxidant capacity of berry skins was measured by generating the radical cation 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) as previously described (Del Castillo

Alonso *et al*. 2016b), using a Perkin-Elmer λ35 spectrophotometer, and was expressed in terms of Trolox equivalent (TE).

Vinification and wine analysis

For each treatment and replicate, grapes were destemmed and crushed and the alcoholic fermentation was carried out following Sampaio *et al*. (2007). Around 3 kg of pomace (must, seed, and skin) were introduced into 2.5 L glass bottles. Potassium metabisulfite (0.09 g kg^{-1}) was added to the samples to give a final total SO_2 concentration of 50 mg L^{-1} and then musts were inoculated with 0.2 g kg^{-1} of commercial *Saccharomyces cerevisiae* r.f. *bayanus* (Enartis, Trecate, Italy). The must was fermented at a controlled temperature of 25 ºC. The alcoholic fermentation finished when reducing sugars were below 2.5 g L^{-1} (two weeks after yeast inoculation). Then, wine was separated from seeds and skins by pressing, and wine analysis was performed.

The bulk level of UVAC, total phenols, individual phenolic compounds, and total antioxidant capacity were analyzed following the same procedures as in berry skins. Color intensity (CI) and Hue were analyzed according to official methods (EEC 1990). For Volatile Organic Compounds (VOCs) extraction (González-Mas *et al*. 2011), 1 mL of wine per sample was transferred to a 10 mL headspace screw cap vial and subjected to headspace solid-phase microextraction (HS-SPME). A 65 µM PDMS/DVB fiber (Supelco, Bellefonte, PA, USA) was used for the analysis. Pre-incubation and extraction were performed at 50 ºC for 10 and 20 min, respectively. Desorption was performed for 1 min at 250 ºC in splitless mode. VOCs trapped on the fiber were analyzed by GC-MS using an autosampler COMBI PAL CTC Analytics (Zwingen, Switzerland), a 6890N GC Agilent Technologies (Santa Clara, CA, USA) and a 5975B Inert XL MSD Agilent, equipped with an Agilent J&W Scientific DB-5 fused silica capillary column (5%-phenyl-95%-dimethylpolysiloxane as stationary phase, 60 m length, 0.25 mm i.d., and 1 µm thickness film). Oven temperature conditions were 40 ºC for 2 min, 5 ºC min^{-1} ramp until 250 ºC and then held isothermally at 250 ºC for 5 min. Helium was used as carrier gas at 1.4 mL min^{-1} constant flow. Mass/z detection was

obtained by an Agilent mass spectrometer operating in the EI mode (ionization energy, 70 eV; source temperature 230 ºC). Data acquisition was performed in scanning mode (mass range *m/z* 35–220). Chromatograms and spectra were recorded and processed using the Enhanced ChemStation software for GC-MS (Agilent). Compound identification was based on both the comparison between the MS for each putative compound with those of the NIST 2005 Mass Spectral library and the match to a GC retention time and Mass Spectra custom library generated using commercially available compounds.

Statistical analysis

The global effect of the treatment (Ambient *vs.* UV-B↑ samples) and year on the variables measured was tested using a 2-way analysis of variance (ANOVA), once proved that the data met the assumptions of normality (Shapiro–Wilks's test) and homoscedasticity (Levene's test). For each year and variable measured, the effect of the treatment was tested using a Student's *t* test. The samples of the different years and treatments were ordinated by Principal Components Analysis (PCA), taking into account the global variables measured in both berry skins and wine. The statistical procedures were performed with SPSS 24.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Radiation received by Ambient and UV-B↑ bunches

The mean values of the maximum daily irradiances received by Ambient bunches during the experimental 5-day periods were notably similar in 2015 and 2017. These irradiances were, respectively, 375 and 363 PAR, 36.5 and 33.7 UV-A, 0.75 and 0.88 UV-B, 0.82 and 0.76 UV_{BE}, and 0.13 and 0.15 UV-B_{BE} (all values in W m^{-2}). In both experimental years, supplemental irradiances received by the UV-B↑ bunches during the 5-day irradiation periods were 2.1 W m^{-2} UV-A, 8.5 W m^{-2} UV-B, 0.95 W m^{-2} UV_{BE} and 1.2 W m^{-2} UV-B_{BE}. Thus, irradiance differences between Ambient and UV-B↑ bunches were mostly due to UV-B and UV-B_{BE}, that increased by around 10-fold in the UV-B↑ bunches (Figure 8.1). However, the total doses of radiation received by the UV-B↑ bunches were only a little higher than those received by the Ambient bunches (Figure 8.1). Specifically, during the 5-day treatment, the UV-B↑ bunches received a supplement of only 0.26 kJ m^{-2} UV-A, 1.25 kJ m^{-2} UV-B, 0.12 kJ m^{-2} UV_{BE} and 0.16 kJ m^{-2} UV-B_{BE} over the levels received by Ambient bunches. UV-B and UV-B_{BE} showed the highest supplements, representing only increases of 1.5% and 1%, respectively, over the doses received by the Ambient bunches.

Phenolic compounds and antioxidant capacity of berry skins and wines

In both 2015 and 2017, a total of 45 phenolic compounds were identified in berry skins and 44 in the resulting wines (Tables 8.1 and 8.2). The same compounds were found in both skins and wines, except two flavonols (the aglycone kaempferol and quercetin-3-*O*-rutinoside), that were only found in skins, and caffeic acid ethyl ester, that was only found in wines. In both skins and wines, the most abundant phenolic group was anthocyanins, followed by flavonols in berry skins and phenolic acids in wines, and then flavanols and stilbenes.

Regarding global variables, in skins, the effect of year was significant on the bulk level of UVAC, total phenols, and the total contents of flavanols, flavonols and phenolic acids, with higher values in 2015 than in 2017, except in the bulk level of UVAC (Table 8.1). In the resulting wines,

the effect of the year was only significant on the bulk level of UVAC, total phenols, and the total contents of flavanols (Table 8.2). The antioxidant capacity was not influenced by the year in skins, but it was in wine, with higher values in 2015 than in 2017. Wine CI and Hue were also influenced by the year, but values were higher in 2017.

Regarding individual compounds, both in skins and wines, more than 60% of the compounds were significantly influenced by the year, even some compounds belonging to the phenolic families whose total contents were not. In general, values were higher in 2015 than in 2017. In skins, procyanidins within flavanols, together with most flavonols (except isorhamnetins and two kaempferols), phenolic acids (except coumaroyl tartaric), and most anthocyanins, showed significant interannual changes (Table 8.1). Conversely, stilbenes did not show those changes. In wines, the behavior of the individual compounds was rather similar to those of skins (Table 8.2).

