

# ANTHOCYANIN COMPOSITION IN CARIGNAN AND GRENACHE GRAPES AND WINES AS AFFECTED BY PLANT VIGOR AND BUNCH UNIFORMITY

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## Abstract

**Aims:** To determine the anthocyanin composition in Carignan and Grenache grapes and wines as affected by vintage, plant vigor and bunch uniformity.

**Methods and results:** Anthocyanin composition of Carignan and Grenache grapes and wines were analysed by chromatographic techniques considering the influence of two different vigor levels over two vintages. The heterogeneity in the distal parts of the bunch was also taken into account. Warm vintage was better for the accumulation of anthocyanins. However, each variety responded differently according to vine vigor. Grenache anthocyanin synthesis decreased in low vigor (weak) vines, whereas Carignan anthocyanin content depended on vigor, berry size, rootstock and vintage. In both varieties, but more significantly in Carignan, there was a tendency to accumulate acylated anthocyanins in bottom berries.

**Conclusion:** Carignan anthocyanin concentration was increased in low vigor plants, where clusters received greater sun exposure, unlike Grenache, where better canopy management in the fruit zone is necessary. Avoiding the poor growing conditions for Grenache in the region and improving the canopy/fruit ratio deserves careful consideration in order to reach optimal anthocyanin content.

**Significance and impact of the study:** Knowledge of anthocyanin accumulation according to both plant vigor and bunch ripeness is of major importance to determine the optimal harvest date for each cultivar and thus improve the quality of wine.

**Key words:** ripeness variability, vigor, vintage, anthocyanins, Carignan, Grenache

## Résumé

**Objectifs:** Déterminer les teneurs en anthocyanes des raisins et des vins de Carignan et de Grenache sous l'influence de la climatologie du millésime, de la vigueur des plantes et de la variabilité dans les grappes.

**Méthodes et résultats:** Les raisins et les vins de Carignan et de Grenache ont été étudiés par des techniques chromatographiques à deux niveaux différents de vigueur pendant deux années. La variabilité dans les parties distales de la grappe a également été prise en compte. Le millésime chaud était favorable à l'accumulation des anthocyanes. Par contre, chaque variété a réagi différemment en fonction de la vigueur de la vigne. La concentration des anthocyanes en Grenache a diminué dans les conditions de faible vigueur, alors que l'accumulation des anthocyanes en Carignan était fonction de la vigueur, du poids de la baie, du porte-greffe et du millésime. Pour les deux cépages, mais de façon plus significative pour le Carignan, les composés acylés s'accumulaient préférentiellement dans les baies inférieures.

**Conclusion:** La synthèse d'anthocyanes dans le Carignan est favorisée en conditions de faible vigueur, favorables à une bonne exposition des grappes, tandis que la faible vigueur affecte négativement le Grenache. Chez ce dernier, la gestion de la surface foliaire dans la zone fructifère est nécessaire pour maintenir une couverture végétale optimale (surface foliaire/récolte) et protéger les grappes.

**Importance et impact de l'étude:** Connaître la composition en anthocyanes des grappes de Carignan et de Grenache en fonction de la vigueur et de la variabilité de la maturité dans les grappes peut aider à gérer la couverture végétale des plantes afin d'obtenir une concentration d'anthocyanes plus élevée dans les raisins et les vins produits.

**Mots clés:** variabilité de la maturité, vigueur, vintage, anthocyanes, Carignan, Grenache

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## INTRODUCTION

Grapes of red varieties contain large amounts of anthocyanin compounds in the skins, and sometimes in the pulp too. These anthocyanins are partially extracted during winemaking. For the European *Vitis vinifera* species, there are five monoglucoside anthocyanins and their corresponding acylate derivatives (acetyl and p-coumaryl); malvidin-3-*O*-glucoside is the most abundant anthocyanin (Pomar *et al.*, 2005), although in some varieties peonidin-3-*O*-glucoside predominates (Mattivi *et al.*, 2006). The evolution of anthocyanins during veraison and ripening corresponds to different enzymatic activities at the cellular level (Castellarin *et al.*, 2011). In He *et al.* (2010)'s review, two synthetic pathways were described from naringenin flavanone: one that generates anthocyanidin cyanidin from the action of F3'H (flavonoid 3'-hydroxylase), DFR (dihydroflavonol 4-reductase) and ANS (anthocyanidin synthase) and another that generates anthocyanidin delphinidin from the action of F3'5'H (flavonoid 3', 5'-hydroxylase), DFR and ANS. The action of UFGT (UDP glucose : flavonoid-3-*O*-glucosyltransferase) generates cyanidin-3-*O*-glucoside from cyanidin and, finally, peonidin-3-*O*-glucoside by the action of OMT (*O*-methyltransferase). UFGT also synthesizes delphinidin-3-*O*-glucoside from delphinidin and the action of OMT generates petunidin-3-*O*-glucoside and malvidin-3-*O*-glucoside. At the end of the ripening process, the synthesis and accumulation of the non-acylated anthocyanins is slowed down or even stopped whereas there is some increase with the acylated derivatives (p-coumarylated and/or acetylated) (González-San José and Díez, 1992, Jordao *et al.*, 1998, Ryan and Revilla, 2003).

Anthocyanin composition depends on the interaction between climate, soil, viticultural practices and genotype (Jackson and Lombard, 1993, Downey *et al.*, 2006), which involves variations in the expression of genes coding for different enzymes (Yamane *et al.*, 2006). Canopy management modifies the growth and structure of the vine, causing changes in exposure to solar radiation and temperature in the fruit zone (Smart, 1985, Smart, 1987, Bergqvist *et al.*, 2001). Dense canopies increase the level of shading in the fruit zone, causing a reduction in the activity of F3'5'H or an increase in the activity of F3'H and hence an increase in the concentration of dioxygenated anthocyanins: peonidin-3-*O*-glucoside and cyanidin-3-*O*-glucoside (Downey *et al.*, 2004). Some authors, however, found quite the opposite result in colder weather conditions, such as in northern Italy (Chorti *et al.*, 2010) and north-eastern United States (Tarara *et al.*, 2008). Temperature is also a factor that alters the biosynthetic pathway of dioxygenated or trioxxygenated (malvidin-3-*O*-glucoside,

petunidin-3-*O*-glucoside, and delphinidin-3-*O*-glucoside) anthocyanins. According to Cohen *et al.* (2008), the proportion of dioxygenated anthocyanins should increase under conditions of low daytime temperatures. Conversely, Guidoni *et al.* (2008) referred to the sensitivity of the enzyme F3'H to temperature in order to justify the high concentrations of dioxygenated anthocyanins found in Nebbiolo grapes during a warm year as opposed to a cool year.

