HOW TERROIR AFFECTS BUNCH UNIFORMITY, RIPENING AND BERRY COMPOSITION IN *VITIS VINIFERA* CVS. CARIGNAN AND GRENACHE

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Abstract

Aims: Vineyards from early and late terroir were evaluated during ripening to determine optimal grape composition, with special emphasis on bunch uniformity.

Methods and results: Four treatments were studied over three years: Carignan and Grenache in two terroir, early and late. Samples were divided into two parts (top and bottom half of the bunch) in order to determine berry weight, sugar content, titratable acidity, total and extractable anthocyanins, total phenols, and seed maturity. The results showed bunch uniformity in Carignan. The kinetics of berry maturity generally showed a straight line pattern in Grenache while in Carignan it varied. For both cultivars, berry weight and yield were higher in the late parcel, whereas anthocyanins were more concentrated in the early terroir.

Conclusion: Pulp maturity is less influenced by the terroir effect than phenolic maturity. Vintage or terroir affect Carignan more consistently than Grenache. Carignan does not achieve complete phenolic ripeness in the late mesoclimate. In warm years, Grenache grapes should be gathered as soon as the pulp reaches the optimal sugar level because the accumulation of anthocyanins does not improve when the harvest is delayed beyond that point.

Significance and impact of the study: The evaluation of phenolic maturity in relation to terroir and bunch uniformity contributes to defining ideal harvest time and optimizing winery management.

Key words: climate, bunch ripeness, berry composition, anthocyanins, phenolic maturity

Résumé

Objectifs : L'uniformité des raisins et la composition de la baie pendant la maturation ont été évaluées dans des régions précoces et tardives.

Méthodes et résultats : Quatre traitements ont été étudiés pendant trois années : Carignan et Grenache dans des terroirs précoces et tardifs. Les grappes ont été analysées séparément (moitié supérieure et inférieure) afin de déterminer le poids des baies, les sucres, l'acidité totale, les anthocyanes totales et extractibles, les phénols totaux et la maturité des pépins. Les résultats montrent que les grappes de Carignan sont plus homogènes que celles du Grenache, tandis que leur cinétique de maturation varie en fonction du paramètre analysé. Les deux cépages ont montré des rendements plus élevés dans le terroir tardif, néanmoins les phénols étaient moins concentrés.

Conclusion : La maturation de la pulpe est moins influencée par le terroir que la maturité phénolique. Les changements produits par le millésime ou terroir affectent notamment le cépage Carignan. Dans les régions tardives, le Carignan n'atteint pas la complète maturité phénolique. La Grenache dans les années chaudes a une augmentation très rapide des sucres pendant la maturation qui n'est pas suivie par les phénols.

Importance et impact de l'étude : La différente composition phénolique des raisins liée au terroir et à l'uniformité des grappes nous permet de préciser la date de la récolte et d'optimiser la qualité des vins produits.

Mots clés : climat, maturité des grappes, composition des baies, anthocyanes, maturation phénolique

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INTRODUCTION

Berry development consists of two consecutive sigmoidal periods, separated by a more moderate phase (Coombe and McCarthy, 2000). Prior to veraison, acids, especially tartaric and malic acids, are synthesized in the pulp and skin (Kliewer, 1965 ; Mato *et al.,,* 2005) and are the precursor compounds to volatile phenols (Adams, 2006). Veraison marks the beginning of grape maturity and is characterized by an increase in berry volume, softening of the berries, acidity reduction, pH increase, sugar accumulation, hormonal variation, synthesis of aromatic substances, and pigment accumulation in the skin (Coombe, 1992 ; Robinson and Davies, 2000 ; Dai *et al.,* 2010). Sugar-induced biosynthesis of anthocyanins is responsible for the increase in total phenolic compounds in the berry during this second period (Pirie and Mullins, 1980 ; Hrazdina *et al.,* 1984 ; González-San José and Díez, 1992). Anthocyanin synthesis begins during veraison, and anthocyanins gradually continue to accumulate in the berry skin throughout grape ripening until a maximum is reached one or two weeks before harvest (Jordao *et al.,* 1998 ; Esteban *et al.,* 2001 ; Vivas de Gaujelac *et al.,* 2001 ; Pérez-Magariño and González-San José, 2004 ; Downey *et al.,* 2006). The main period of tannin synthesis in the seeds occurs immediately after fruit set, with the maximum level observed around veraison (Downey *et al.,* 2003 ; Ristic and Iland, 2005). Proanthocyanidin composition differs between seeds and skin. The level of extractable tannins was observed to decrease between veraison and harvest in both seeds and skin. Physiologically, the decreasing extractability of tannins, particularly from grape skins, represents a decrease in the overall bitterness and astringency of tannins in the grape berry and is probably part of the seed dispersal strategy that includes sugar accumulation and anthocyanin biosynthesis in the berry. Kennedy *et al.,* (2001) observed a correlation between the increase in proanthocyanidin mean degree of polymerization during ripening and increases in the level of anthocyanins associated with the proanthocyanidin fraction. Many factors such as cultivar/rootstock, climate, soil composition and wine-growing practices, in short, the "terroir" (Vaudour, 2002 ; Van Leeuwen *et al.,* 2004 ; Van Leeuwen and Seguin, 2006), define, directly or indirectly, vine growth, vigour and vine physiology, which in turn influence fruit composition (Jackson and Lombard, 1993). Climate is decisive for the development of the vineyard, limiting and modifying the biological processes through its multiple interactions (Jones *et al.,* 2005). The topography and orientation of the parcels are the main factors of climate variability in a specific region, and as such, edaphoclimatic parameters influence the ripening process and the quality of grapes (Nadal *et al.,* 2008). Climate variations, and especially temperature (Buttrose *et al.,* 1971 ; Kliewer, 1977 ; Bergqvist *et al.,* 2001) and soil moisture (Conradie *et al.,* 2002 ; Bodin and Morlat, 2006), are particularly important for wine production and determine vintage-to-vintage quality differences (Jones and Davis, 2000 ; Pereira *et al.,* 2006 ; Keller *et al.,* 2008). Soil characteristics such as texture, depth and pH influence the effective root depth and the water and nutrient holding capacity and determine the water stress and the nutritional level of the plant (Matthews and Anderson, 1988 ; De Andrés-de-Prado *et al.,* 2007). Cultural techniques, trellis-training system, pruning, fertilization and pesticide treatments also affect physiological maturity (Haselgrove *et al.,* 2000 ; Bavaresco *et al.,* 2008).

In terms of canopy microclimate, sunlight, temperature and humidity influence the vine's activity, determining parameters such as photosynthetic activity, stomatal conductance and transpiration (Flexas *et al.,* 2002 ; Spayd *et al.,* 2002 ; Escalona *et al.,* 2003 ; Koundouras *et al.,* 2006). Canopy management is essential in optimizing the microclimate surrounding the vine, because it reduces potential disease problems, increases photosynthesis efficacy, improves colour development in berries, and enhances the accumulation of sugars in grapes (Intrieri *et al.,* 1997 ; Hunter, 2000). It is possible to alter these parameters and open up the canopy to increase sunlight penetration and ventilation, and to use various canopy management practices such as leaf removal and crop thinning to achieve optimum leaf area and fruit weight ratio (Howell, 2001 ; Kliewer and Dokoozlian, 2005 ; Poni *et al.,* 2009 ; Diago *et al.,* 2010). Sunlight-exposed berries generally achieve a higher content of sugar, anthocyanins, and phenolic compounds but lower levels of acidity and berry weight compared to non-exposed fruits (Dokoozlian and Kliewer, 1996 ; Downey *et al.,* 2004 ; Cortell *et al.,* 2007). According to the results of many studies on the flavonoid composition of grapes and wine (reviewed in Downey *et al.,* 2006), total phenolics are higher in wines made from sun-exposed grapes compared to wines made from comparatively shaded grapevines. Several studies (Kliewer, 1970 ; Mori *et al.,* 2004 ; Yamane *et al.,* 2006) have shown how high temperatures (>35ºC) inhibited anthocyanin accumulation and subsequent