As for the significant effects of the radiation treatment on global variables, the total contents of flavonols and phenolic acids were higher in UV-B↑ than in Ambient samples, both in berry skins and the resulting wines. The antioxidant capacity showed the same effects. Other global variables, such as the bulk level of UVAC and total phenols, were significantly higher in UV-B↑ than in Ambient samples only in berry skins but not in wines. The remaining variables showed more subtle or diffuse results. Stilbenes changed only in skins, increasing in UV-B↑ samples in one year but decreasing in the other. Regarding individual compounds, at least half of the flavanols, flavonols and phenolic acids were significantly influenced by the treatment in both skins and wines, whereas anthocyanins and stilbenes showed different behaviour in skin and wines. Only one anthocyanin in skins and seven in wines showed significant differences between treatments.

Figure 8.2 shows the differences in the main phenolic groups in Tempranillo berry skins and the resulting wines as influenced by the year of experimentation and the radiation treatment applied. In general, significant differences between Ambient and UV-B↑ samples were more frequent in 2015 (nine differences in skins and nine in wine) than in 2017 (four and seven,

respectively). Quercetins were the only group of compounds showing the same behavior in skin and wine in both years, increasing in UV-B↑ samples with respect to Ambient samples (around 1.3-fold in skins and 1.3-2.0-fold in wines). Myricetins and isorhamnetins showed similar changes to quercetins, although some increases were not significant. Kaempferols significantly increased in skins in both years, whereas in wines they increased by 3-fold in 2015 but did not show significant differences in 2017. Syringetins only increased significantly in UV-B↑ skin samples, in comparison with Ambient samples, in 2015. Catechins and procyanidins showed similar changes in skin and wine, with important and significant increases in UV-B↑ samples in 2015 (14-24% in skin and 40-60% in wine) but not in 2017, when increases were not significant (in skins) or did not occur at all (in wines). Changes in anthocyanins were diffuse, with no significant difference between Ambient and UV-B↑ samples in skins in any of the two years, and only scattered significant increases in UV-B↑ wine samples in 2017 (40% increase in peonidins, 100% in cyanidins, and almost 400% in delphinidins). Cinnamic acids showed erratic changes in skins, but in wines they significantly increased in UV-B↑ wine samples in both years. Benzoic acids significantly increased in UV-B↑ skin samples in both years, in comparison with Ambient samples, but in wine they increased in 2015 and decreased in 2017. Finally, resveratrol did not show any significant difference between UV-B↑ and Ambient wine samples in any of the years, and in skin samples they showed similar results in 2017 but decreased by 19% in UV-B↑ samples in 2015.

The skin and wine samples of the two radiation regimes applied (Ambient and UV-B↑) in both years of experimentation (2015 and 2017) were ordinated by PCA using the global variables that were measured in both berry skins and wine. The accumulated variance by the first three axes was 91.8% (57.8% for axis I, 21.2% for axis II, and 12.8% for axis III). The plot using the first two axes is shown in Figure 8.3. There was a clear separation between Ambient and UV-B↑ samples along axis I. All the Ambient samples (berry skins and wines of both experimental years) were grouped towards the negative part of axis I, whereas the UV-B↑ samples were grouped mostly

towards the positive part. Taking into account the loading factors of the positive part of axis I, UV-B↑ samples of both skins and wines had higher contents of flavanols (procyanidins and catechin) and flavonols (myricetins, quercetins and kaempferols), together with higher antioxidant capacities, than Ambient samples of skins and wines. The most significant loading factors of axis II were the contents in total phenols and syringetins. Consequently, both Ambient and UV-B↑ wines of 2015 were placed towards the negative part of axis II, whereas wines of 2017 were placed to the positive part due to their higher contents of total phenols and syringetins. The separation between the skins of 2015 and 2017 along axis II was less marked than that of wines, particularly for Ambient samples, which were almost overlapped. Overall, the grouping power of axis I was stronger than that of axis II, and thus the effect of the radiation treatment (enhanced UV-B) was more robust than the effect of the year.

Volatile organic compounds (VOCs) of wines

A total of 27 VOCs, belonging to five different chemical groups, were identified in the wines elaborated in 2017 from grapes exposed to the two radiation treatments applied (Ambient and UV-B↑): four fatty acids, six alcohols, 15 esters, one aldehyde and one lactone (Table 8.3). The most abundant VOCs were alcohols (68.1% of the total in Ambient wines and 64.8% in UV-B↑ wines), followed by esters (29.0% and 32.3%, respectively). The remaining compounds were much less abundant. None of the groups showed significant changes due to the radiation applied to the bunches, but some individual compounds significantly increased in UV-B↑ wines in comparison with Ambient wines: one fatty acid (hexanoic acid), one alcohol (2-methyl-1-propanol), three esters (ethyl dodecanoate, ethyl ethanoate and methyl hexanoate), and one aldehyde (n-nonaldehyde). In particular, ethyl dodecanoate increased around 80-fold. Conversely, the alcohol 2-phenylethanol decreased in UV-B↑ wines.

DISCUSSION

This is the first study, to our knowledge, that analyzes the phenolic composition in both grape skins and the resulting wines as influenced by artificially enhanced UV-B under field commercial conditions. Previously, the effects of enhanced UV-B on grapes had been studied using grapes produced in special cuttings (not commercial grapevines), culturing plants under more or less controlled conditions, or exposing grapes to UV radiation after having been taken from the plants (Zhang *et al*. 2013, Martínez-Lüscher *et al*. 2014, Loyola *et al*. 2016, Liu *et al*. 2018). In addition, it is remarkable that our study is also the first evaluating the influence of enhanced UV-B on the VOC characteristics of wine. Thus, our study opens new possibilities to realistically introduce the artificial application of UV-B radiation as an additional agricultural practice under field commercial conditions, in order to take advantage of the positive effects that enhanced UV-B can cause on the quality of grapes and the resulting wine. Given the novelty of our results, they are not easily comparable to other previous results in the context of UV research on grapevine.

Dose or irradiance?

We compared the effects of ambient and enhanced UV-B levels on grape and wine characteristics. Given that the lamp used only supplied UV-B to the bunches during 25 s, the dose applied was relatively low in comparison with the UV-B dose received naturally by the bunches (at most, 1.5% increase over ambient levels). Thus, it is plausible to think that the effects shown by the grape skins that received enhanced UV-B (and the effects shown by the resulting wine) should be attributed rather to the UV-B irradiance peaks supplied by the lamp than to the overall UV-B dose received. In addition, other environmental factors and culture conditions were similar between Ambient and UV-B↑ samples, which emphasizes the role of UV-B irradiance peaks as the main factor causing the effects that were found in both skins and wines.

The effect of the year vs. the effect of enhanced UV-B

The year of experimentation significantly influenced 36 variables of grape skins (out of 53) and 34 of wines (out of 54). This outstanding influence could be expected, because many factors change from year to year (temperature, precipitation, incidence of pests and diseases, etc.), and these changes may strongly modify the phenolic characteristics of grapes and, consequently, those of the resulting wines (Teixeira *et al*. 2013). In particular, the year significantly affected crucial variables for grape and wine quality, such as total phenols, total flavanols, total flavonols, more than 60% of individual phenolics, wine color, etc. Hence, the application of enhanced UV-B to the grapes will not have the same effects in every year, and, obviously, the quality of grapes and wine will strongly depend on the year of cultivation.