The distribution of anthocyanins between the acylated and non-acylated forms is altered by the combination of temperature and solar radiation. This is because acyltransferase activity increases with temperature (Haselgrove *et al.*, 2000, Spayd *et al.*, 2002). This combination is complex because it can be synergistic if the temperature is moderate or antagonistic if the temperature is extreme (Tarara *et al.*, 2008). Downey *et al.* (2004) observed that under conditions of moderate temperature and shading, the proportion of p-coumarylated anthocyanins was increased with respect to non-acylated and acetylated anthocyanins. However, if the temperatures were too high, the concentration of total anthocyanins would decrease due to the degradation of non-acylated and acetylated anthocyanins, which are less stable than p-coumarylated anthocyanins (Rodríguez-Saona *et al.*, 1999). This decrease was more pronounced in shaded berries. This suggests that while high temperatures can induce anthocyanin degradation, solar radiation can induce anthocyanin biosynthesis. It also means that there are probably two systems of regulation and accumulation of anthocyanins: on one hand, a system that generates anthocyanins progressively during the ripening period and, on the other hand, a system induced by radiation exposure. However, in warm regions, the prolonged exposure of bunch to direct sunlight and, consequently, to high temperatures, should be avoided in order to prevent the slowdown or shutdown of anthocyanin synthesis and maximize berry color (Spayd *et al.*, 2002, Nadal and Lampreave, 2007, Chorti *et al.*, 2010).

It is well known that the optimum temperature for anthocyanin synthesis is around 30°C, and that higher values up to 35°C inhibit it. Therefore, modification of the vine microclimate through canopy management can prevent excessive sunlight and high temperatures from reaching the bunch and improve anthocyanin content (Downey *et al.*, 2003). Moreover, dense canopies can cause uneven berry ripening within the bunch. This heterogeneity can lead to variations in the final composition of the grape in terms of sugar, acidity, aroma and color (Kasimatis *et al.*, 1975, Tarter and Keuter, 2005, Pagay and Cheng, 2010). It is not an easy task to determine the ideal harvest date, when grapes have reached their optimum composition for a particular

style of wine. In this context, it is necessary to evaluate the ripeness of the pulp and skin to improve the knowledge of the genetic potential of each variety. In red varieties, wine quality depends on the proper ripening of the skin and seeds and the nature of their phenolic compounds. Authors such as Amrani Joutei and Glories (1994) and Ribéreau-Gayon *et al.* (2000) have also suggested using the anthocyanin concentration as a tool for the determination of harvest dates. The extraction of anthocyanins in wine depends mainly on fermentation temperature, skin maceration time (Pérez-Magariño and González-San José, 2004) and winemaking techniques such as the addition of pectolytic enzymes (Amrani Joutei and Glories, 1995). It also depends on the type of molecule: non-acylated anthocyanins are more easily extracted, whereas p-coumarylated anthocyanins are extracted in small amounts in wine (Roggero *et al.*, 1984, Mateus *et al.*, 2001). As for aging, the acylated compounds improve the stability of wine color over the non-acylated compounds (Boulton, 2001).

The main goal of this study is to evaluate the anthocyanin composition in grapes and wines made from *Vitis vinifera* L. cv Carignan and Grenache grown in a Mediterranean climate, as influenced by vine vigor and vintage. As a secondary goal, the ripeness heterogeneity of the distal parts of the bunch is considered.

## MATERIALS AND METHODS

### 1. Site description, plant material and experimental design

The study was carried out in 2007 and 2008 in the Terra Alta Designation of Origin or wine appellation (DO), in the Tarragona area (Spain). Climate, soils and plant material were described in Edo-Roca *et al.* (2013).

Ten-year-old Grenache (Gre) and Carignan (Car) vines were grown in non-irrigated trials. The study was conducted with two different levels of vigor, low (L) and high (H), resulting in four parcels: L-Car; H-Car; L-Gre; and H-Gre. Plants were trained as bush vines and pruned to 5-7 buds in low vigor plants and 9-11 buds in high vigor plants. Soils of low vigor parcels were shallow and had moderate stoniness and good drainage capacity; soils of high vigor parcels were deeper and had higher water holding capacity.

Three plot replications of each vigor/variety combination were randomly distributed in the vineyards, with each elementary plot consisting of 30 vines.

### 2. Fruit sampling and analysis

In order to analyse grape maturity, samples of whole bunch were collected from the four treatments as

described in Edo-Roca *et al.* (2013). Sugars, acids and phenolics were determined in each top and bottom half of the bunch. Carignan clusters are inverted-cone-shaped and their top parts represented up to 60 % of the total weight of the bunch. However, in Grenache, this percentage was increased up to 65 % because the shoulders are located at the top position of the cluster. For each distal part of the bunch, a sample of 100 berries was picked up to determine the sugar level (°Brix), acidity (g·L<sup>-1</sup> tartaric acid) and pH. Another sample of 300 berries was used to analyse phenolics (Nadal, 2010).

## 3. Vine measurements

### 3.1 Vigor

Two vines per replicate were used for vigor measurements, with a total of six vines per treatment. Number of shoots per vine, diameter and length of shoots, number of clusters per vine, berry weight, bunch weight, yield, pruning weight and total leaf area (TLA) were measured in each replicate. Furthermore, the Ravaz index (yield/pruning weight) and the TLA/yield ratio were calculated. TLA was calculated as described in Edo-Roca *et al.* (2013).

### 3.2 Canopy microclimate

Photosynthetically active radiation (PAR) was measured with an AccuPar PAR/LAI ceptometer model LP 80. PAR readings were taken above (rad<sub>abv</sub>) and below (rad<sub>blw</sub>) grapes to evaluate the percentage of non-intercepted radiation on each vine canopy treatment. An additional radiation reading (rad<sub>ref</sub>) was collected to update the above canopy PAR reference in order to reflect spatial changes. In each level (above and below), PAR was measured at every 45°-angle interval in each plane, placing the probe radially, above and below the clusters in a horizontal plane (parallel to the soil surface) centered at the trunk. The average of eight measurements above and eight measurements below was calculated. All measurements were taken at 10 am, on clear, sunny days. The percentage (%) of above canopy radiation collected for each vine (I<sub>abv</sub>) was calculated with both reference radiation (rad<sub>ref</sub>) and radiation above the vine (rad<sub>abv</sub>) using the following equation:  $I_{abv} (\%) = [(rad_{abv})/(rad_{ref})]$ . A similar equation was used when sampling below canopy radiation:  $I_{blw} (\%) = [(rad_{blw})/(rad_{ref})]$ .

## 4. Winemaking and wine analysis

Three replicated wines were made for each treatment, separating the top and bottom half of the bunch, according to red traditional procedures. For each replicate, 10 kg of fruit was harvested, hand-crushed,

and then inoculated with Vitilevure Grenache yeast (Martin Vialatte Oenologie) according to the manufacturer's guidelines. The pomace (must and crushed skins) was punched down two times per day to extract color. Alcoholic fermentation took place at 24°C over 10 days to completely consume the sugars in the medium. After that, 20 g·hL<sup>-1</sup> of sulphur dioxide was added to avoid oxidation, before bottling. The wine did not undergo malolactic fermentation; hence, the wine obtained was young, without any oak or aging process. Chemical wine analyses of alcohol degree (Alc % vol), total acidity (TA), and pH were determined according to OIV methods (OIV, 1990). Total phenol index (TPI) was measured as described by Ribéreau-Gayon *et al.* (2000). Anthocyanin extraction (%) was calculated using the wine anthocyanins/grape anthocyanins ratio.

## 5. Determination and identification of anthocyanins by RRLC-DAD-TOF/MS

### 5.1 Chemicals

All solvents were of HPLC grade. Water, methanol and trifluoroacetic acid were purchased from J.T. Baker (Phillipsburg, NJ, USA). Standard of malvidin-3-*O*-glucoside was purchased from Sigma Aldrich (St. Louis, MO, USA).