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coloration of berry skin. Sugar concentration and phenolic compounds have been closely related to vine water status during grape maturation (Ojeda *et al.,* 2002 ; Conde *et al.,* 2007). In general, water deficit has a clear positive effect on sugar content and berry phenolic composition. The higher temperatures and water deficit predicted in the context of climate change will simultaneously affect vine growth and berry size (Bindi *et al.,* 1996 ; Schultz, 2000 ; Webb *et al.,* 2007). Berry size plays an important role in the composition of grape and is a determining factor in wine grape quality (Matthews and Nuzzo, 2007). Since anthocyanins and other phenolic compounds accumulate in the skin, smaller berries have a relatively greater solute to solvent ratio than larger berries (Coombe *et al.,* 1987). Although other studies do not agree, the effect of vine water status on the concentration of skin tannins and anthocyanins is greater than the effect of fruit size *per se* on these variables (Roby *et al.,* 2004). Over-ripening of the berry affects the final concentration of anthocyanins : although dehydration leads to a concentration of phenols, the anthocyanins can often be degraded and/or polymerized (Nadal *et al.,* 2004 ; Mori *et al.,* 2007). Berry weight is heterogeneous across the population of berries in a bunch and on the same vine (Ojeda *et al.,* 1999 ; May, 2000), which ultimately affects the nature of the phenolic compounds and their concentration in the resulting wine (Choné *et al.,* 2001 ; Ojeda *et al.,* 2002). Gray and Coombe (2009), evaluating the variation of berry size, found that both 'floral differentiation' and 'timing of harvest' are crucial stages in achieving a harvest of homogeneous berry size. Even though berry size variation is presumed to have an impact on juice composition and wine quality, they also remind us that this presumption is still scientifically unproven. Viticulturists try to achieve synchronization of berry maturation in the same bunch to obtain an optimal harvest and a higher quality wine. As a result, a wine's style is directly related to the quality of compounds accumulated in the grape when it is harvested (Main *et al.,* 2002 ; Nadal and Hunter, 2007 ; Holt *et al.,* 2008 ; Jensen *et al.,* 2008). Monitoring of ripening is important in determining the ability of a region to produce a crop within the confines of its climate regime. Various authors (Barbeau *et al.,* 2004 ; Gil and Yuste, 2004 ; Deloire and Hunter, 2005) have studied the kinetics of grape ripening in different cultivars and ecosystems, but there is a lack of studies regarding the kinetics undergone by berries within a single berry (Kasimatis *et al.,* 1975 ; Tarter and Keuter, 2005 ; Pagay and Cheng, 2010). Heterogeneity in the ripening of a single bunch may lead to variations in the final composition of the whole grape, and as such, the level of uniformity may push the harvest date forward or backward in order to obtain optimum berry composition. The main goal of this study was to evaluate the uniformity of the bunch and its ripening kinetics in order to reach the optimal grape ripeness. The maturity of Carignan and Grenache grown in Terra Alta Denomination of Origin in Tarragona (Spain) was assessed in two terroirs, characterized as early and late mesoclimate over three years.

MATERIALS AND METHODS

1. Site details

The study was carried out in the Terra Alta DO, which is located in the precoastal mountains, in the province of Tarragona (Spain). This region has a Mediterranean climate with a continental tendency. The annual average temperature is 14.5ºC, the annual rainfall is approximately 470 mm, and the annual evapotranspiration (ET_0) is 1036mm. The Terra Alta DO is characterized as region IV by the Winkler Index (Amerine and Winkler, 1944), thermal integral calculated from April 1st to October 31st, and as HI +1 CI-1 DI +1 (temperate warm, temperate nights, moderately dry) according to the *Geoviticulture Multicriteria Climatic Classification System* (Tonietto and Carbonneau, 2004). The typical soils of the region, called *panal* (a mixture of silt and limestone), belong to the Entisols order according to the American Soil Taxonomy (Soil Survey Staff, 1998). The common white varieties of *Vitis Vinifera* cultivated in the Terra Alta DO are Grenache, Macabeu and Parellada, and the most common red varieties are Grenache and Carignan. The Terra Alta DO produces dry white and red wines with alcohol levels ranging between 12.5 and 15 % and liqueur wines with alcohol levels ranging between 15 and 20 %.

2. Experimental design

The experiment was conducted over three consecutive years (2006, 2007 and 2008) in four different commercial vineyards in the Terra Alta DO. Ten-year-old Carignan and red Grenache bush vines were grown in non-irrigated trials. The vineyards for each cultivar were chosen based on the common date of harvest in two different locations, called early and late ripeness parcels, each characterized by a specific terroir. The Carignan vineyards (Car) were located in the village of Vilalba, while the red Grenache

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vineyards (Gre) were located in the village of Batea. In total, early-Car (lat. 41.12º, long. 0.36º and alt. 370m), late-Car (lat. 41.13º, long. 0.36º and alt. 305m), early-Gre (lat. 41.11º, long. 0.21º and alt. 236m) and late-Gre (lat. 41.10º, long. 0.34º and alt. 422m) treatments were established. Three plot replications of each of these four combinations were randomly distributed in the vineyards, with each replication consisting of 30 grapevines. Two vines per replicate were chosen for vegetative measurements, whereas all 30 plants were used for maturity control. We also analyzed the composition of the distal (top and bottom) parts of the bunch to evaluate bunch uniformity during the ripening process dynamics in each treatment. Parcels of Carignan were planted at a density of 1.4 m x 2.8 m and parcels of Grenache at a density of 1.2 m x 2.8 m. Both cultivars were grafted on limestone resistant rootstocks : 110R for early treatments (resistant to limestone at 17 %) and 41B for late treatments since the high calcium content in these soils does not allow the successful use of rootstocks other than 41B (resistant at 40 %). The plants spur-pruned to two buds. Cultural practices were those commonly used in the region : the soil was ploughed at the beginning of spring and after harvest, vines were green pruned in May, and basal leaf removal took place at the end of June/beginning of July. In order to prevent diseases, vines were sprayed four (Gre) and eight (Car) times against oidium, once against mildew and twice against *Botrytis*. The vines were also sprayed for *Lobesia botrana* for the second and third generation.

3. Meteorological data

Table 1 shows the annual data for temperature (ºC), rainfall (mm) and ET_0 (mm), provided by the Meteocat weather station located in Batea (http://www.ruralcat.net/agrometeo/html/agrometeobc90.htm ; lat. 41.09º, long. 0.32º and alt. 382m). The annual accumulated degree days (GDD) and the Winkler Index (Amerine and Winkler, 1944) were calculated for each year. Rainfall is also shown as the average of two seasonal periods (from March 1st to May 31st, and from March 1st to September 30th) in order to facilitate discussion of the results. In each treatment, a HOBO weather station (www.onset-comp.com) was placed in order to characterize the climate and understand the specific climatic conditions in the four established parcels. Temperature, relative humidity (%) and solar radiation ($W \cdot m^2$) were recorded every hour. Accumulated GDD and ET_0 were calculated.

4. Sampling

Grape samples were collected from both cultivars during ripening and between 6 and 9 days after the start of veraison. Field sampling consisted of randomly gathering four grapes from three replicates per treatment. After collection, bunches were transported to the laboratory in plastic bags under refrigerated conditions. Samples from each replicate were divided in two parts, top and bottom half of the bunch. For each part, a sample of 100 berries was used for sugar level, acidity and pH analysis to determine the most suitable harvest date, and another sample of 300 berries was used to analyze phenolic maturity.