The application of artificially enhanced UV-B to the bunches influenced skins and wines less markedly than the year. However, the influence of enhanced UV-B was still notable, since it affected 23 variables of skins and 27 of wine. This was expected because ambient solar levels of UV-B can modify the phenolic characteristics of grape skins and wine, as several filtering experiments have demonstrated (Koyama *et al*. 2012, Carbonell-Bejerano *et al*. 2014, Martínez-Lüscher *et al*. 2014, Del-Castillo-Alonso *et al*. 2015, 2016a, Liu *et al*. 2018), and thus it could be anticipated that the effects of enhanced UV-B over the ambient levels would also have significant effects, as it has been found in previous studies using enhanced UV-B (Zhang *et al*. 2013, Martínez-Lüscher *et al*. 2014, Loyola *et al*. 2016, Liu *et al*. 2018).

The most significant effects were obtained for the total contents of flavonols and phenolic acids, together with the antioxidant capacity, that increased in UV-B↑ samples both in berry skins and the resulting wines. Other variables, such as the bulk level of UVAC and total phenols, also increased in UV-B↑ samples, but only in berry skins and thus this increase was not conserved in wines. It was not surprising that flavonols showed the most striking response since they are very reactive to UV-B, particularly quercetins and kaempferols, both in grape skins and the resulting wines (see the previous chapter of this Thesis and the references therein). The increase in

flavonols would improve the quality of grapes and wine through several aspects, such as their role in wine copigmentation (stabilizing anthocyanins and wine color) and their importance as bioactive healthy compounds in both grapes and wine (Price *et al*. 1995, Flamini *et al*. 2013). It should be remarked that, in our study, quercetins were the only group of compounds increasing in UV-B↑ samples in skin and wine in both years of experimentation, thus showing the most consistent response to enhanced UV-B. Myricetins, isorhamnetins and kaempferols also showed similar changes to quercetins. Thus, for these compounds, changes found in grape skins are maintained in the resulting wines.

Other variables showed diffuse and/or not-significant changes. This occurred with stilbenes and anthocyanins. For stilbenes, this lack of response is logical because they increase in grapes in response to both biotic and abiotic stressors, including pathogen attacks, and they also vary along berry development, but rarely respond to UV wavelengths other that UV-C (González-Barrio *et al*. 2009, Jug and Rusjan 2012, Flamini *et al*. 2013, Del-Castillo-Alonso *et al*. 2016a). For anthocyanins, their responses to UV-B are complex and depend on the interaction of internal (variety, developmental stage of the berry) and environmental (temperature) factors (Del-Castillo-Alonso *et al*. 2016a, Jordan 2017).

The results described above were summarized by PCA, confirming the strong influence of enhanced UV-B on the phenolic characteristics of both grapes and the resulting wines. In particular, most of the flavonols mentioned above (quercetins, myricetins and kaempferols) acted as important and significant loading factors separating Ambient and UV-B↑ samples, together with some flavanols (procyanidins and catechin). In addition, the higher antioxidant capacities of UV-B↑ skins and wines contributed to the separation of these samples from Ambient samples, suggesting that enhanced UV-B could contribute to increase the antioxidant capacity of grapes and wines and, thus, their healthy properties (Heim *et al*. 2002).

We also demonstrated that, among the 27 VOCs analyzed, six of them (the fatty acid hexanoic acid, the alcohol 2-methyl-1-propanol, three esters (particularly ethyl dodecanoate, but also

ethyl ethanoate and methyl hexanoate), and the aldehyde n-nonaldehyde) increased in the 2017 wines elaborated with grapes exposed to enhanced UV-B. These compounds contribute to the aroma characteristics of wine, and thus can be important in vinification. No comparative results can be found in the literature regarding the effects of UV radiation on this kind of compounds, and thus more research is needed to increase our knowledge about this topic.

Practical UV-B manipulation under commercial conditions: present and future

We have settled some experimental bases to manipulate the enhancement of UV-B and use it as an additional agricultural practice to increase grape and wine quality at a crop scale. In this sense, one of our main goals has been that we propose a mechanical method to apply enhanced UV-B, which strongly facilitates the agricultural labor. The application of enhanced UV-B could especially be important in at least two aspects: 1) in countries or years with insufficient natural sunlight and, consequently, insufficient UV-B; under these conditions, enhanced UV-B could improve the quality of both grapes and wines; and 2) enhanced UV-B would be an advantage under every climatic conditions to potentially decouple phenolic and sugar maturity, that is, to increase useful phenolic compounds in berries without increasing sugar content and, consequently, alcoholic degree. This second effect should be confirmed in future studies, since sugar content in berries and alcoholic degree in wines were not measured in the present study (although it is already known from other chapters of this Thesis that ambient UV-B levels received by grapes did not influence the alcoholic degree of the resulting wines).

Future perspectives for the use of enhanced UV-B in viticulture and enology are promising, and several challenges remain to be solved: 1) to determine the moment of application in the season; critical periods for the production of many useful compounds are veraison and the weeks before harvest, but more data are needed in this regard because each compound may have a different production dynamics along the season; 2) to define the best moment of the day for UV-B application; it must be taken into account that the treatment consists of a UV-B supplement over the ambient levels received by the plants, so that the moments of the day with

the highest irradiances should *a priori* be avoided; 3) to establish the adequate irradiance to be applied in each case, and whether it is better to give one or more applications; 4) to corroborate that UV-B-radiated berries produce better wines, incorporating specific wine tastings by experts; and 5) if the effects of enhanced UV-B on grapes are conserved not only in young but also in aged wines, because ageing in barrels strongly modifies wine compounds and could mask the UV-B influence.

TABLES AND FIGURES

Table 8.1. Phenolic composition and antioxidant capacity of Tempranillo berry skins as influenced by the year of experimentation (2015 vs. 2017) and the radiation treatment applied (Ambient vs. enhanced UV-B, UV-B↑). Values are means ± standard errors (n=3). For each variable, the statistical significance of a 2-way ANOVA for year and radiation treatment, together with the interaction between both main factors, are shown. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant. For each year and variable, different letters mean significant differences (at least $p < 0.05$) between Ambient and UV-B↑ samples (Student's t). UVAC, UV-absorbing compounds. $AUC_{280–400}$, area under the absorbance curve in the interval 280–400 nm. DW, dry weight. GAE, gallic acid equivalents. TE, Trolox equivalent.