### 5.2 Grape and wine preparation

The phenolic maturity of grapes was analysed according to the modified Glories method (Nadal, 2010). The extract (at pH=1; total anthocyanins) was filtered through a 0.22- $\mu$ m PVDF filter before carrying out the analysis of anthocyanins by RRLC-DAD-TOF/MS (rapid resolution liquid chromatography coupled with diode array detection and electrospray ionization time-of-flight mass spectrometry). The same procedure was followed for the wine samples.

### 5.3 Instrumentation

The analysis of grape and wine anthocyanins was performed on a Rapid Resolution Liquid Chromatography RRLC 1200 (Agilent Technologies, USA) comprising a degasser G1379B, a binary pump G1312B, an autosampler G1367C, a thermostatted column compartment G1316B and a diode array detector (DAD) G1316B. The RRLC was coupled to a TOF mass spectrometer G6220A (Agilent Technologies, USA) equipped with an electrospray interface.

### 5.4 Chromatographic conditions

A volume of 5  $\mu$ L of each sample was injected onto a Zorbax Eclipse Plus C18 column (150 x 2.1 mm, 3.5  $\mu$ m; Agilent Technologies) at 50°C. A gradient consisting of solvent A (water/trifluoroacetic acid,

99.8/0.2) and solvent B (methanol/trifluoroacetic acid, 99.8/0.2) was applied at a flow rate of 0.4 mL/min as follows: 10-30 % B from 0 to 7 min, 30-50 % B from 7 to 15 min, 50-100 % B from 15 to 20 min, 100 % B isocratic for 2 min. Malvidin-3-*O*-glucoside was used as a standard at 535 nm to identify and quantify eluting anthocyanins.

### 5.5 Mass spectrometry

Anthocyanin ionization was carried out by electrospray in positive mode. Nitrogen was used as drying gas at 12 L/min and also as nebulizing gas at an inlet pressure of 50 psi and a temperature of 350°C. Quantification was carried out in scan mode from m/z 100 to 1000.

## 6. Statistical analysis

Statistical analysis of data was performed using analysis of variance (ANOVA) to determine statistically significant differences at a significance level of  $p \leq 0.05$ . The *Tukey* test was applied to compare the four established treatments. All statistical analyses were performed using SPSS 17.0 program for Windows.

## RESULTS AND DISCUSSION

### 1. Climate

Terra Alta is a grape growing area with a Mediterranean climate characterized by warm summers and very low rainfall. Based on a 10-year data set (2000-2009), this area can be classified as Region IV of the Winkler scale (Amerine and Winkler, 1944). If the Winkler scale is calculated separately for each year, the area can be classified as Region V in 2007 and as Region IV in 2008. Monthly  $T_m$  (mean temperature) and  $T_{max}$  (maximum temperature) in the spring and summer months (May, June and July) were 1.3°C and 2.0°C higher in 2007 than in 2008, respectively (Table 1).

Regarding total annual rainfall and taking the 10-year average as reference (470 mm), the year 2007 was significantly drier (384 mm) than 2008 (588 mm). In addition, total rainfall from April to September was 180 mm greater in 2008 compared to 2007 and accumulated growing degree days (GDD) were higher in 2007 (2040°C) than in 2008 (1921°C) (data not shown). These data allow us to clearly differentiate the 2007 and 2008 vintages: 2007 was warm and dry, while 2008 was characterized by a temperate climate with higher rainfall and lower temperatures.

### 2. Vine vigor

The vigor treatments tested in this study, low (L) and high (H), were defined by the vegetative measurements and the yield components of each parcel (Tables 2 and

**Table 1. Climatic conditions in 2007 and 2008**

	Tm (°C)		Tmax (°C)		Tmin (°C)		Rainfall (mm)		ET <sub>0</sub> (mm)	
	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Jan	6.4	7.2	11.3	11.5	2.2	3.2	15.6	20.8	25.2	28.3
Feb	9.5	9.3	14.7	13.8	5.3	5.4	14.0	17.8	37.0	37.9
Mar	10.2	10.5	16.2	16.1	5.4	5.9	32.5	7.6	72.9	76.3
Apr	13.3	13.9	18.8	20.5	8.6	8.3	120.8	27.0	81.0	106.2
May	17.5	15.6	23.7	20.7	12.2	11.6	38.8	216.7	131.6	101.9
Jun	21.3	19.9	28.3	25.9	15.3	14.9	7.1	52.5	149.3	144.6
Jul	23.5	23.1	30.4	29.8	17.6	17.5	5.1	21.6	172.4	166.0
Aug	22.9	23.5	30.1	30.3	16.9	17.8	7.1	17.4	149.7	149.4
Sep	19.7	19.1	26.4	25.1	14.3	14.3	14.4	41.8	108.0	98.1
Oct	15.0	14.7	20.2	19.8	11.0	10.4	53.6	59.3	64.4	58.8
Nov	9.2	8.4	14.3	12.5	5.0	4.9	6.2	55.3	42.0	33.7
Dec	6.2	5.3	10.1	8.4	2.8	2.5	69.1	50	24.9	18.6
Annual	14.6	14.2	20.4	19.5	9.7	9.7	384.3	587.8	1058.0	1020.0

Temperature and ET<sub>0</sub> data correspond to monthly averages and rainfall data to monthly totals. Tm, mean temperature; Tmax, maximum temperature; Tmin, minimum temperature; ET<sub>0</sub>, potential evapotranspiration.

4). The high vigor vines (H-Car and H-Gre) generally had three or four shoots more than the weak or low vigor plants (L-Car and L-Gre) and a higher vegetative growth, which was reflected in greater length and diameter of shoots (Table 2). The H vines grew 30-40 cm longer than the L vines, and shoot thickness was between 1.6 and 1.9 mm greater in the H vines except for H-Car/2008. Fertility and TLA were statistically higher in the H vines than in the L vines.

Regarding the “bush vine” canopies, it is worth mentioning the specific type of geometrical shape for each individual treatment (Figure 1). The trunk of the weak L-Car (Fig. 1a) and L-Gre vines (Fig. 1c) was 30- to 32-cm long, and the short, upright shoots took on a tetrahedral shape. The grapes were exposed to high levels of solar radiation, as corroborated by measurements of PAR (70 % in L-Car and 68 % in L-Gre; Table 3). On the other hand, the H-Car vines (Fig. 1b) had 40- to 44-cm long trunks and longer shoots than the L-Car vines. They also took on a tetrahedral shape, although more voluminous than the L-Car vines, with more numerous lateral shoots and a denser canopy. The higher density of leaves in the area above the grapes (fruit zone) resulted in lower sun exposure (59 % radiation) when compared with the L-Car vines (70 % radiation) (Table 3). As for Grenache, the H-Gre vines (Fig. 1d) had smaller trunks (25- to 30-cm long), with

very long shoots that drooped towards the ground, giving the vine an umbrella-like shape. Radiation received above the fruit zone was 67 % (center of the umbrella). However, on the sides of the umbrella, there were several layers of densely packed leaves, increasing the level of shade inside the canopy and thus decreasing the percentage of radiation received below the cluster area (28 %) (Table 3).