5. Methods

In order to evaluate vine growth, the total leaf area was calculated from the length of the main nerve, extrapolating a straight line (Carbonneau, 1976 ; Smart and Robinson, 1991 ; Cuevas, 2001). The main nerve of every leaf was measured in four shoots per vine. Two vines per replicate were used for measuring the leaf area, with a total of six vines per treatment. Sugar content and acidity of grape juice was determined periodically by classical analyses (sugar level, total acidity and pH), according to O.I.V. (1990). The sugar content was determined using a refractometer equipped with a temperature control set at 20ºC and expressed as Brix degrees. Titratable acidity was measured by titration with a base to an end point of pH 8.2 (20ºC), expressing the results as $g \cdot L^{-1}$ tartaric acid. Furthermore, the modified Glories method (Nadal, 2010) was used to analyze the phenolic maturity of the sample of 300 berries. The easily extractable anthocyanins were macerated at pH 3.6 instead of pH 3.2. Maceration in a pH 1 and pH 3.6 buffers lasted one hour. The extracted solutions were analyzed to obtain : a) the total (T Ant) and extractable (E Ant) anthocyanins and b) the total phenol index (TPI) by measuring the absorbance at 280nm. Anthocyanins were determined using the bisulfite decoloration method at 520nm. The extractability index and seed maturity index were calculated as follows :

$$\text{Extractability index (\%AE)} = \frac{(\text{T Ant} - \text{E Ant})}{\text{T Ant}} \times 100$$

$$\text{Seed maturity index (\%SM)} = \frac{(\text{TPI (pH3.6)}) - (\text{E Ant} \times 0.04)}{\text{TPI (pH3.6)}} \times 100$$

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6. Statistics

Each treatment was replicated three times and the data obtained was statistically analyzed using ANOVA (analysis of variance) and the Tukey comparison test. The means were compared using the SPSS 17.0 program for Windows (SPSS Inc. 233 South Wacker Drive, 11th Floor Chicago, IL 60606-6412, EE.UU) with three significance levels ($p \leq 0.001$; $p \leq 0.05$; $p \leq 0.1$).

RESULTS

1. Edaphoclimatic characteristics of parcels

Table 1 shows the data of temperature, rainfall, ET_0, accumulated GDD and Amerine and Winkler heat summation for 2006, 2007, 2008 and the ten-year averages for these measurements (2000-2009). Average temperature and ET_0 in 2008 (14.2ºC, 1021 mm) were lower than in previous years, as demonstrated by the data shown for the 2006 (15.3ºC, 1070 mm) and 2007 vintages (14.6ºC, 1058 mm). Furthermore, annual rainfall was more abundant in 2008 (588 mm) than in 2006 or 2007 (338 mm and 384 mm, respectively) and was higher than the average of the last decade (470 mm). Taking into account these results, the 2008 vintage could be considered a temperate year. On the other hand, the higher Winkler Index and GDD values in 2006, compared to the values in 2007 and 2008, led us to consider 2006 a very warm year.

The resulting data registered by the weather stations positioned in each parcel showed differences between early and late treatments during berry development. Accumulated GDD, average temperature and evapotranspiration from July 1st to the harvest were calculated and graphed in order to clarify the intensity of these differences (figures 1 and 2).

The GDD recorded during summer in the early parcels was 100ºC higher than in the late parcels. Average temperature and evapotranspiration were also higher in the early treatments (ranging from 3 to 5 units).

According to SINEDARES (Herrero *et al.,* 1993, adapted from American Soil Taxonomy (Soil Survey Staff, 1990)), soil classification indicates horizons Ap-Bw-C and Ap-Bkm-C for profiles of shallow soils, i. e., early-Car and early-Gre, respectively (table 2). Early-Gre soil is differentiated by gravel and pebbles situated along the profile, which lends good drainage capacity. Soils from late parcels are much deeper and the texture is lighter than those located in the early parcels, with soil profiles of Ap-Bw-2Bw-C (late-Car) and Ap-Bw-C (late-Gre). A lack of stones in the deeper soils indicates less drainage capacity

Table 1. Yearly meteorological data of the experimental site (Terra Alta DO) and long-term (10 years, 2000-2009) climatic data.

	Temperature (ºC)							Rainfall (mm)			ET_0 (mm)
	Average	Max	Min	Annual GDD	A-W	Seasonal[a]	Seasonal[b]	Annual	Seasonal[a]	Seasonal[c]	Annual
2006	15.3	21.5	10.3	2415	2243	483	1273	338	26	213	1070
2007	14.6	20.4	9.7	2040	1944	375	1158	384	192	226	1058
2008	14.2	19.5	9.7	1921	1839	339	1122	588	251	385	1021
2000-2009	14.7	20.6	9.8	2136	2022	377	1220	470	164	283	1036

ET_0: evapotranspiration; GDD: growing degree days (base 10ºC), calculated from the average daily temperature; A-W: heat summation calculated according to Amerine and Winkler (from April 1st to October 31st). [a] from March 1st to May 31st, [b] from June 1st to August 31st, [c] from March 1st to September 30th.

Table 2. Soil characteristics for the selected parcels in Terra Alta DO.

Plot	Soil horizon	Soil depth (m)	Classification	Texture	pH	Limestone (%)
early-Car	Ap-Bw-C	1,0	Typic Xerofluvent	Sandy loam	8.7	13.1
late-Car	Ap-Bw-2Bw-C	> 4,0	Typic Xerofluvent	Loam	8.8	9.7
early-Gre	Ap-Bkm-C	0,2 - 0,6	Xeric petrocalcid	Sandy loam	8.6	13.5
late-Gre	Ap-Bw-C	0,4 - 1,0	Typic Xerorthent	Silty loam	8.6	16.4

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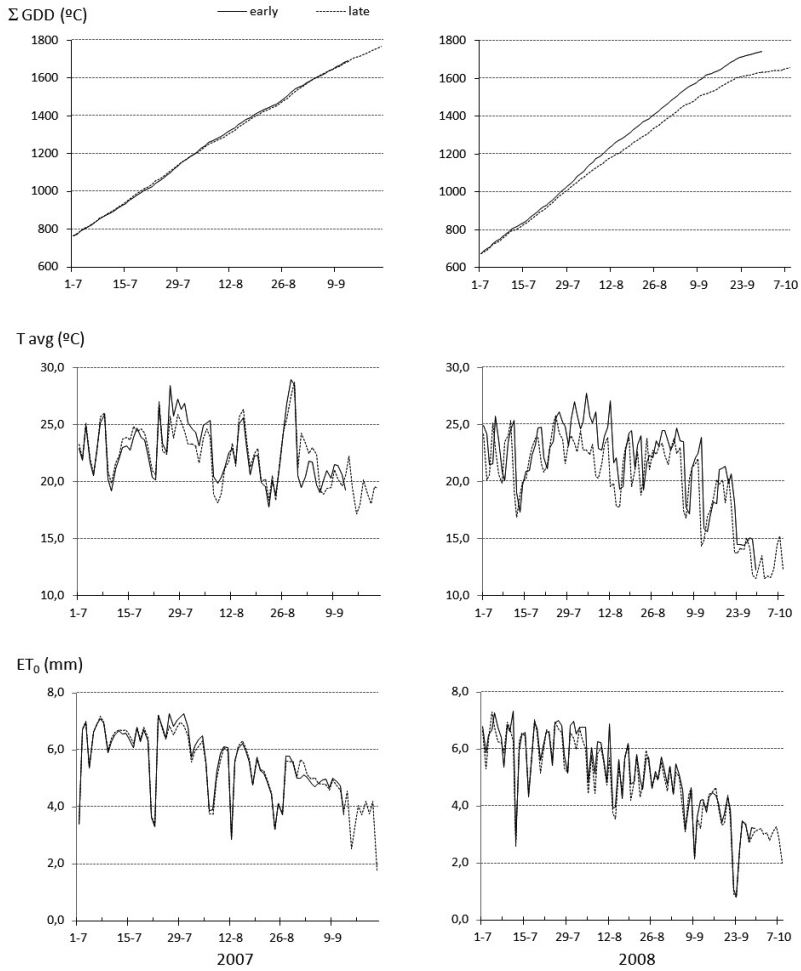


Figure 1. Accumulated growing degree days (∑=GDD, ºC), average temperature (Tavg, ºC) and evapotranspiration (ET_0, mm) according to Hargreaves, from July 1st to harvest date of Carignan parcels (early and late) in Terra Alta DO.

compared with soils of the early parcels. The combination of greater soil depth and higher clay and silt content in the late parcels provides higher water-holding capacity than the shallower and stony soils of the early treatments.