	2015				2017				statistical significance		
Berry skin	**Ambient**		**UV-B↑**		**Ambient**		**UV-B↑**		**year**	**treat**	**Y*T**
UVAC ($AUC_{280-400}$ mg^{-1} DW)	58 ± 3	a	72 ± 1	b	101 ± 1	a	127 ± 1	b	**	*	ns
Total phenols (GAE, mg g^{-1} DW)	208 ± 7	a	238 ± 11	b	202 ± 8		209 ± 9		*	*	ns
Antioxidant capacity (µmol TE g^{-1} DW)	6073 ± 188	a	7247 ± 337	b	4975 ± 294	a	8289 ± 409	b	ns	***	**
Flavanols (µg g^{-1} DW)											
Catechin	78 ± 4	a	90 ± 2	b	87 ± 1		90 ± 3		ns	ns	ns
Epicatechin	9.4 ± 0.2	a	14 ± 0	b	9 ± 0	a	13 ± 0	b	ns	**	ns
Catechin gallate	8.1 ± 0.2	a	12 ± 0	b	6.1 ± 0.4		6.8 ± 0.6		**	*	ns
Epigallocatechin	3.7 ± 0.2	b	2.2 ± 0.1	a	4.2 ± 0.1	b	3.7 ± 0.2	a	ns	*	ns
Gallocatechin	39 ± 0		43 ± 5		27 ± 1	a	34 ± 0	b	*	ns	ns
Epigallocatechin gallate	16 ± 1	b	9.6 ± 0.6	a	19 ± 5		16 ± 4		ns	ns	ns
Procyanidin B1	97 ± 1	a	125 ± 0	b	84 ± 1		94 ± 8		**	**	ns
Procyanidin B2	10 ± 1		9.6 ± 0.5		16 ± 1		15 ± 0		***	ns	ns
Total flavanols	260 ± 1	a	306 ± 1	b	252 ± 3	a	273 ± 6	b	*	ns	ns
Flavonols (µg g^{-1} DW)											
Myricetin	69 ± 3	a	86 ± 3	b	43 ± 2	a	57 ± 1	b	***	***	ns
Myricetin-3-*O*-glucoside	2092 ± 168		2707 ± 148		1847 ± 109		1857 ± 4		*	ns	ns
Myricetin-3-*O*-glucuronide	195 ± 15	a	256 ± 14	b	183 ± 10		169 ± 7		***	*	ns
Laricitrin-3-*O*-glucoside	271 ± 9	a	311 ± 8	b	152 ± 9		157 ± 10		**	ns	*
Quercetin	3.4 ± 0.1		2.5 ± 0.3		2.8 ± 0.1	b	2.0 ± 0.2	a	*	**	ns
Quercetin-3-*O*-galactoside	147 ± 12		167 ± 19		111 ± 11		111 ± 15		**	ns	ns
Quercetin-3-*O*-glucoside	653 ± 34		800 ± 68		452 ± 26		447 ± 42		*	ns	ns
Quercetin-3-*O*-glucuronide	779 ± 54	a	1136 ± 26	b	414 ± 52	a	651 ± 78	b	**	**	ns
Quercetin-3-*O*-rutinoside	70 ± 4	a	96 ± 3	b	28 ± 7	a	63 ± 6	b	*	*	ns
Kaempferol	0.28 ± 0.03	b	0.16 ± 0.03	a	0.16 ± 0.01	a	0.24 ± 0.01	b	ns	ns	ns
Kaempferol-3-*O*-glucoside	67 ± 1		70 ± 2		84 ± 3		96 ± 5		*	ns	ns
Kaempferol-3-*O*-galactoside	14 ± 1	a	23 ± 2	b	17 ± 0	a	30 ± 2	b	ns	**	ns
Isorhamnetin-3-*O*-glucoside	73 ± 2		74 ± 3		73 ± 2		74 ± 1		ns	ns	ns
Isorhamnetin-3-*O*-glucuronide	12.0 ± 0.2	a	16.5 ± 1.1	b	8.8 ± 1.9	a	16.6 ± 1.7	b	ns	*	ns
Syringetin	2.5 ± 0.0	a	4.9 ± 0.3	b	3.0 ± 0.1		2.9 ± 0.1		**	***	***
Syringetin-3-*O*-glucoside	61 ± 2		68 ± 2		76 ± 2		78 ± 6		**	ns	ns
Total flavonols	4510 ± 147	a	5821 ± 141	b	3493 ± 103	a	3812 ± 114	b	**	*	ns
Phenolic acids (µg g^{-1} DW)											
Protocatechuic acid	68 ± 1	a	75 ± 3	b	34 ± 0	a	55 ± 5	b	***	**	*
Gallic acid	14 ± 1	a	17 ± 1	b	16 ± 1	b	12 ± 0	a	*	ns	***
Caffeoyl tartaric acid	185 ± 10		167 ± 2		146 ± 3	a	331 ± 35	b	*	**	**
Coumaroyl tartaric acid	127 ± 2	b	100 ± 5	a	92.3 ± 8.9		108 ± 8		ns	ns	ns
Total phenolic acids	393 ± 5	b	360 ± 9	a	288 ± 10	a	506 ± 25	b	***	**	**
Stilbenes (µg g^{-1} DW)											
Resveratrol	1.7 ± 0.1		1.9 ± 0.1		1.0 ± 0.1	a	1.8 ± 0.0	b	ns	*	ns
Resveratrol-3-*O*-glucoside	54 ± 1	b	43 ± 2	a	46 ± 1		46 ± 1		ns	**	**
Total stilbenes	56 ± 0	b	45 ± 2	a	47 ± 1		48 ± 1		ns	*	ns

Anthocyanins (mg g^{-1} DW)	Ambient	UV-B↑	Ambient	UV-B↑	year	treat	Y*T
Cyanidin-3-*O*-glucoside	1.4 ± 0.1	1.4 ± 0.0	1.8 ± 0.2	1.9 ± 0.2	*	ns	ns
Delphinidin-3-*O*-glucoside	21 ± 2	19 ± 2	13 ± 1	13 ± 1	**	ns	ns
Malvidin-3-*O*-glucoside	70 ± 7	56 ± 5	60 ± 4	64 ± 4	ns	ns	ns
Peonidin-3-*O*-glucoside	9.4 ± 0.6	8.8 ± 0.5	6.4 ± 0.5	6.8 ± 0.5	**	ns	ns
Petunidin-3-*O*-glucoside	26 ± 1	22 ± 2	15 ± 1	15 ± 1	***	ns	ns
Cyanidin-3-*O*-(6'-acetyl)glucoside	0.05 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	ns	*	ns
Delphinidin-3-*O*-(6'-acetyl)glucoside	0.31 ± 0.02	0.29 ± 0.02	0.23 ± 0.01	0.24 ± 0.02	**	ns	ns
Malvidin-3-*O*-(6'-acetyl)glucoside	10.3 ± 0.9	8.26 ± 0.91	19.2 ± 0.3	18.9 ± 0.2	***	ns	ns
Peonidin-3-*O*-(6'-acetyl)glucoside	0.19 ± 0.01	0.18 ± 0.01	0.16 ± 0.00	0.14 ± 0.01	**	ns	ns
Petunidin-3-*O*-(6'-acetyl)glucoside	0.68 ± 0.02	0.66 ± 0.06	0.56 ± 0.03	0.52 ± 0.02	**	ns	ns
Cyanidin-3-*O*-(6'-*p*-coumaroyl)glucoside	0.03 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	*	ns	ns
Delphinidin-3-*O*-(6'-*p*-coumaroyl)glucoside	6.7 ± 0.6	5.9 ± 0.5	1.4 ± 0.1	1.4 ± 0.1	***	ns	ns
Malvidin-3-*O*-(6'-*p*-coumaroyl)glucoside	44 ± 4	38 ± 3	49 ± 3	61 ± 3	**	ns	ns
Peonidin-3-*O*-(6'-*p*-coumaroyl)glucoside	3.2 ± 0.1	3.1 ± 0.1	3.5 ± 0.2	3.6 ± 0.2	ns	ns	ns
Petunidin-3-*O*-(6'-*p*-coumaroyl)glucoside	9.6 ± 0.8	7.3 ± 0.8	5.9 ± 0.5	5.9 ± 0.4	**	ns	ns
Total anthocyanins	202 ± 13	171 ± 13	177 ± 10	192 ± 8	ns	ns	ns