Regarding the yield components, high vigor plants had grape yields (kg fruit-vine<sup>-1</sup>) two times higher than low vigor plants and statistically greater berry weights (Table 4). In Carignan, bunches tended to be heavier in H-Car in both vintages, while in Grenache differences were only perceived in 2008. The greatest growth rates of H-Car and H-Gre were linked to a higher yield and pruning weight. The Ravaz index and TLA/yield parameters were taken into account to provide information about the balance between vegetative and reproductive growth. Significant differences between treatments in Ravaz index values were observed during the dry vintage (i.e., 2007) in both varieties; the L vines gave a higher index than the H vines. The TLA/yield ratio varied depending on the vintage and vigor in Carignan, showing the highest value (1.8 m<sup>2</sup>·kg<sup>-1</sup>) in low vigor vines and in temperate year (L-Car/2008). The Grenache treatments showed ratios between 0.9 and 1.4.

**Table 2. Growth measurements for vine vigor classification**

	# of shoots	Length of shoots (cm)	Diameter of shoots (mm)	# of clusters·vine <sup>-1</sup>	Total leaf area (m <sup>2</sup> ·vine <sup>-1</sup> )
2007					
L-Car	6.3 (0.6) b	73.7 (9.4) b	9.8 (1.3) b	9.3 (1.2) b	3.0 (0.4) b
H-Car	9.3 (1.2) a	115.3 (19.5) a	11.7 (1.0) a	12.0 (1.7) a	4.0 (0.7) a
2008					
L-Car	5.3 (0.6) b	102.9 (23.2) b	10.5 (1.5)	12.0 (2.7)	3.9 (0.6) b
H-Car	10.7 (1.5) a	131.1 (24.2) a	9.8 (0.9)	13.7 (3.5)	5.7 (1.0) a
2007					
L-Gre	5.3 (1.5) b	102.3 (19.5) b	11.6 (2.3) b	6.5 (1.5) b	2.6 (0.9) b
H-Gre	9.3 (1.5) a	133.7 (12.3) a	13.4 (1.0) a	13.3 (1.2) a	5.9 (0.9) a
2008					
L-Gre	7.0 (1.7)	68.4 (19.6) b	10.0 (1.8) b	10.7 (3.8) b	3.4 (0.8) b
H-Gre	10.0 (2.7)	114.3 (28.9) a	11.6 (1.3) a	16.0 (1.0) a	5.3 (1.1) a

Mean value and standard deviation. ANOVA analysis and significant differences by *Tukey* test ( $p \leq 0.05$ ) are indicated by different letters (a, b). #: number. Treatments are low (L) and high (H) vigor Carignan (Car) and Grenache (Gre) vines.



**Figure 1. Specific geometrical shape of vine canopy in the different vigor treatments. a: Low vigor Carignan vine (L-Car), b: High vigor Carignan vine (H-Car), c: Low vigor Grenache vine (L-Gre), and d: High vigor Grenache vine (H-Gre).**

### 3. Berry composition

Referring to the whole bunch, the values of sugar content ( $^{\circ}$ Brix), total acidity (TA) and total phenol index (TPI) showed significant differences between L and H treatments, except for TPI in Car/2008 and Gre/2007 (Table 5). The smaller berries, which were typically found in L-Car and L-Gre, tended to have higher  $^{\circ}$ Brix and TPI values. Actually, the grapes in low vigor

treatments were well exposed to sunlight compared with high vigor treatments. Several authors have noted the relationship between lower berry volume and higher sugar concentration (Koundouras *et al.*, 2006, Conde *et al.*, 2007), although others have associated the increase of sugar content in heavier berries with increased photosynthetic activity (Matthews and Anderson, 1988, Santesteban and Royo, 2006). Reynolds *et al.* (1986) found higher concentrations of soluble solids in grapes

**Table 3. Percentage of non-intercepted solar radiation into the vine canopy**

	I <sub>abv</sub> (%)	I <sub>blw</sub> (%)
L-Car	70 (17) a	39 (18) a
H-Car	59 (11) b	26 (16) b
L-Gre	68 (11)	39 (17) a
H-Gre	67 (15)	28 (15) b

Mean value and standard deviation. ANOVA analysis and significant differences by *Tukey* test ( $p \leq 0.05$ ) are indicated by different letters (a, b). I<sub>abv</sub>, above the fruit zone; I<sub>blw</sub>, below the fruit zone. Treatments are low (L) and high (H) vigor Carignan (Car) and Grenache (Gre) vines.

that were well exposed to sunlight. This discrepancy between different studies is due to multiple factors (e.g., climate, soil and cultural techniques) that influence the berry composition in each wine region.

Concerning the heterogeneity in bunch ripeness, the differences in °Brix values ranged from 0.4 (L-Car/2007) to 1.5 units (L-Gre/2008) between berries from the top and bottom half of the bunch (Table 5). In Carignan, top berries were usually well ripe in L-Car, while bottom berries accumulated more sugars in H-Car, depending on vintage. Grenache berries accumulated more sugars on the top half of the bunch, regardless of vigor and vintage. TA tended to reach higher concentration in the bottom half of the bunch, with significant differences in H-Car/2007 and L-Gre/2007 treatments. TPI values did not differ between top and bottom berries. As a result,

heterogeneity was more pronounced in terms of sugar content than acidity.

There are relatively few studies on the heterogeneity of berry ripeness within a bunch. In this respect, the work of Kasimatis *et al.* (1975) on the Thompson Seedless variety grown in California stands out. They also observed a decrease in sugar content from the top to the bottom of the bunch. By contrast, Tarter and Keuter (2005) found that Cabernet Sauvignon berries at the bottom of the bunch achieved higher soluble solid concentrations than those at the top. When grown in cool climate vineyards (New York), Pagay and Cheng (2010) observed a higher accumulation of soluble solids at the bottom of Concord and Cabernet Franc grape bunch. So, variety and climate factors deserve special attention because they have a noteworthy effect on the heterogeneity of berry ripening.

#### 4. Anthocyanin composition in grapes

The chromatographic determination of anthocyanins showed higher concentrations of these compounds in the warm and dry vintage, in both Carignan and Grenache (Table 6). Vineyards whose vines are stressed usually favor phenol synthesis (Sivilotti *et al.*, 2005), although in hot climates the opposite can be observed (Ryan and Revilla, 2003).

##### 4.1 Vigor

###### a. Carignan

In general, the L-Car berries accumulated more anthocyanins ( $\text{mg} \cdot \text{kg}^{-1}$  fruit;  $\text{mg} \cdot \text{berry}^{-1}$ ) than their

**Table 4. Yield components of low (L) and high (H) vigor Carignan (Car) and Grenache (Gre) vines**

	Berry weight (g)	Bunch weight (g)	Crop ( $\text{kg} \cdot \text{vine}^{-1}$ )	Pruning weight (g)	Ravaz index	TLA/yield ( $\text{m}^2 \cdot \text{kg}^{-1}$ )
2007						
L-Car	1.6 (0.1) b	317 (91) b	2.9 (0.4) b	282 (61) b	10.7 (1.5) a	1.0 (0.2) a
H-Car	2.3 (0.3) a	425 (84) a	5.1 (1.0) a	710 (16) a	7.3 (1.2) b	0.8 (0.1) b
2008						
L-Car	2.0 (0.1) b	188 (35) b	2.3 (0.5) b	420 (124) b	5.4 (1.2)	1.8 (0.6) a
H-Car	2.2 (0.1) a	359 (128) a	4.9 (1.0) a	734 (107) a	6.7 (2.7)	1.3 (0.6) b
2007						
L-Gre	1.6 (0.1) b	397 (119)	2.6 (0.6) b	264 (111) b	10.6 (3.7) a	1.0 (0.4)
H-Gre	2.0 (0.2) a	327 (102)	4.4 (0.4) a	659 (55) a	6.6 (0.5) b	1.4 (0.3)
2008						
L-Gre	1.5 (0.1) b	269 (59) b	2.9 (1.0) b	318 (65) b	9.0 (3.2)	1.2 (0.1) a
H-Gre	1.9 (0.1) a	380 (134) a	6.1 (0.4) a	744 (77) a	8.2 (0.5)	0.9 (0.2) b