2. Bunch uniformity and kinetics of berry ripeness - Carignan

a. Brix

Monitoring of ripening in 2006 and 2007 showed significant differences in sugar content between the top and bottom half of the bunch in both early (figures 3.a and 3.c) and late locations (figures 3.b and 3.d). The dynamics of sugar accumulation in berries in 2006 and 2007 (figures 3.a-d) was similar in both treatments assessed. Kinetics is divided in the two periods with different slopes. The temperate vintage (figures 3.e-f) also presented kinetics divided in two phases, but with a slower increase of sugars during maturation. The accumulation was milder in the period after veraison and more gradual with a steep gradient until harvest.

b. Titratable acidity (TA)

There were no statistical differences between the distal parts of the bunch in 2006 and 2008 (figures 3.a-b and 3.e-f). The trend was similar to that observed for sugar content. The kinetics in the distal parts was similar in each treatment, and resembled a straight line. Nonetheless, the speed of acid decrease varied widely. The kinetics of decreasing acids in 2008 (figures 3.e-f) displayed two phases with very high slopes after veraison

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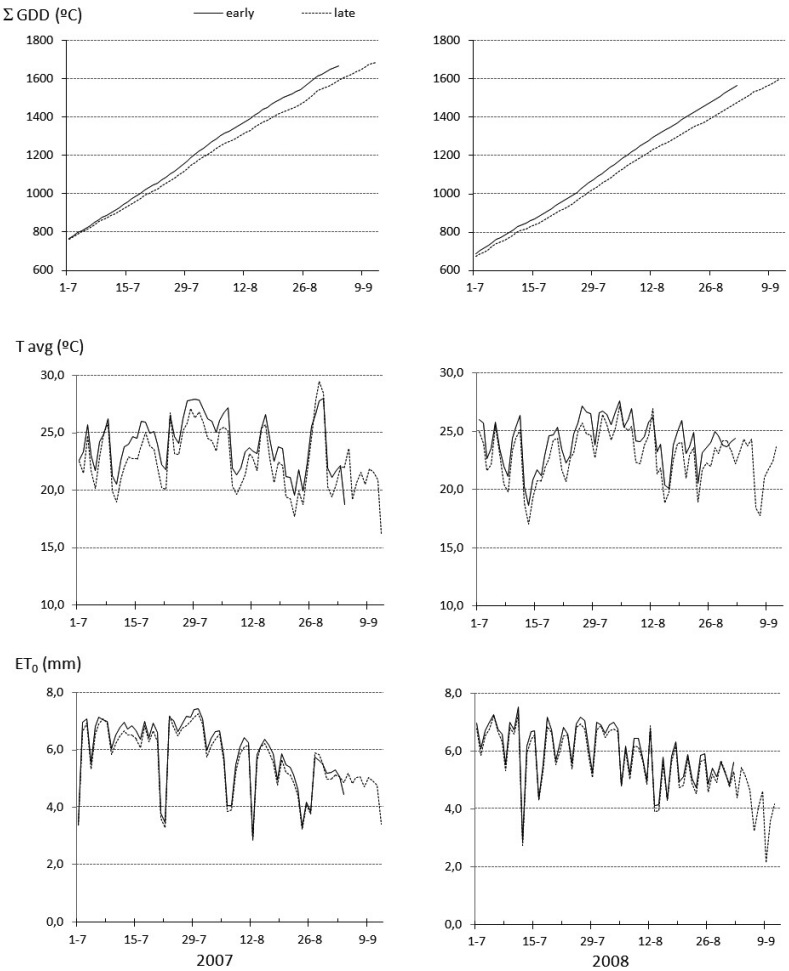


Figure 2. Accumulated growing degree days (∑GDD, ºC), average temperature (Tavg, ºC) and evapotranspiration (ET_0, mm) according to Hargreaves, from July 1st to harvest date of Grenache parcels (early and late) in Terra Alta DO.

followed by a moderate decrease in the second period of ripening.

c. Berry weight

Statistical analysis shows an absence of significance between the distal parts despite some differences of up to 15 % between the berry weights in some treatments, due to the wide range of values between different berries (figures 3.a-f). The general trend in the berry weight kinetics was an increase during ripening, except for the early treatment in 2006 (figure 3.a), when the berry size did not increase in the last weeks before the harvest. The slopes of the straight lines ranged from m = 0.12 to m = 0.26.

d. Extractable anthocyanins (pH 3.6)

A significant difference in E Ant between the top and bottom half of the bunch was observed only in 2006 in the early parcel, reaching at harvest 736 mg/L-top and 872 mg/L-bottom (figure 4.a). However, the concentration of E Ant in the late parcel was between 13 % and 48 % higher in the top half during ripening (figure 4.b). Practically no significant differences were found between the distal parts in 2007 (figures 4.c-d) and 2008 (figures 4.e-f). In general, the concentration of anthocyanins at the end of ripening barely increased. The anthocyanins in the early parcel were constantly higher than the concentration obtained in the late parcel. In the three years, the kinetics of anthocyanin accumulation in the late treatment (figures 4.b, 4.d and 4.f) was much slower than in the early treatment.

e. Seed maturity (%SM)

There were consistent statistical differences between the top and bottom half at harvest only in

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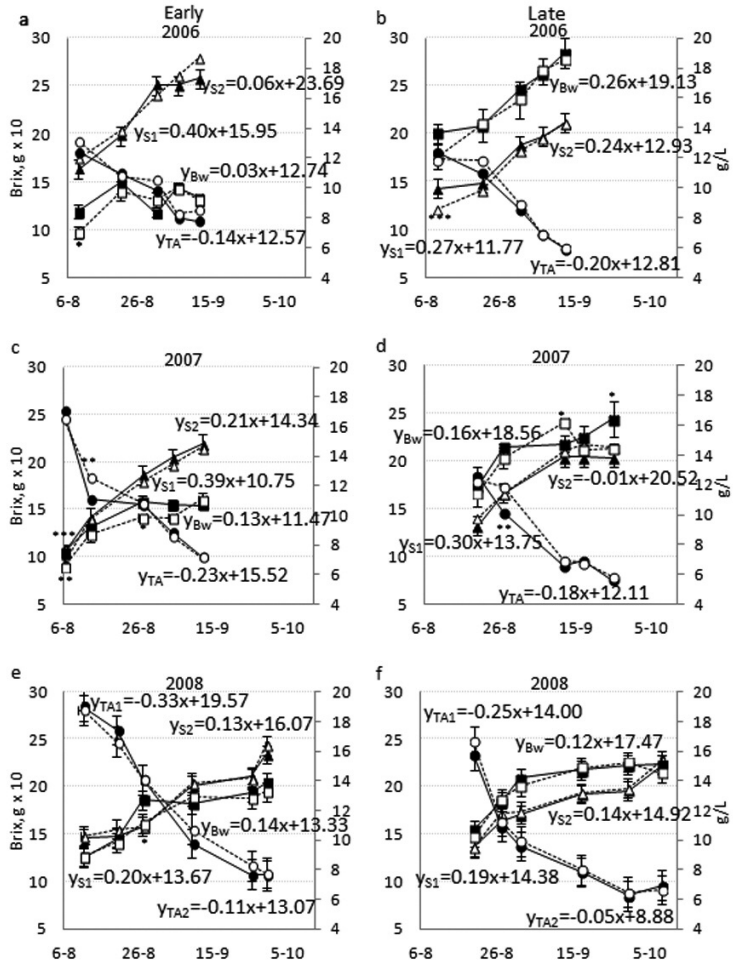