Table 8.2. Color, phenolic composition and antioxidant capacity of Tempranillo wines as influenced by the year of experimentation (2015 vs. 2017) and the radiation treatment applied to the bunches from which they were elaborated (Ambient vs. enhanced UV-B, UV-B↑). Values are means ± standard errors (n=3). For each variable, the statistical significance of a 2-way ANOVA for year and radiation treatment, together with the interaction between both main factors, are shown. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant. For each year and variable, different letters mean significant differences (at least p.< 0.05) between Ambient and UV-B↑ samples (Student's t). UVAC, UV-absorbing compounds. $AUC_{280–400}$, area under the absorbance curve in the interval 280–400 nm. GAE, gallic acid equivalents. TE, Trolox equivalent.

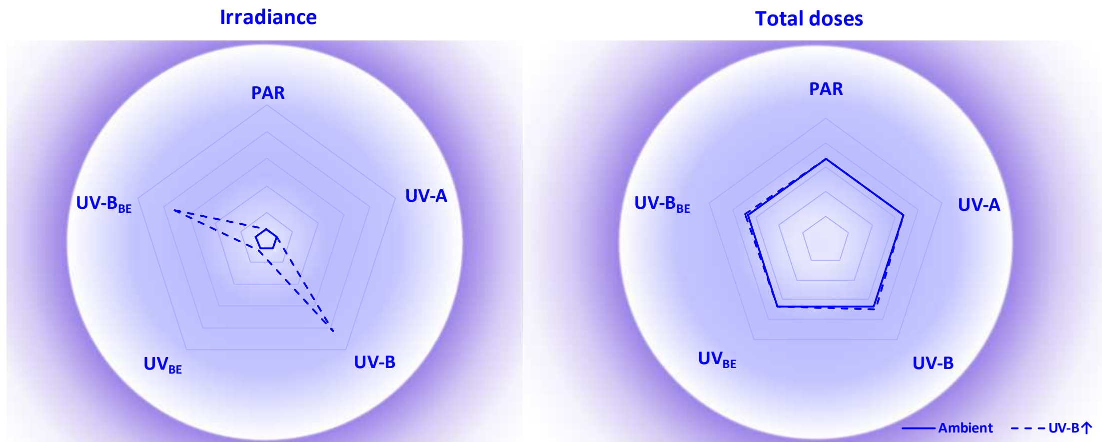
	2015				2017				statistical significance		
Wine	Ambient		UV-B↑		Ambient		UV-B↑		year	treat	Y*T
Color intensity (CI)	6.9 ± 0.6		7.0 ± 0.4		13 ± 0		14 ± 1		***	ns	ns
Hue	0.53 ± 0.00		0.51 ± 0.01		0.58 ± 0.02		0.59 ± 0.02		**	ns	ns
UVAC ($AUC_{280-400}$)	1275 ± 56		1406 ± 104		2215 ± 27		2119 ± 107		***	ns	ns
Total phenols (GAE, g L^{-1})	1.4 ± 0.0		1.7 ± 0.1		2.1 ± 0.0		2.0 ± 0.1		**	ns	ns
Antioxidant capacity (mM TE)	32 ± 2	a	45 ± 3	b	20 ± 0		18 ± 1		***	*	*
Flavanols (mg L^{-1})											
Catechin	10 ± 1	a	16 ± 1	b	9.5 ± 0.6		7.7 ± 0.3		**	*	**
Epicatechin	4.6 ± 0.1	a	6.0 ± 0.1	b	5.4 ± 0.2	b	3.7 ± 0.2	a	**	ns	***
Catechin gallate	0.03 ± 0.01		0.08 ± 0.02		0.03 ± 0.01	a	0.05 ± 0.01	b	ns	**	ns
Epigallocatechin	13 ± 1		16 ± 1		13 ± 1		13 ± 1		ns	ns	ns
Gallocatechin	1.2 ± 0.2	a	1.9 ± 0.1	b	1.4 ± 0.2	a	2.2 ± 0.1	b	ns	**	ns
Epigallocatechin gallate	0.02 ± 0.00		0.03 ± 0.00		0.02 ± 0.00		0.02 ± 0.00		ns	ns	ns
Procyanidin B1	4.8 ± 0.4	a	8.0 ± 0.3	b	5.6 ± 0.1		5.4 ± 0.0		*	**	**
Procyanidin B2	0.97 ± 0.11		1.22 ± 0.04		1.8 ± 0.2		1.5 ± 0.0		**	ns	*
Total flavanols	34 ± 2	a	49 ± 3	b	37 ± 1	b	33 ± 1	a	***	ns	ns
Flavonols (mg L^{-1})											
Myricetin	4.6 ± 0.5	a	10.6 ± 1.2	b	4.9 ± 0.2		5.2 ± 0.1		*	**	**
Myricetin-3-*O*-glucoside	2.6 ± 0.1		3.3 ± 0.2		2.2 ± 0.2		2.3 ± 0.2		**	*	ns
Myricetin-3-*O*-glucuronide	1.7 ± 0.1	a	2.5 ± 0.1	b	1.4 ± 0.1		1.6 ± 0.0		**	**	*
Laricitrin-3-*O*-glucoside	0.66 ± 0.05		0.72 ± 0.05		0.71 ± 0.03		0.74 ± 0.05		ns	ns	ns
Quercetin	2.3 ± 0.2	a	6.3 ± 0.4	b	2.2 ± 0.0		2.1 ± 0.3		***	***	***
Quercetin-3-*O*-galactoside	14 ± 0	a	23 ± 2	b	10 ± 1		13 ± 0		**	**	*
Quercetin-3-*O*-glucoside	7.0 ± 0.8	a	14 ± 1	b	3.1 ± 0.6		3.8 ± 0.2		***	**	**
Quercetin-3-*O*-glucuronide	7.8 ± 0.2	a	14 ± 1	b	7.2 ± 0.6	a	11 ± 1	b	**	***	*
Kaempferol-3-*O*-glucoside	0.08 ± 0.02	a	0.26 ± 0.05	b	0.06 ± 0.01		0.06 ± 0.01		**	**	**
Kaempferol-3-*O*-galactoside	0.02 ± 0.00	a	0.04 ± 0.00	b	0.01 ± 0.00		0.02 ± 0.00		***	***	**
Isorhamnetin-3-*O*-glucoside	0.14 ± 0.01		0.19 ± 0.03		0.13 ± 0.01		0.13 ± 0.02		ns	ns	ns
Isorhamnetin-3-*O*-glucuronide	2.1 ± 0.2	a	3.2 ± 0.2	b	2.1 ± 0.4		3.0 ± 0.3		ns	*	ns
Syringetin	0.26 ± 0.02		0.23 ± 0.01		0.32 ± 0.02	b	0.20 ± 0.02	a	ns	**	*
Syringetin-3-*O*-glucoside	2.3 ± 0.1		2.5 ± 0.1		4.7 ± 0.1		4.8 ± 0.1		***	ns	ns
Total flavonols	46 ± 1	a	81 ± 4	b	39 ± 1	a	48 ± 0	b	ns	*	ns
Phenolic acids (mg L^{-1})											
Protocatechuic acid	0.76 ± 0.09		0.76 ± 0.06		0.73 ± 0.05		0.70 ± 0.04		ns	ns	ns
Gallic acid	14 ± 1	a	18 ± 1	b	16 ± 1	b	12 ± 0	a	*	ns	**
Caffeic acid ethyl ester	0.19 ± 0.01		0.18 ± 0.01		0.26 ± 0.02		0.23 ± 0.01		**	ns	ns
Caffeoyl tartaric acid	42 ± 1	a	49 ± 1	b	19 ± 1		23 ± 1		***	**	ns
Coumaroyl tartaric acid	11 ± 1		13 ± 0		10 ± 1		14 ± 1		ns	*	ns
Total phenolic acids	68 ± 1	a	81 ± 1	b	45 ± 2	a	50 ± 1	b	ns	**	*
Stilbenes (mg L^{-1})											
Resveratrol	0.04 ± 0.01		0.03 ± 0.00		0.03 ± 0.01		0.04 ± 0.00		ns	ns	ns
Resveratrol-3-*O*-glucoside	1.3 ± 0.1		1.2 ± 0.0		1.4 ± 0.1		1.5 ± 0.0		**	ns	ns
Total stilbenes	1.4 ± 0.1		1.3 ± 0.0		1.4 ± 0.1		1.7 ± 0.1		ns	ns	ns