Mean value and standard deviation. ANOVA analysis and significant differences by *Tukey* test ( $p \leq 0.05$ ) are indicated by different letters (a, b). TLA, total leaf area

**Table 5. Chemical characteristics of clusters (whole cluster and top and bottom berries) from low (L) and high (H) vigor Carignan (Car) and Grenache (Gre) vines at harvest**

	Cluster heterogeneity			Vine vigor		
	°Brix	TA (g·L <sup>-1</sup> tartaric)	TPI	°Brix	TA (g·L <sup>-1</sup> tartaric)	TPI
2007						
L-Car				21.8 (0.2)	7.2 (0.1)	50.2 (1.1)
top	21.9 (0.2) a	7.3 (0.1)	50.0 (2.1)			
bottom	21.5 (0.1) b	7.2 (0.1)	50.7 (0.8)			
H-Car				21.3 (0.1)	5.8 (0.1)	44.3 (1.8)
top	20.3 (0.1) b	5.6 (0.1) b	44.0 (1.6)			
bottom	21.5 (0.0) a	5.8 (0.1) a	44.8 (2.3)			
				*	*	*
2008						
L-Car				23.8 (0.9)	7.7 (0.1)	49.9 (3.1)
top	23.4 (1.0)	7.7 (0.2)	48.9 (2.0)			
bottom	24.4 (0.7)	7.7 (0.2)	51.4 (4.7)			
H-Car				22.5 (0.4)	6.8 (0.3)	44.6 (2.0)
top	22.3 (0.2)	7.0 (0.5)	45.0 (1.0)			
bottom	22.8 (1.0)	6.7 (0.3)	44.1 (4.1)			
				*	*	NS
2007						
L-Gre				26.0 (0.1)	4.9 (0.1)	53.4 (0.9)
top	26.2 (0.2) a	4.7 (0.1) b	54.4 (1.1)			
bottom	25.4 (0.4) b	5.3 (0.2) a	51.8 (0.5)			
H-Gre				25.7 (0.1)	5.7 (0.1)	51.3 (0.8)
top	25.9 (0.1) a	5.7 (0.1)	52.2 (0.7)			
bottom	25.5 (0.1) b	5.8 (0.1)	49.1 (1.1)			
				*	*	NS
2008						
L-Gre				23.2 (0.1)	4.3 (0.4)	50.2 (1.9)
top	23.6 (0.2) a	4.1 (0.7)	50.8 (2.2)			
bottom	22.1 (0.1) b	4.7 (0.2)	49.1 (1.1)			
H-Gre				22.0 (0.5)	5.7 (0.3)	44.2 (0.7)
top	22.6 (0.9)	5.6 (0.4)	44.9 (0.5)			
bottom	21.3 (1.4)	6.0 (0.2)	43.2 (1.2)			
				*	*	*

Mean value and standard deviation. ANOVA analysis and significant differences between the top and bottom half of the bunch (*Tukey* test,  $p \leq 0.05$ ) are indicated by different letters (a, b). Significant differences between low (L) and high (H) vigor treatments (*Tukey* test,  $p \leq 0.05$ ) are indicated by (\*). NS: not significant. TA, total acidity; TPI, total phenol index.

vigorous counterparts, in both vintages (Table 6). The skin/pulp ratio corroborates that anthocyanin biosynthesis was favored in the berries of weak vines (L-Car), which were smaller than the berries of high vigor vines (H-Car). Furthermore, light and temperature in the lowest vegetative density may have had a favorable effect on the synthesis and accumulation of anthocyanins. As mentioned, the radiation intercepted by the canopy showed a difference of 11 % in the fruit zone (70 % in L-Car vs. 59 % in H-Car) and 13 % below the fruit zone (39 % in L-Car vs. 26 % in H-Car) (Table 3). In fact, authors such as Smart (1985), Bergqvist *et al.* (2001) and Spayd *et al.* (2002) claimed that sun-exposed berries contained higher levels of soluble solids and anthocyanins. Regarding the rootstock, although 110R is known to favor vine vigor (Williams, 2010), the H-Car/41B vines growing in deeper soils showed greater canopy development than the L-Car/110R vines. This fact, together with the low water holding capacity of the soil, suggested a possible effect of the rootstock on anthocyanin content.

Concerning the synthesis of acylated glucosides, Carignan gave a different response depending on vintage and vigor (Table 6a). It reached higher concentrations ( $\text{mg}\cdot\text{kg}^{-1}$  fruit) in L-Car/2007 and H-Car/2008, while the concentration of acylated anthocyanins per berry ( $\text{mg}\cdot\text{berry}^{-1}$ ) was significantly higher in H-Car in both vintages. The 2008 data for p-coumarylated anthocyanins showed a greater concentration in the vigorous treatment ( $85.1 \text{ mg}\cdot\text{kg}^{-1}$  in H-Car/2008 vs.  $71.9 \text{ mg}\cdot\text{kg}^{-1}$  in L-Car/2008) (data not shown).

Concerning the individual non-acylated glucosides, low vigor favored the synthesis of trioxxygenated (dp-glc, mv-glc and pt-glc) and dioxygenated (cy-glc and pn-glc) glucosides in both warm (2007) and temperate (2008) vintages compared to high vigor (Table 6b). However, the synthesis of trioxxygenated was favored over that of dioxygenated anthocyanins in the warm vintage, while in the temperate vintage, mv-glc, pt-glc and pn-glc were the most favored. This result contradicts previous studies. For instance, Downey *et al.* (2004) and Ristic *et al.* (2007) noted an increase in the synthesis of dioxygenated glucosides (pn-glc and cy-glc) in shaded Shiraz grapes, while the accumulation of trioxxygenated glucosides decreased.

## b. Grenache

Grapes from H-Gre had greater anthocyanin content ( $\text{mg}\cdot\text{kg}^{-1}$ ;  $\text{mg}\cdot\text{berry}^{-1}$ ) than those from L-Gre (Table 6). As a consequence, berry size did not display any influence on berry anthocyanin content. Regarding the rootstock, H-Gre/41B grew much better in silty loam soils than L-Gre/110R in sandy loam soils with lower

water supply. Nevertheless, Ezzahouani and Williams (1995) did not find significant differences in yield and must composition between 41B and 110R growing in the same vineyard. Thus, rootstock and soil characteristics might have a joint effect on the anthocyanin content in berries. So, the balanced vigor of the H-Gre vines has a positive effect on Grenache quality. As mentioned above, even though leaf area was greater in H-Gre than L-Gre vines (Table 2), the fruit zone showed similar radiation in both vigor treatments (Table 3). However, in the zone below, L-Gre vines received 11 % more radiation than H-Gre. Therefore, the lower anthocyanin synthesis in L-Gre berries could be due to a general depression in plant growth that inhibited secondary metabolism. Grenache is an isohydric variety that is very resistant to drought (Schultz, 2003). The lower berry weight produced by the L-Gre vines (25 % lower than the H-Gre vines) might be due to changes in plant water balance and could adversely affect the synthesis of anthocyanins. These results suggest a greater sensitivity to high temperatures in weak Grenache vines (L-Gre).