Figure 3. a-f. Evolution of pulp composition and berry weight of Carignan. Early and late parcels during 2006, 2007 and 2008 vintages. Brix top; Brix bottom; TA top; TA bottom; Bw top; Bw bottom. S: sugar concentration (Brix), TA: titratable acidity (g/L tartaric acid), Bw: berry weight (g). *, ** and * indicate significance at p ≤ 0.1, p ≤ 0.05 and p ≤ 0.001, respectively.**

2006 (figures 4.a-b). The percentage attained in the top half was 12 % higher than on the opposite half. No significant differences were found in 2007 and 2008 (figures 4.c-f). In general, seed maturity (figures 4.a-f) decreased gradually between veraison and harvest.

3. Bunch uniformity and kinetics of berry ripeness - Grenache

a. Brix

Figures 5.a-f show the sugar content in the top and bottom half of the bunch for the early and late treatments in 2006, 2007 and 2008. The significant differences between distal parts during ripening were minimal (5 %). The kinetics of the sugar content in the early parcel (figures 5.a, 5.c and 5.e) showed an ascending linear trend until the harvest.

In late locations, the increase in sugars was gradual in 2006 but accumulation stopped one week before harvest (figure 5.b).

The 2007 vintage was characterized by a gradual accumulation followed by a sharp increase of 10 Brix degrees in the last fortnight (figure 5.d). In 2008, sugar content (figures 5.e-f) followed a linear and constant increasing trend during ripening**.**

b. Titratable acidity

The evolution of TA showed few significant differences between the distal parts (figures 5.a-f). Regarding kinetics, very small decreases in acidity were observed in warm years throughout the maturity process. The kinetics of ripening in the temperate year (2008) is represented by two periods in both early and late, with the turning

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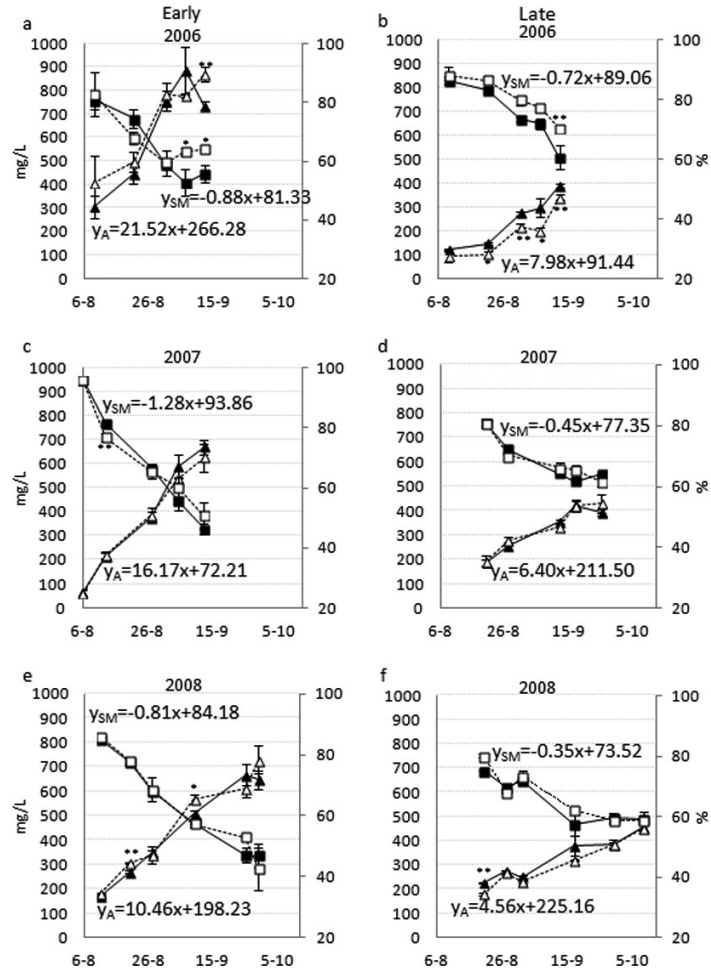


Figure 4. a-f. Evolution of phenolic ripening of Carignan. Early and late parcels during 2006, 2007 and 2008 vintages. E Ant top; E Ant bottom; %SM top; %SM bottom. A: extractable anthocyanins (mg/L), SM: seed maturity index. *, ** and * indicate significance at p ≤ 0.1, p ≤ 0.05 and p ≤ 0.001, respectively.**

point on 12th August in the early parcel and 26th August in the late parcel.

c. Berry weight

Significant differences were observed at harvest in 2006-early parcel and 2007-late parcel, either in the top half or in the bottom half (figures 5.a-d).

d. Extractable anthocyanins (pH 3.6)

Figures 6.a-f show the changes in E Ant content for 2006, 2007 and 2008. The distal parts of the bunch did not statistically differ in the 2007 vintage. In the early treatment, the distal parts presented significant differences at harvest in 2006, with the highest concentration of anthocyanins in the top half (figure 6.a). The same trend was observed in the late treatment (figure 6.b), with the two bunch parts statistically different, with percentages of 25 % during ripening and 40 % at harvest. Differences of 30 % were also observed in the last week of ripening in 2008 (figure 6.f).

e. Seed maturity (%SM)

Slight differences between the two parts of the bunch (<10%) were observed. The % SM in Grenache (figures 6.a-f) showed a common pattern of gradual decrease from veraison to harvest in both treatments and in all three years.

4. Berry composition at harvest

Table 3 shows the results for Carignan at harvest, comparing the early and late parcels in the top half on one hand and in the bottom half on the other.

Statistical analysis was performed jointly with data from the three years. In the early treatment, and in both distal parts, higher values of Brix, total acidity (TA), total anthocyanins (T Ant), extractable anthocyanins (E Ant) and total phenol index (TPI)

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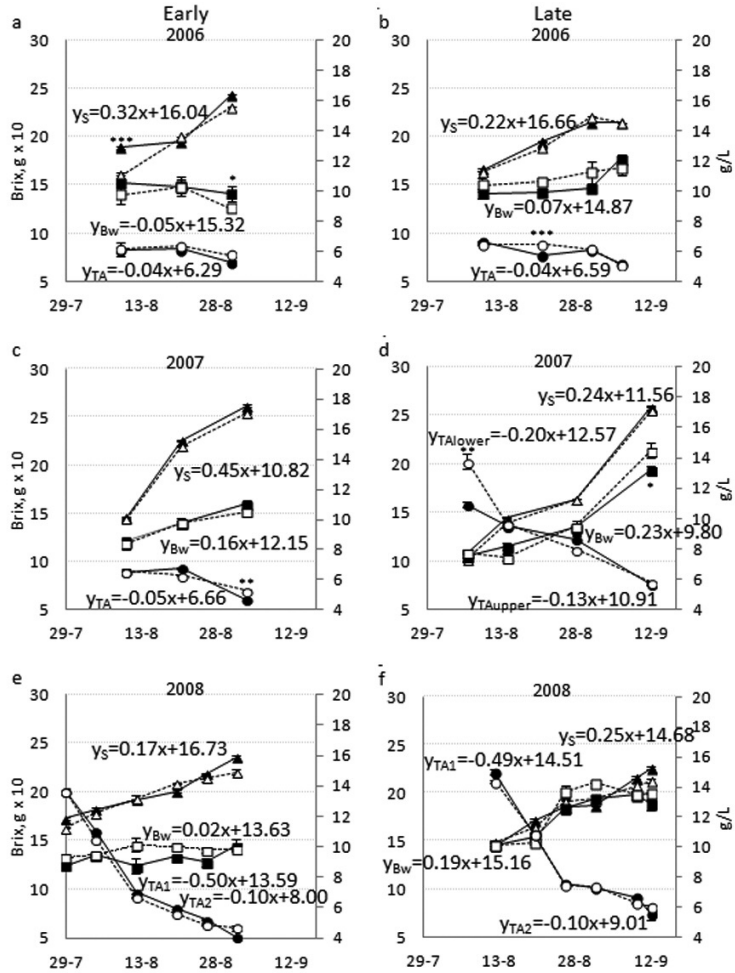