Anthocyanins (mg L^{-1})	Ambient		UV-B↑		Ambient		UV-B↑		year	treat	Y*T
Cyanidin-3-*O*-glucoside	1.0 ± 0.0	a	1.4 ± 0.1	b	0.42 ± 0.13		2.85 ± 1.00		**	ns	ns
Delphinidin-3-*O*-glucoside	40 ± 3		44 ± 1		14 ± 4	a	72 ± 6	b	*	ns	ns
Malvidin-3-*O*-glucoside	308 ± 7		295 ± 9		258 ± 13		261 ± 9		ns	*	*
Peonidin-3-*O*-glucoside	14 ± 0		15 ± 1		19 ± 1	a	27 ± 0	b	*	ns	*
Petunidin-3-*O*-glucoside	53 ± 7		64 ± 5		66 ± 14		91 ± 8		ns	ns	ns
Cyanidin-3-*O*-(6'-acetyl)glucoside	1.1 ± 0.0		1.0 ± 0.0		1.1 ± 0.1		1.2 ± 0.1		***	ns	ns
Delphinidin-3-*O*-(6'-acetyl)glucoside	2.3 ± 0.3		2.3 ± 0.2		0.70 ± 0.10		0.72 ± 0.06		**	*	*
Malvidin-3-*O*-(6'-acetyl)glucoside	28 ± 3	a	37 ± 3	b	32 ± 2		33 ± 3		***	***	**
Peonidin-3-*O*-(6'-acetyl)glucoside	1.9 ± 0.1		2.1 ± 0.1		1.4 ± 0.1		1.4 ± 0.1		ns	*	ns
Petunidin-3-*O*-(6'-acetyl)glucoside	3.4 ± 0.3	b	2.3 ± 0.1	a	4.0 ± 0.4		4.4 ± 0.3		***	**	**
Cyanidin-3-*O*-(6'-*p*-coumaroyl)glucoside	1.8 ± 0.2		2.5 ± 0.2		1.5 ± 0.2		2.1 ± 0.1		***	ns	ns
Delphinidin-3-*O*-(6'-*p*-coumaroyl)glucoside	3.8 ± 0.4		3.5 ± 0.3		1.1 ± 0.1		1.0 ± 0.3		*	ns	**
Malvidin-3-*O*-(6'-*p*-coumaroyl)glucoside	19 ± 2		19 ± 1		18 ± 3		23 ± 3		ns	***	***
Peonidin-3-*O*-(6'-*p*-coumaroyl)glucoside	3.1 ± 0.3		3.0 ± 0.2		1.7 ± 0.1		2.3 ± 0.1		ns	**	*
Petunidin-3-*O*-(6'-*p*-coumaroyl)glucoside	3.9 ± 0.5		3.6 ± 0.2		2.2 ± 0.2		2.9 ± 0.2		*	ns	ns
Total anthocyanins	485 ± 13		496 ± 33		420 ± 21	a	526 ± 24	b	ns	ns	ns

Table 8.3. Effect of the radiation treatment applied to Tempranillo bunches (Ambient vs. enhanced UV-B, UV-B↑) in 2017 on the Volatile Organic Compounds (VOCs) of the resulting wines. Values, in percentages of the total VOCs content, are means ± standard errors (n=3). For each variable, the statistical significance of the Student's t test performed is shown (**, $p < 0.01$; *, $p < 0.05$; ns, not significant), and different letters mean significant differences between Ambient and UV-B↑ samples.