Regarding the acylated compounds, there were no significant differences between vigor treatments during the warm vintage (Table 6a). In the temperate vintage, the concentration of acylated anthocyanins ( $\text{mg}\cdot\text{kg}^{-1}$ ;  $\text{mg}\cdot\text{berry}^{-1}$ ) was statistically higher in H-Gre vines. The positive effect of shading on the synthesis of acylated glucosides is well known (Haselgrove *et al.*, 2000, Downey *et al.*, 2006, Tarara *et al.*, 2008) and was again observed here with both varieties.

Regarding the individual non-acylated anthocyanins, mv-glc and pn-glc were mainly accumulated in H-Gre treatments, in both warm and temperate vintages (Table 6b). However, in the temperate vintage, the accumulation of non-acylated glucosides at harvest decreased, irrespective of vigor, with mv-glc and pn-glc remaining the most abundant.

By contrast, studies such as those by Cortell and Kennedy (2006) and Cortell *et al.* (2007) with Pinot noir (similar to Grenache in terms of low color content) revealed that high sun exposure had a positive influence on berry total anthocyanin accumulation and that anthocyanin composition in shaded berries was shifted towards dioxygenated anthocyanins. However, Chorti *et al.* (2010) observed a decrease of dioxygenated and an increase of trioxxygenated anthocyanins in shaded Nebbiolo grapes. Other works such as that by Guidoni *et al.* (2008) with Nebbiolo referred to the sensitivity of the enzyme F3'H to temperature to explain the high concentrations of dioxygenated glucosides in a warm vintage compared to a cool vintage. Together, this confirms that anthocyanin composition is different

**Table 6. Grape anthocyanin concentration in low (L) and high (H) vigor Carignan (Car) and Grenache (Gre) vines at harvest**  
**(A) Non-acylated and acylated anthocyanins - (B) Individual non-acylated anthocyanin (mg·kg<sup>-1</sup>)**

A		non-acylated (mg·kg <sup>-1</sup> )	acylated (mg·kg <sup>-1</sup> )	non-acylated (mg·berry <sup>-1</sup> )	acylated (mg·berry <sup>-1</sup> )
	2007				
	L-Car	529.4 (6.4) a	154.2 (2.3) a	0.83 (0.01) a	0.24 (0.01) b
	H-Car	346.1 (13.6) b	139.1 (8.2) b	0.79 (0.04) b	0.32 (0.01) a
	2008				
	L-Car	410.7 (31.3) a	93.7 (3.7) b	0.82 (0.08) a	0.19 (0.01) b
	H-Car	237.8 (3.7) b	102.3 (2.9) a	0.52 (0.01) b	0.22 (0.01) a
	2007				
	L-Gre	137.5 (39.8) b	23.9 (6.4)	0.24 (0.02) b	0.04 (0.01)
	H-Gre	209.8 (13.3) a	26.7 (1.0)	0.41 (0.03) a	0.05 (0.003)
	2008				
	L-Gre	84.0 (1.8) b	9.1 (0.5) b	0.12 (0.01) b	0.01 (0.10) b
	H-Gre	145.3 (11.4) a	12.9 (2.2) a	0.28 (0.04) a	0.03 (0.01) a

B		dp-glc	mv-glc	cy-glc	pt-glc	pn-glc
	2007					
	L-Car	85.5 (2.0) a	339.9 (2.3) a	5.8 (0.6) a	77.8 (2.1) a	20.4 (1.3) a
	H-Car	55.7 (0.9) b	227.7 (9.9) b	3.2 (0.2) b	50.9 (2.8) b	8.6 (0.6) b
	2008					
	L-Car	6.7 (0.2) a	350.4 (30.9) a	4.8 (0.1) a	26.6 (0.7) a	22.3 (0.7) a
	H-Car	5.0 (0.1) b	210.3 (3.5) b	1.7 (0.1) b	11.1 (0.1) b	9.8 (0.1) b
	2007					
	L-Gre	8.0 (3.1)	106.7 (30.0) b	1.6 (0.4) b	10.3 (4.3) b	10.9 (3.7) b
	H-Gre	12.5 (0.9)	157.9 (10.2) a	2.8 (0.4) a	18.1 (1.3) a	18.2 (1.0) a
	2008					
	L-Gre	0.7 (0.1)	67.7 (1.6) b	0.3 (0.0) a	4.6 (0.2) b	10.8 (0.1) b
	H-Gre	0.7 (0.1)	122.1 (10.7) a	0.2 (0.1) b	5.7 (0.4) a	16.5 (0.3) a

Mean value and standard deviation. ANOVA analysis and significant differences by *Tukey* test ( $p \leq 0.05$ ) are indicated by different letters (a, b). dp-glc: delphinidin-3-*O*-monoglucoside; mv-glc: malvidin-3-*O*-monoglucoside; cy-glc: cyanidin-3-*O*-monoglucoside; pt-glc: petunidin-3-*O*-monoglucoside; pn-glc: peonidin-3-*O*-monoglucoside. 2007, warm, dry vintage; 2008, temperate vintage.

depending on the variety, the growing area and the environmental conditions.

#### 4.2 Bunch ripeness uniformity

As observed with different vigor treatments and vintages, noticeable heterogeneity in anthocyanin accumulation was observed in Carignan bunch (top vs. bottom berries) (Table 7). The concentration of non-acylated anthocyanins was higher in top berries (Car) in

the warm vintage, while the opposite was found in the temperate vintage (Table 7a). In terms of non-acylated anthocyanins accumulated per berry, the same pattern was observed, although the differences were significant only in H-Car vines. In both vintages, bottom Car berries tended to have a higher concentration of acylated compounds (mg·kg<sup>-1</sup>) than top berries, although the statistical analyses revealed fewer differences between top and bottom berries when the accumulation per berry (mg·berry<sup>-1</sup>) was considered.

Conversely, the synthesis of anthocyanins in Grenache bunch was quite uniform. Significant differences were only found in the L-Gre treatment, in both vintages. The accumulation of non-acylated anthocyanins was higher in the top berries in 2007 and in the bottom berries in 2008. The same pattern was observed for acylated anthocyanins, although not statistically significant.

Regarding the individual non-acylated anthocyanins, there were few statistically significant differences within a bunch in both varieties (Table 7b). In fact, only H-Car showed a larger heterogeneity in temperate year. However, mv-glc tended to reach higher concentration in

the top berries in the warm vintage and in bottom berries in the temperate vintage, irrespective of vigor.

From these results, it can be noted that the uniformity of anthocyanin accumulation within bunches depends on the variety, as each variety responded differently to the vigor and vintage effects.