Figure 5. a-f. Evolution of pulp composition and berry weight of Grenache. Early and late parcels during 2006, 2007 and 2008 vintages. Brix top; Brix bottom; TA top; TA bottom; Bw top; Bw bottom. S: sugar concentration (Brix), TA: titratable acidity (g/L tartaric acid), Bw: berry weight (g). *, ** and * indicate significance at $p \leq 0.1$, $p \leq 0.05$ and $p \leq 0.001$, respectively.**

were always obtained. However, berry weight (Bweight) and seed maturity (%SM) were greater in the late parcel.

In the same way, the results of Grenache at harvest are shown in table 4 with the top and bottom half of the bunch compared separately.

No significant differences in Brix, T Ant, E Ant or % SM were observed. Nevertheless, TPI was significantly higher in the early treatment, while TA and Bweight were higher in the late parcel.

DISCUSSION

Although Terra Alta DO is classified as region IV by the Amerine-Winkler's scale, it is classified as region V when considering the data obtained for 2006 (2243ºC), as region IV for 2007 (1944ºC), and as region III for 2008 (1839ºC). This highlights the higher temperatures registered at the end of summer in both 2006 and 2007 vintages compared to 2008. Additionally, differences between vintages were mainly found in annual rainfall, which was much higher in 2008 (588 mm) than the last 10-year-average recordings (470 mm). Consequently, 2008 was a 'gentle' vintage that notably affected the berry composition results. Concerning the terroir, climatic data recorded by the weather stations in each location also revealed differences between the early (warm) and late (temperate) vineyard parcels. The harvest was delayed in the late parcels due to this climatic effect. Therefore, berry composition showed plenty of differences depending on the location (early or late).

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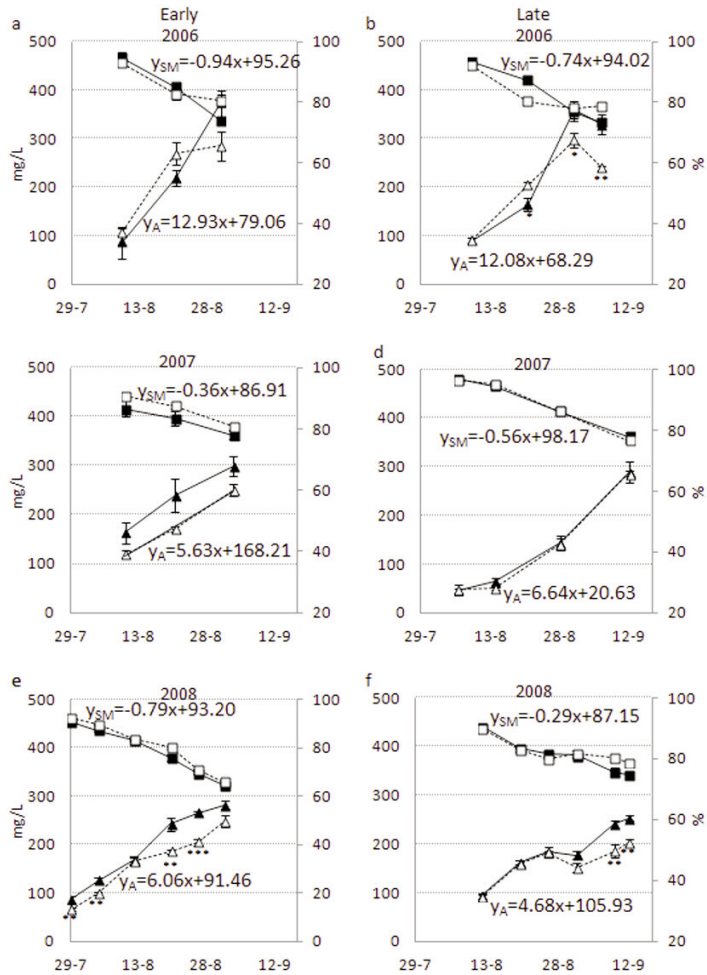


Figure 6. a-f. Evolution of phenolic ripening of Grenache. Early and late parcels during 2006, 2007 and 2008 vintages. E Ant top; E Ant bottom; %SM top; %SM bottom. A: extractable anthocyanins (mg/L), SM: seed maturity index. *, ** and * indicate significance at $p \leq 0.1$, $p \leq 0.05$ and $p \leq 0.001$, respectively.**

1. Bunch uniformity and kinetics of berry ripeness. Carignan

a. Pulp

Both the accumulation of sugars and the decrease in acids in the distal parts of a single bunch during the ripening process are considered uniform, since the differences found were less than 5 %, except in 2006, where the difference reached 10 % in the early treatment. Concerning grape maturity, the 2006 and 2007 vintages showed similar behaviour in the kinetic accumulation of compounds in pulp, displaying a pattern characterized by two straight lines with a turning point in late August. Sugar accumulation was faster in the first period of ripening, from veraison to the end of August. The high maximum temperatures registered in the last days of August 2006 and 2007, ranging between 36-38ºC (with low humidity of 35-40 % and absence of rains), may have contributed to the decrease in the speed of sugar accumulation toward the end of the process. Nevertheless, the high temperatures led to a decline in berry size due to dehydration, which in turn led to an increase in sugar content (figure 3.a), as shown by the values at harvest (table 3). Studies like those by Freeman and Kliewer (1983) in Carignan and by Kennedy *et al.,* (2002) in Cabernet Sauvignon in California found that dry years accelerate the accumulation of sugars in the berry. In comparison with the temperate vintage, the accumulation of sugars was more gradual. This ripening dynamics could be explained by the climatic conditions in 2008: milder temperatures, higher rainfall and less ET_0 than in

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Table 3. Berry composition and berry weight for *Vitis vinifera* cv. Carignan
Average value of three vintages (standard deviation). Titratable acidity (TA) (g/L tartaric acid); berry weight (Bweight) (g) x 10; total (T Ant) and extractable (E Ant) anthocyanins (mg/L); total phenolic index (TPI) and seed maturity index (%SM). *, ** and * indicate significance $p \leq 0.1$, $p \leq 0.05$ and $p \leq 0.001$, respectively.**

	top		bottom	
	early	late	early	late
Brix	23.71(1.77)	21.27(0.86)	24.63(2.81)	21.83(0.89)
	**		**	
TA	7.57(0.27)	6.15(0.66)	7.82(0.60)	6.15(0.42)
	***		***	
Bweight x 10	16.40(3.16)	25.09(3.42)	16.23(2.95)	23.52(3.29)
	***		***	
T Ant	1033(198)	724(76)	1090(285)	685(84)
	***		***	
E Ant	685(57)	414(40)	742(129)	408(59)
	***		***	
TPI	55.40(10.30)	43.04(3.90)	66.24(23.00)	44.89(2.67)
	**		**	
%SM	49.65(6.48)	61.32(4.44)	52.36(11.76)	63.57(5.33)
	***		**	

the preceding years (see table 1). The milder climate and increased rainfall registered in 2008 affected Carignan maturity, delaying harvesting and allowing the grapes to reach their expected ripeness (figures 3.e-f). The acids decreased in the warmest years according to a linear kinetic model. This trend was characterized by a fast decrease of acids after veraison and a slow diminution before harvest. On the contrary, the moderate climatological conditions in 2008 led to a delay in the combustion of acids. This behaviour is reflected in the kinetics, which shows two segments with a turning point two weeks after veraison at the end of August, coinciding with the highest maximum temperatures. Our results indicate a remarkable variability in the kinetic pattern mainly due to the intra-annual climate changes. Barbeau *et al.,* (2004), when evaluating the level of acids in the maturity process, found that it varied randomly and that it was difficult to find a relationship between the acidity and the climatic variables from one year to another. In terms of winemaking, the acidity of Carignan at harvest remained at a good level for wine quality, while the sugar content did not achieve the optimal concentration in the late treatment (remarkable in gentle vintages).