Volatile Organic Compounds (VOCs)	Wine 2017 Ambient		UV-B↑		statistical significance
Fatty acids					
Ethanoic acid	1.8 ± 0.2		1.9 ± 0.3		ns
Hexanoic acid	0.42 ± 0.02	a	0.54 ± 0.01	b	*
n-Nonanoic acid	0.14 ± 0.03		0.17 ± 0.01		ns
Octanoic acid	0.38 ± 0.01		0.55 ± 0.05		ns
Total acids	2.8 ± 0.6		3.2 ± 0.3		ns
Alcohols					
2,3-Butanediol	0.52 ± 0.02		0.54 ± 0.04		ns
1-Hexanol	1.8 ± 0.1		2.0 ± 0.1		ns
2-Methyl-1-butanol	9.6 ± 0.4		9.5 ± 0.3		ns
3-Methyl-1-butanol	37.5 ± 1.2		37.1 ± 1.8		ns
2-Methyl-1-propanol	1.6 ± 0.1	a	2.1 ± 0.1	b	*
2-Phenylethanol	17.1 ± 0.8	b	13.6 ± 0.5	a	*
Total alcohols	68.1 ± 2.3		64.8 ± 2.1		ns
Esters					
Diethyl succinate	5.0 ± 0.0		4.2 ± 0.3		ns
Ethyl butanoate	0.58 ± 0.04		0.59 ± 0.07		ns
Ethyl decanoate	0.24 ± 0.02		0.28 ± 0.02		ns
Ethyl dodecanoate	0.03 ± 0.00	a	2.6 ± 0.3	b	**
Ethyl ethanoate	8.2 ± 0.0	a	10.2 ± 0.3	b	*
Ethyl heptanoate	0.03 ± 0.00		0.03 ± 0.01		ns
Ethyl hexadecanoate	2.8 ± 0.8		2.7 ± 0.3		ns
Ethyl hexanoate	7.6 ± 0.8		7.2 ± 0.2		ns
Ethyl nonanoate	0.04 ± 0.01		0.04 ± 0.01		ns
Ethyl octanoate	2.8 ± 0.2		2.8 ± 0.3		ns
Ethyl tetradecanoate	0.04 ± 0.01		0.06 ± 0.02		ns
2-Methylbutyl acetate	0.24 ± 0.02		0.22 ± 0.01		ns
3-Methylbutyl acetate	1.2 ± 0.1		1.2 ± 0.0		ns
Methyl hexanoate	0.01 ± 0.00	a	0.02 ± 0.00	b	*
2-Phenylethyl acetate	0.15 ± 0.03		0.13 ± 0.01		ns
Total esters	29.0 ± 1.7		32.3 ± 1.8		ns
Other compounds					
Hydroxybutyric acid lactone	0.09 ± 0.01		0.07 ± 0.01		ns
n-Nonaldehyde	0.05 ± 0.01	a	0.08 ± 0.01	b	*
Other compounds	0.14 ± 0.00		0.16 ± 0.01		ns

Figure 8.1. Differences in irradiances (left) and total doses (right) of photosynthetically active radiation (PAR), UV-A, UV-B, biologically effective UV (UV_{BE}), and biologically effective UV-B (UV-BBE), between the plants that received ambient solar radiation (Ambient, solid line) and those that received a UV-B supplement (UV-B↑, dashed line).



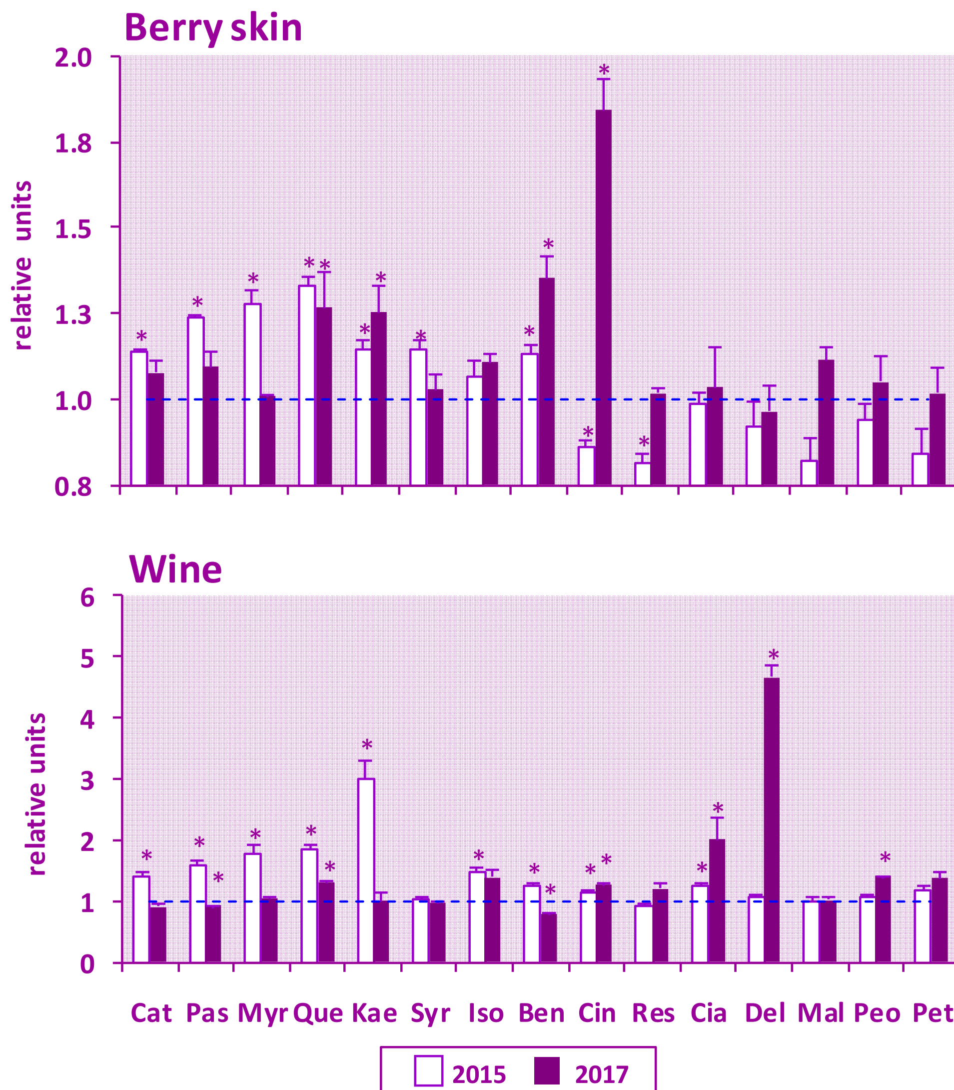


Figure 8.2. Differences in the main phenolic groups in Tempranillo berry skins (top) and the resulting wines (bottom) as influenced by the year of experimentation (2015 vs. 2017) and the radiation treatment applied (Ambient vs. enhanced UV-B, UV-B↑). All the results are expressed in relative units, taking the Ambient samples as the unit value. Asterisks over the bars mean significant differences between Ambient and UV-B↑ samples (at least $P < 0.05$) for a specific year. Cat, catechin. Pas, procyanidins. Myr, myricetins. Que, quercetins. Kae, kaempferols. Syr, syringetins. Iso, isorhamnetins. Ben, benzoic acids. Cin, cinnamic acids. Res, resveratrol. Cya, cyanidins. Del, delphinidins. Mal, malvidins. Peo, peonidins. Pet, petunidins. For each variable, means ± standard errors are shown.