## 5. Wine composition

Slight differences in wine composition were found between vigor treatments (Table 8). Specifically, significant differences were observed in total phenols in

**Table 7. Anthocyanin concentration in berries (top vs. bottom half of the bunch) from low (L) and high (H) vigor Carignan (Car) and Grenache (Gre) vines**

**(A) Non-acylated and acylated anthocyanins - (B) Individual non-acylated anthocyanin (mg·kg<sup>-1</sup>)**

A	non-acylated (mg·kg <sup>-1</sup> )	acylated (mg·kg <sup>-1</sup> )	non-acylated (mg·berry <sup>-1</sup> )	acylated (mg·berry <sup>-1</sup> )
2007				
L-Car				
top	547.5 (8.9) a	146.6 (5.1) b	0.84 (0.02)	0.23 (0.01) b
bottom	501.0 (12.0) b	167.1 (11.6) a	0.80 (0.06)	0.27 (0.03) a
H-Car				
top	350.8 (12.9) a	133.1 (2.6)	0.85 (0.09) a	0.32 (0.04)
bottom	317.9 (16.2) b	140.5 (18.2)	0.68 (0.05) b	0.30 (0.04)
2008				
L-Car				
top	381.6 (16.9) b	88.3 (4.1) b	0.78 (0.02)	0.18 (0.01)
bottom	452.7 (52.1) a	101.5 (3.1) a	0.89 (0.16)	0.20 (0.02)
H-Car				
top	215.7 (1.9) b	95.5 (3.7) b	0.48 (0.01) b	0.21 (0.01) b
bottom	273.0 (12.4) a	113.2 (1.7) a	0.59 (0.03) a	0.24 (0.003) a
2007				
L-Gre				
top	164.6 (26.4) a	29.3 (10.4)	0.26 (0.03) a	0.05 (0.01)
bottom	128.2 (10.5) b	26.0 (2.1)	0.19 (0.02) b	0.04 (0.003)
H-Gre				
top	214.6 (19.2)	26.5 (1.5)	0.42 (0.04)	0.05 (0.005)
bottom	192.5 (6.7)	26.1 (1.5)	0.41 (0.03)	0.06 (0.001)
2008				
L-Gre				
top	73.1 (5.7) b	8.2 (0.9) b	0.11 (0.02) b	0.01 (0.002)
bottom	106.8 (6.2) a	10.9 (0.4) a	0.15 (0.01) a	0.02 (0.001)
H-Gre				
top	138.3 (26.9)	11.6 (3.6)	0.26 (0.06)	0.02 (0.01)
bottom	154.5 (9.1)	14.6 (0.3)	0.31 (0.01)	0.03 (0.003)

**B**

	dp-glc	mv-glc	cy-glc	pt-glc	pn-glc
2007					
L-Car					
top	90.0 (3.0) a	349.3 (5.0) a	6.3 (0.6)	80.7 (2.2) a	21.4 (1.0)
bottom	78.3 (1.7) b	325.3 (4.6) b	5.1 (0.5)	73.4 (2.8) b	18.9 (2.5)
H-Car					
top	56.7 (0.9) a	229.8 (9.3)	3.3 (0.3)	52.4 (2.2)	8.6 (0.6)
bottom	50.7 (1.6) b	210.7 (11.1)	2.9 (0.4)	45.4 (4.1)	8.1 (0.6)
2008					
L-Car					
top	6.7 (0.3)	321.2 (16.2)	4.8 (0.1)	26.2 (0.8)	22.7 (0.9)
bottom	6.6 (0.1)	392.7 (52.1)	4.8 (0.04)	27.0 (0.6)	21.6 (0.7)
H-Car					
top	4.9 (0.0)	189.6 (1.9) b	1.8 (0.02) a	10.5 (0.02) b	8.9 (0.0) b
bottom	5.1 (0.2)	243.2 (12.0) a	1.4 (0.03) b	12.1 (0.3) a	11.2 (0.1) a
2007					
L-Gre					
top	10.3 (3.6)	126.7 (18.6)	2.0 (0.3)	12.7 (4.8)	12.8 (2.8)
bottom	5.2 (0.3)	102.5 (10.3)	1.3 (0.4)	8.5 (0.7)	10.7 (0.4)
H-Gre					
top	13.3 (1.4) a	160.4 (14.5)	3.2 (0.7)	18.9 (2.0) a	18.8 (1.4)
bottom	10.8 (0.4) b	147.3 (5.0)	2.1 (0.1)	15.8 (0.6) b	16.4 (1.0)
2008					
L-Gre					
top	0.7 (0.1)	56.4 (5.1) b	0.4 (0.01)	4.7 (0.4)	10.9 (0.1)
bottom	0.7 (1·10 <sup>-2</sup> )	91.2 (5.8) a	ND	4.4 (0.1)	10.5 (0.4)
H-Gre					
top	0.7 (0.2)	113.5 (24.3)	0.2 (0.1)	5.9 (1.0)	18.0 (1.4) a
bottom	0.7 (0.1)	133.5 (7.2)	0.1 (0.01)	5.6 (0.5)	14.5 (1.3) b

Mean value and standard deviation. ANOVA analysis and significant differences by *Tukey* test ( $p \leq 0.05$ ) are indicated by different letters (a, b). dp-glc: delphinidin-3-*O*-monoglucoside; mv-glc: malvidin-3-*O*-monoglucoside; cy-glc: cyanidin-3-*O*-monoglucoside; pt-glc: petunidin-3-*O*-monoglucoside; pn-glc: peonidin-3-*O*-monoglucoside. ND, not determined. 2007, warm, dry vintage; 2008, temperate vintage.

the temperate vintage, leading to a significant decrease in total phenol index in the H-Car/2008 and H-Gre/2008 treatments. Furthermore, the H-Gre/2008 treatment led to wines with a lower level of alcohol than L-Gre/2008, and the acidity level was reduced in L-Gre/2007 wines compared to H-Gre/2007 wines.

Comparing both vintages, the alcoholic degree was only lower in H-Gre/2008, showing a lack of bunch ripeness in this treatment. In the same year, the Carignan harvest date was delayed by two weeks due to the slow ripening process, thus achieving higher berry sugar content than

in 2007 (because of the absence of rainfall during the last weeks of the maturation process).

## 6. Anthocyanin composition in wine

Carignan and Grenache wines showed the highest concentrations of total anthocyanins in the dry and warm vintage, as observed in the grapes (Table 9). However, the nature of the anthocyanins led to variability in the extraction ratios (data not shown) and, as a consequence, the anthocyanin composition of the wines varied with respect to the grapes. In that respect, De Villiers *et al.* (2004) found that non-acylated glucosides were more

**Table 8. Chemical characteristics of Carignan and Grenache wines according to low (L) and high (H) vigor treatments**

	Alc % vol	TA (g·L <sup>-1</sup> tartaric)	pH	TPI
2007				
L-Car	11.8 (1.0)	5.7 (0.2)	3.3 (0.1)	24.1 (3.6)
H-Car	11.9 (0.4)	5.4 (0.3)	3.3 (0.1)	23.5 (3.8)
2008				
L-Car	14.3 (0.6)	5.6 (0.3)	3.1 (0.1) b	48.0 (2.7) a
H-Car	14.1 (0.1)	5.2 (0.2)	3.4 (0.1) a	41.4 (1.1) b
2007				
L-Gre	15.1 (0.9)	4.4 (0.4) b	3.6 (0.1)	29.8 (10.8)
H-Gre	14.0 (1.4)	5.4 (0.3) a	3.4 (0.1)	28.0 (6.2)
2008				
L-Gre	15.1 (0.3) a	5.4 (0.3)	3.6 (0.1)	35.7 (1.3) a
H-Gre	13.0 (0.4) b	5.9 (0.4)	3.4 (0.1)	28.9 (2.4) b

Mean value and standard deviation. ANOVA analysis and significant differences by *Tukey test* ( $p \leq 0.05$ ) are indicated by different letters (a, b). Alc, alcoholic degree; TA, total acidity; TPI, total phenol index. 2007, warm, dry vintage; 2008, temperate vintage.

easily extracted, followed by acetylglucosides and, finally, p-coumarylated glucosides, which are the most difficult to extract from grapes to wine. In this study, the non-acylated compounds were also found at higher concentrations than their acylated counterparts.