b. Phenolic maturity

The grapes were uniform in regard to phenolic ripening in 2007 and 2008. Significant ripeness differences between the distal parts were found only in the warmest year (2006), probably due to the different rate of accumulation observed during the ripening process. The concentration of anthocyanins gradually increased until one week before the harvest date. In the last week of ripening, there can be a decline in the speed of anthocyanin accumulation depending on climate and vintage. This effect was only observed in the warmest year (2006) in the top half of the bunch. The difference in anthocyanin content at harvest between early and late treatments is almost certainly due to the speed of anthocyanin accumulation in the early stages of ripening. Actually, the accumulation of anthocyanins in late parcel was slower after

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Table 4. Berry composition and berry weight for *Vitis vinifera* cv. Grenache
Average value of three vintages (standard deviation). Titratable acidity (TA) (g/L tartaric acid); berry weight (Bweight) (g) x 10; total (T Ant) and extractable (E Ant) anthocyanins (mg/L); total phenolic index (TPI) and seed maturity index (%SM). *, ** and * indicate significance at $p \leq 0.1$, $p \leq 0.05$ and $p \leq 0.001$, respectively.**

	top		bottom	
	early	late	early	late
Brix	24.73(1.16)	23.32(2.02)	23.53(1.51)	22.78(2.19)
	ns		ns	
TA	4.67(0.62)	5.45(0.31)	5.25(0.47)	5.63(0.42)
	**		*	
Bweight x 10	14.87(1.27)	18.64(0.90)	13.96(1.16)	19.40(2.41)
	***		***	
T Ant	456(71)	414(94)	359(74)	352(82)
	ns		ns	
E Ant	318(51)	290(43)	261(35)	243(37)
	ns		ns	
TPI	57.33(3.59)	47.20(5.68)	56.53(3.61)	44.11(4.88)
	***		***	
%SM	74.02(3.74)	75.32(2.78)	77.65(4.99)	78.04(1.28)
	ns		ns	

veraison and this pattern was also observed during ripening, resulting in a lower concentration at harvest. The level of anthocyanins reached in late parcels was insufficient for obtaining good quality in red wines. This incomplete maturity could be attributed to different factors such as vigour and canopy management. The vines in the late parcel showed less exposed berries, corresponding to higher solar radiation intercepted by the canopy (data not shown), than those in the early parcel, which is consistent with the differences in vine growth. The data for total leaf area (TLA) were 3.4 $m^2 \cdot vine^{-1}$ in the early parcel, compared to 4.8 $m^2 \cdot vine^{-1}$ in the late parcel. Smart (1985), Downey *et al.,* (2004) and Cortell and Kennedy (2006) found that the clusters located within the canopy, which receive less radiation, synthesize smaller amounts of anthocyanins. Bergqvist *et al.,* (2001) showed that Cabernet Sauvignon grapes with more exposure to radiation presented more colour and a higher concentration of anthocyanins.

2. Bunch uniformity and kinetics of berry ripeness. Grenache

a. Pulp

Given that the differences found in the accumulation of sugars in the distal parts of the bunch were less than 5 %, ripening can be deemed uniform. Depending on the treatment, the final yield of sugars in the early parcel was 10 % higher than in the late parcel in the driest year (2006); the same trend was observed in 2007 and 2008 although the difference did not exceed 5 %. In the 2006 and 2007 vintages, the acids decreased according to a linear kinetics, displaying very low concentration during ripening in vines growing in the early and late parcels. The temperate year presented a kinetic model divided into two periods. Regarding berry weight, no variability was evidenced in the distal parts of the bunch in all treatments. Berry growth stopped soon after veraison only in the driest year. Also evident was the influence of the terroir on the berry weight parameter, which was higher in the late parcel.

b. Phenolic maturity

In general, the results obtained in the different treatments - 2006 and 2008 - showed a heterogeneous accumulation of anthocyanins within the same bunch, which was very marked in 2008 during the maturity period. The concentration of anthocyanins at the end of ripening was higher in the top half of the bunch, with values ranging between 14-30 % (figures 6.a-f). This may be due to the shape of the bunch in Grenache: the top half

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is bigger and consequently more exposed to solar radiation than the bottom half. In the early parcel, the speed of anthocyanin accumulation was faster than in the late parcel throughout the entire process. The kinetics of accumulation of sugars and anthocyanins was similar except in 2006, which presented a decline in anthocyanins in the last ripening control. It is well known that the synthesis of anthocyanins is directly related to the accumulation of sugars (Hardie and Considine, 1976 ; Pirie and Mullins, 1980). However, anthocyanin synthesis can be diminished by environmental factors, as evidenced in the warmest vintage (2006). Furthermore, biosynthesis can even be blocked due to thermal or physiological conditions in warm years (González-San José and Diez, 1992 ; Spayd *et al.,* 2002). This evident decline in E Ant at the end of ripening and the halt in the accumulation of anthocyanins two weeks before harvest (figures 6.a-b) could be explained by the dryness and high temperatures that occurred during that year, due to the sensibility of Grenache under these environmental conditions (Schultz, 2003). The GDD from fruit set through late August 2006 (seasonal[b] 1273ºC, table 1) was higher than the corresponding GDD in 2007 and 2008 (1158ºC and 1122ºC, respectively). Actually, the average temperature in July was quite high in 2006 (26.6ºC) compared to 2007 (23.5ºC) and 2008 (23.1ºC), which could influence the veraison process. Furthermore, one week before harvest, the maximum temperatures reached an unusual 40ºC on three consecutive days. Nadal *et al.,* (2004) found a relationship in Syrah (grown in Stellenbosch) between the dehydrated berries on overripe grapes and the halt in synthesis or degradation of phenolic compounds, and therefore the decline in the concentration of anthocyanins in the berry. Cacho *et al.,* (1992) in Somontano County (north of Spain) found that the concentration of anthocyanins in Grenache was highly variable and greatly dependent on vintage. When comparing terroirs, anthocyanin content was slightly higher in the early parcel.

Concerning seed maturity, the few differences found revealed a uniform ripening of the seeds on a single bunch. Whatever the climatology of the year, warm or temperate, Grenache did not reach the optimal seed ripeness because of the high % SM index (70-80 %) attained at harvest for both early and late treatments. In fact, the incomplete seed maturity could be due to a characteristic of this variety and may depend on a genetic factor rather than a climatic factor. Grenache seed composition should be further investigated in order to determine whether this finding can be stated, since our possible hypotheses can even raise new considerations.