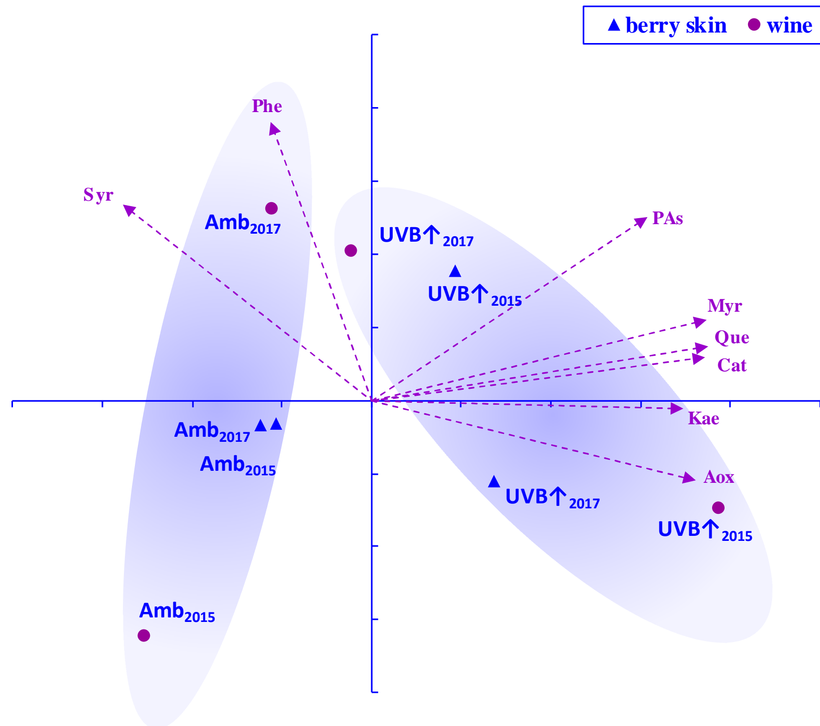


Figure 8.3. Ordination, through Principal Components Analysis (PCA), of the Ambient (Amb) and enhanced UV-B (UV-B↑) Tempranillo berry skin (triangles) and wine (circles) samples of the two experimental years (2015 and 2017), on the basis of the global variables measured in both berry skins and wine. Significant loading factors for the positive and negative parts of each axis are shown as arrows. Axis I is the horizontal one, and axis II is the vertical one. Each tick-mark on axes I and II represents 1 unit. Aox, antioxidant capacity. Cat, catechin. Kae, kaempferols. Myr, myricetins. PAs, procyanidins. Phe, total phenols. Que, quercetins. Syr, syringetins.

Chapter 9. Conclusions

CONCLUSIONS

The overall conclusion of the present Doctoral Thesis is that the effects of UV radiation on grapevine have been studied 1) integrating different scales, from molecular aspects to field approaches; 2) integrating the influences of internal (genetic) and environmental factors; and 3) including proposals to improve the quality of grapes and the resulting wine through and adequate UV management at a crop scale in the field. These innovative approaches clearly showed the complexity of the UV effects on grapevine, although some responses were well characterized, such as the induction of flavonols in grapes and the resulting wines.

This overall conclusion can be divided in several partial conclusions, paralleling the objectives pursued:

-·UV effects on grapes may be wavelength-specific, with ambient UV-B causing stronger effects than ambient UV-A. However, synergic effects between both wavelength bands were observed, for example increasing flavonols.

-·Ambient solar UV-B is essential for the synthesis of a number of phenolic compounds (particularly quercetins and kaempferols) contributing to grape and wine quality, and with potential use as nutraceuticals. This was demonstrated from genes to metabolites. Conversely, the responses of other phenolic compounds (anthocyanins, flavanols, stilbenes and acids) and volatile organic compounds (VOCs) were more diffuse.

- Realistically enhanced UV-B doses (as predicted from global change models) led to rather subtle changes in comparison with ambient UV-B in the long-term, but changes were globally significant as shown by multivariate analysis. In this line, high UV-B peak irradiances caused stronger changes. Thus, irradiance peaks may be more important than total doses to induce positive changes contributing to improve the quality of both grapes and the resulting wines. This opens new possibilities of UV management at a crop scale.

- UV natural latitudinal gradients were useful to detect the interactions between UV, temperature and water availability in the regulation of the responses of grape phenolic compounds to UV, and the critical phenological stages to increase those responses.

- UV responses of grapevine depend on the variety used, with Tempranillo apparently more UV-responsive than Graciano or Pinot noir, although studies under the same conditions should be performed to confirm this hypothesis.

- The physiology of grapevine leaves was well adapted to the ambient UV levels typical of Mediterranean conditions, thus experiencing eustress ("good stress") rather than distress ("destructive stress") through, mainly, increasing both UV screening and antioxidant capacity. Thus, ambient UV was needed for an adequate leaf photoprotection. Overall, in line with the modern concepts of UV research, UV represents a regulatory factor rather than a generic stressor.

- Leaves and grape skins seem to have different regulation mechanisms of their phenolic metabolism in response to UV, with kaempferols as the only compounds showing similar responses.

- Among the three berry components (skin, flesh and seeds), skin was the most UV-responsive, probably because it receives the highest UV impact.

- Veraison was the phenological stage of the berry which showed the strongest responses to UV-B (increase of flavonols, especially quercetins, and of *VvFLS4* expression). Also, pea size was a UV-responsive stage and this should be further explored. Nevertheless, overall natural evolution of phenolic compounds from pea size to harvest was not modified by UV under the experimental conditions used in our study.

- The effects of UV on grapes and the resulting wines were modulated by the specific year of application.

- Grape variables were more UV-responsive than leaf variables, with phenolic compounds (and related genes) showing much clearer responses than VOCs. Some of these responses were conserved in the resulting wines, thus affecting their sensorial characteristics. In addition, morphology of grapes was also affected by, specifically, UV-B.

- The methanol-soluble fraction of phenolic compounds was more UV-responsive than the –insoluble fraction, which was insensitive to UV.

- For the first time to our knowledge, the UV effects on the detailed phenolic composition of grapevine were studied along the whole way of grapevine commercial exploitation from the grapes to the wine. This allowed to show that some UV effects on grapes were conserved in the resulting wines, whereas other effects could be modified during winemaking, leading to losses of interesting compounds that, otherwise, could be recovered by changing viniculture practices.

- We developed a mechanical procedure to apply enhanced UV-B to the grapes under field conditions at a crop scale, providing new possibilities of UV radiation management contributing to improve the quality of both grapes and the resulting wines. This could particularly be applied in zones or years with insufficient natural UV-B.

- UV research under field conditions shows some technical constraints leading to a relatively high variability, probably because 1) low UV amounts (particularly of UV-B) can trigger a cascade of responses; 2) the interaction of UV and temperature; and 3) the intrinsic variability of grape characteristics. In addition, the optical characteristics of commercially available filters, and the spectral characteristics of lamps, are not always optimal.

- Although some progress has been achieved in the study of the effects of UV radiation on grapevine, further research is needed to progressively better understand the mechanisms underlying these effects and their consequent applications.

The conclusions mentioned above were commented in detail in the different chapters of the Thesis, which can be consulted for a more thorough information.

Chapter 10. References

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