### 6.1 Vigor

The wine results showed few significant differences between treatments (Table 9) when compared with those found in grapes (Table 6a). The Carignan wines only showed significant differences between vigor treatments in 2008, with higher total anthocyanin concentration in L-Car. In Grenache wines, there were no differences in non-acylated anthocyanin concentration between treatments in both vintages. However, the acylated glucosides, despite showing higher concentrations in the warm vintage, only differed statistically between vigor treatments in 2008, showing slightly higher contents in H-Gre.

### 6.2 Bunch ripeness uniformity

The composition of Carignan and Grenache wines made from the top and bottom half of clusters separately are not shown because there was practically no significant difference between these wines. Only a certain heterogeneity in the anthocyanin composition was observed (Table 10). In Carignan, the concentration of acylated glucosides was higher in wines made from the bottom half of the bunch but only in the L treatment for both vintages (L-Car/2007 and L-Car/2008). The

significant differences in H-Car/2008 showed a higher concentration of non-acylated and acylated anthocyanins in wines made from the top half of clusters. In Grenache, the concentration of non-acylated anthocyanins in H-Gre/2007 and H-Gre/2008 was higher in wines made from the top half of clusters. However, the acylated glucosides accumulated better in the wines made from the bottom half of clusters in the temperate vintage (L-Gre/2008 and H-Gre/2008). Taken together, anthocyanin composition in wine showed a certain heterogeneity for both Carignan and Grenache, which was more evident in the temperate than in the warm vintage, and in high vigor than in weak vigor conditions. However, this variability was lower in wines than in grapes.

## CONCLUSIONS

Vigor and vintage significantly affect the anthocyanin composition of the grapes and wines from both varieties. The synthesis and accumulation of anthocyanins is more favorable in a warm vintage, although the response in Carignan and Grenache is different according to plant vigor. Carignan grapes accumulate more anthocyanins in low vigor vines. Conversely, anthocyanin accumulation in Grenache increases in high vigor vines. The low vigor of Grenache together with the high temperatures of warm and dry years (common in a Mediterranean climate) probably leads to a slowing down of the secondary metabolism of berry ripening. On the other hand,

**Table 9. Anthocyanin concentration in Carignan and Grenache wines according to low (L) and high (H) vigor treatments**  
**Mean value (mg·L<sup>-1</sup>) and standard deviation. ANOVA analysis and significant differences by *Tukey* test (p≤0.05) are indicated by different letters (a, b). 2007, warm, dry vintage; 2008, temperate vintage.**

	non-acylated	acylated		non-acylated	acylated
2007			2007		
L-Car	177.3 (47.7)	46.3 (8.5)	L-Gre	102.2 (18.9)	12.5 (1.3)
H-Car	197.4 (24.8)	50.3 (10.1)	H-Gre	91.8 (3.6)	10.1 (1.8)
2008			2008		
L-Car	238.2 (12.1) a	9.5 (0.2) a	L-Gre	73.7 (24.7)	2.4 (0.4) b
H-Car	175.5 (17.6) b	4.1 (0.6) b	H-Gre	84.8 (2.1)	3.8 (0.4) a

Mean value (mg·L<sup>-1</sup>) and standard deviation. ANOVA analysis and significant differences by *Tukey* test (p≤0.05) are indicated by different letters (a, b). 2007, warm, dry vintage; 2008, temperate vintage.

**Table 10. Anthocyanin concentration in Carignan and Grenache wines according to berry position within the cluster (top vs. bottom half of the cluster)**

	non-acylated	acylated		non-acylated	acylated
2007			2007		
L-Car			L-Gre		
top	184.7 (48.2)	35.9 (5.9) b	top	108.0 (16.1)	12.7 (1.3)
bottom	165.7 (52.6)	63.6 (14.3) a	bottom	90.9 (32.9)	12.2 (1.6)
H-Car			H-Gre		
top	206.2 (18.0)	52.4 (12.1)	top	99.2 (7.0) a	10.1 (3.7)
bottom	171.2 (40.7)	43.8 (8.3)	bottom	76.4 (3.6) b	9.8 (2.3)
2008			2008		
L-Car			L-Gre		
top	254.0 (24.0)	6.7 (2.1) b	top	69.1 (24.1)	1.5 (0.3) b
bottom	215.4 (64.1)	13.6 (2.6) a	bottom	83.3 (25.9)	4.3 (0.4) a
H-Car			H-Gre		
top	221.1 (0.8) a	5.0 (0.3) a	top	104.8 (6.6) a	3.4 (0.3) b
bottom	102.9 (46.8) b	2.7 (1.2) b	bottom	58.2 (3.8) b	4.2 (0.5) a

Mean value (mg·L<sup>-1</sup>) and standard deviation. ANOVA analysis and significant differences by *Tukey* test (p≤0.05) are indicated by different letters (a, b). 2007, warm, dry vintage; 2008, temperate vintage.

Carignan is more affected by rootstock and berry size than Grenache. An interaction between rootstock and the soil water holding capacity is observed: 110R in shallow soils favors the synthesis of anthocyanins in Carignan berries, while 41B favors Grenache. However, the differences observed in grapes almost vanished in wines; only L-Car wines showed statistically higher anthocyanin content than H-Car wines in 2008.

The cluster heterogeneity reveals a tendency to accumulate acylated anthocyanins in berries positioned

at the bottom of the bunch, which is more pronounced in Carignan than in Grenache. Sugar and anthocyanin content within bunches depends on the variety as well as on the vigor and vintage effects. Grenache ripened more uniformly than Carignan, showing only differences in weak vines. In addition, the top berries had higher anthocyanin content than the bottom berries in 2007, contrary to 2008. Carignan ripeness is less predictable; warm conditions and extended maturation can improve the sugar and anthocyanin content. In the wines obtained from top and bottom berries, this

variability practically disappears in warm vintage. However, a larger heterogeneity still remains in temperate year, more likely affecting high vigor than low vigor treatment.

The current study highlights different performances for each variety: Grenache is very much influenced by vigor, as anthocyanin accumulation was favored in balanced high vigor vines, while Carignan anthocyanin content varies under the combined effect of vigor, rootstock, berry size and vintage. In warm and dry Mediterranean climates, creating an ideal microclimate for vines helps increase anthocyanin synthesis. Specifically, this means that canopy management in Carignan must be aimed at maximizing grape exposure to sunlight, whereas the opposite must be achieved with Grenache, where the grapes should be protected from sunlight. Moreover, the Carignan harvest date may be delayed in more temperate vintages and under high vigor conditions in order to improve bunch uniformity and achieve higher sugar and anthocyanin levels. In any case, phenolic maturity must be supervised to avoid undesirable overripeness, which counts against grape quality.

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