3. Berry composition at harvest. Carignan

Considering the average of the three years (table 3), sugar content and acidity were 12 % and 25 % higher in the early parcel, respectively. Carignan grapes in the late location evidenced a greater crop and larger berries, with less sugar and acid content, probably because of the dilution effect, compared to those from the early treatment (García-Escudero *et al.,* 1995 ; Nadal and Arola, 1995 ; Calò *et al.,* 1996). The berry weight was 50 % greater in the late parcel (2.4 g vs. 1.6 g in the early) and the yield reached 4.6 kg·vine^{-1} compared to 2.3 kg·vine^{-1} in the early parcel. The deeper soil in the late parcel is able to store more water than the shallow soil in the early parcel and this influences vine vigour and berry size. In fact, the pruning weight per vine in the late parcel was 722 g, while in the early parcel it was 351 g.

Taking each vintage separately, the results for berry weight in the late parcel exceeded those in the early parcel by over 100 % in 2006 and by only 10 % in 2008. It is apparent that the warmer and drier year resulted in the largest difference in berry weight between early and late locations. In terms of phenolic composition, the results revealed heterogeneity in the anthocyanin content of a bunch because the berries on the bottom remained less ripe at harvest. Bigger differences were shown when comparing treatments : total and extractable anthocyanins were higher in warm terroir. Grapes in the early parcel showed a 43 % higher concentration of T Ant in the top half and 59 % in the bottom half than those obtained in the late parcel. A similar trend was observed with E Ant : the values were higher in the early parcel and the differences more marked, being 65 % higher in the top half and 82 % in the bottom half bunch than those in the late parcel. Vines in warm parcel with less access to water achieve lower berry weights and higher anthocyanin content (Ojeda *et al.,* 2002). The differences found in anthocyanins (700 mg/L in early, 400 mg/L in late), seed maturity and total phenol index between early and late treatments enable us to apply different vinification techniques for each terroir. Regarding seed maturity, Ribéreau-Gayon *et al.,* (2000) established that up to 60 % of % SM cause failure to ripen and high levels of astringency. The high % SM values in the late parcel (60-70 %) suggest that Carignan in this parcel also failed to complete the

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phenolic ripening of seeds. Moreover, the Brix values (21.6 degrees) did not attain the minimum established by the Terra Alta DO Wine Regulatory Council. In consequence, the thermal integral needed to obtain optimal maturity in Carignan grapes were insufficient for completing the maturity of the pulp and phenolic ripeness in temperate years and late terroir. The results of grape composition at harvest provide us with a perfect definition of the two styles of Carignan wine in the Terra Alta DO. In a late location, it is possible to produce young wines with a lower alcohol content (21.3 degree Brix equal to 12.3 % vol), lower acidity (6.15 g/L), low phenolic compounds, and in summary, a lighter and fresher style. A second type of wine can be produced from grapes in a early terroir with a higher alcohol content (24 degree Brix equal to 14.1 % vol), higher acidity (7.5 g/L), 700 mg/L of anthocyanins, and low % SM values (42-55 %), which compose the ideal conditions for producing a reserve wine.

4. Berry composition at harvest. Grenache

Grenache belongs to the medium-late group regarding maturity date, with optimal ripening usually achieved during the first fortnight of September in Terra Alta DO. Both the high level of sugars and the lack of differences in Brix between early and late treatments signified that the maturity of the pulp is completed in both locations. Considering the average for the three years (table 4) and unlike the Carignan results, the acidity of Grenache was 10-15 % lower in the early parcel. Late parcels maintained better levels of acidity, assuring wine quality compared to the warm terroirs. Gil and Yuste (2004) found a similar trend in Grenache and Tempranillo cultivated in two mesoclimatically different zones, with lower acidity in the warmer region. The higher berry weight in the late treatment resulted in higher percentages, 25 % in the top half bunch and 40 % in the bottom half bunch, compared to the early parcels. In our study, the higher berry weight in the late parcel could be attributed to the vine's vigour. Grapevines in the late treatment had much vigour, as shown by the greater pruning weight (702 g·vine^{-1}) compared to the early treatment (291 g·vine^{-1}), and they produced a larger crop (early = 2.5 kg·vine^{-1}, late = 5.9 kg·vine^{-1}) probably due to the soil depth. Actually, the deeper soil in the late location is able to hold more water, thereby reducing the effects of drought in this parcel.

The higher acidity achieved in the late terroir suggests that gentle temperatures favour the grape composition for making Grenache premium wines in Terra Alta DO (warm Mediterranean climate). Despite the crop in the late parcel was higher no difference in sugar or anthocyanin content attained at harvest was found. The intra-vintage variability observed in Grenache (figures 5 and 6) is canceled out when the three-year-average is considered. The high % SM has confirmed the immaturity of seeds found in each vintage separately considered (figures 6.a-f).

CONCLUSIONS

The analyses performed during ripening on Carignan and Grenache varieties revealed that berry composition is more influenced by the terroir effect (early and late ripeness) than by bunch uniformity. The highest concentrations of anthocyanins and total phenols at harvest are found in the early parcel. In both cultivars, the yields per vine and berry weight were higher in the late locations. The higher vine vigour in late parcels is probably linked to both the milder temperatures and the higher water-holding capacity of these environments.

Berry composition in the top and bottom parts of Carignan bunches shows uniformity during ripening in both the pulp and the skin. In Grenache, the accumulation of sugars and acids is homogenous along the maturity process; however, anthocyanin synthesis is heterogeneous within the same bunch in 50 % of the treatments considered, regardless of the year and terroir (early/late). The top half of the bunch showed higher concentrations of anthocyanins.

The kinetics of sugar accumulation differs depending on the variety. The sugar accumulation in the berries is faster and is represented by a straight line in Grenache, whereas in Carignan this increase occurs in two phases. In general, the speed of sugar accumulation is more marked with higher slopes as found in early rather than in late treatment. The turning point in the increase of sugars is attributed to the high temperatures usually recorded on the third or fourth week of August, approximately three weeks before harvest. In both cultivars, the acids decreased according to a linear kinetics, displaying very low concentration at early stages of ripeness in warm vintages. Nevertheless, the kinetics in temperate vintages is characterized by a model involving two phases with different gradients and a turning point coinciding with the highest maximum temperatures. The acidity varies independently of location (early vs. late parcels),

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which is not explained by berry size, and changes considerably depending on climatic conditions. Dry and warm years only affect the anthocyanins in Grenache close to the end of ripening, one or two weeks before harvest. The severe climate conditions at the end of maturity greatly affect anthocyanin synthesis, bringing sugar and anthocyanin accumulation to a halt. Meanwhile, the kinetics of accumulation of anthocyanins in Carignan follows a straight line model with different slopes depending on the climate, as in the late location where the gradient value is always too low. Moderate climate in late terroir led to a decrease of anthocyanins in the berries.

The final composition at harvest, assessed over the three years as a whole, shows that the Carignan grapes in the late treatment did not complete their ripening cycle and, consequently, their anthocyanin and sugar content was below the desirable level required for premium red wines. Concerning Grenache, the high seed maturity index reached at harvest denotes some risk of astringency in the potential wines obtained, regardless of the terroir and vintage. The lack of seed maturity suggests that winemaking practices, such as pomace management, should be carefully considered during all the fermentation period. Depending on the style of the wine, the extraction time during vinification must be shorter in order to prevent excessive astringency in the finished wine. According to this result, it would not be beneficial to postpone the date of harvest, as quality would not be improved once pulp ripeness is complete. Nevertheless, the seed composition should be investigated more thoroughly to confirm this hypothesis.

In Mediterranean climates with warm summers (tending towards continentality such as the Terra Alta DO), the synthesis of anthocyanins in the early ripeness locations is halted in Grenache in dry vintages. Global climate change in dry and warm regions could affect the kinetics of sugars and anthocyanins differently, depending on the variety. Irrigation and canopy management strategies to improve berry ripening in severe dry and warm terroirs should be implemented according to the weather conditions of the vintage and the needs of each variety.

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