Research Article

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Water stress and ripeness effects on the volatile composition of Cabernet Sauvignon wines

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Abstract

BACKGROUND: Controlled water deficits affect grape berry physiology and the resulting wines, with volatile composition being the one of the affected parameters. However, there is a potential disconnect between aromatic maturity and sugar accumulation. Accordingly, the effects of three different water status levels over two growing seasons (2014 and 2015) and two different harvest dates on the aroma compounds from Cabernet Sauvignon wines were studied. Volatile compounds were determined using headspace solid phase microextraction coupled with gas chromatoghraphy/mass spectrometry.

RESULTS: Around 45 volatile compounds were determined in the wines and, among these, esters were affected the most, presenting lower concentrations when the most restrictive water treatment was applied in both years. By contrast, volatile acids presented the highest concentrations when the lowest level of irrigation was applied. On the other hand, a delay in harvesting produced an increase in the total amount of volatile compounds in samples from the most restrictive water treatment. These results are coincident with a principal component analysis that indicated a great separation between years, deficit irrigation treatments and harvest dates.

CONCLUSION: The results of the present study suggest that a low water supply had a negative effect on the aromatic potential of wines at a similar ripening stage. However, this effect could be countered by harvesting at a later date.

Keywords: Cabernet Sauvignon; deficit irrigation; harvest date; volatile compounds; wine aroma

INTRODUCTION

In grapevines, environmental factors such as climate and soil, as well as plant material and field practices (i.e. variety, ripeness at harvest and irrigation practices), are known to influence grape composition.[1–6] Regarding irrigation, controlled water deficits are a common viticultural practice worldwide, particularly in red grape varieties cultivated in Mediterranean climates because of their well-known effects on wine.[7–13] Water deficits positively impact the grape through lower berry sizes, with a concomitant increase in the skin to pulp ratio, concentrating secondary metabolites.[14] Additionally, a water deficit reduces plant vigour, improves the microclimate of the fruiting zone and, most importantly, increases the activity of the secondary metabolism in grapes, which is mediated by abscisic acid.[15–17] Wine quality is a multi-faceted construct, defined by different parameters, amongst which aroma has been suggested as one of the most important.[18] Wine volatile compounds may originate from the grapes, must treatment and the fermentation process, as well as the aging process.[19] Terpenes are primary/varietal compounds that originate from the grape. These compounds represent part of the potential aroma of the berries and are mainly present bound to sugar as glycosides (90%), being chemically or enzymatically released in the wine during fermentation.[20] Some of these compounds have a relative low odor threshold, and have an influence regarding the sensory profile of the wine. Ethyl esters are mainly enzymatically synthesized by yeast during alcoholic fermentation.[21] In Cabernet Sauvignon wine, esters have been positively related in sensory studies to the overall aroma of the wine, including red fruits and dark fruit, amongst others.[18] Moreover, some alcohols such as 2-phenylethanol (rose-like aroma) have been described as comprising impact odorants of Cabernet Sauvignon.[22] In this sense, some studies have investigated the effects of different irrigation practices on the grape with respect to free aroma compounds and glycosidically bound aroma compounds (potential aroma), as well as on the volatile composition of wines. These studies have reported that a water deficit in the vines has a positive effect on the aromatic potential of Cabernet Sauvignon grapes,[23] as well as Merlot grapes,[24] as a result of increases in the concentrations of

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aromatic precursors. This is in agreement with a study reported by Ou *et al.*,[25] who observed that Merlot wine produced from grapevines under deficit irrigation was affected similarly with respect to the concentrations of terpene alcohols and norisoprenoids, which are both present in the grapes as glycosidically bound aroma compounds. Regarding the free aroma compounds, water stress resulted in a decrease of major volatile compounds in Merlot grapes, including hexanal, trans-2-hexenal and 1-hexanol.[24] Qian *et al.*[26] observed that Merlot wine produced from deficit-irrigated vines had increased amounts of vitispirane, β-damascenone, guaiacol, 4-methylguaiacol, 4-ethylguaiacol and 4-vinylguaiacol compared to wines produced from well-watered vines. However, no effect was observed on the concentrations of esters and terpenes.

Some sensory studies have also reported a positive effect of restrictive grapevine irrigation on wine quality in Cabernet Sauvignon grapes and wines[23] and in Monastrell grapes.[27] Similarly, after sensory evaluation of Cabernet Sauvignon wines, Chapman *et al.*[28] reported that vines under water deficit lead to wines with fruitier and less vegetal aromas compared to those from well-irrigated vines.

It should be emphasized, however, that the composition and aromatic profile of grapes and wines depends not only on the irrigation practices in the vineyards, but also, amongst many other factors, on the ripeness of the berries. It is generally recognized that grape maturity is governed by sugar content[29] but not necessarily coupled to the evolution of the grape berry's aromatic metabolism.[30,31]

The timing of the so-called 'optimal ripeness' of grapes, leading to a particular type of wine, is generally influenced by several factors, such as grape cultivar, climate, topography, seasonal weather conditions and vineyard management practices.[32]

Advanced grape maturity and greater sun exposure favour the accumulation of varietal compounds in the berry.[33] Additionally, lower canopy densities may produce an increase in glycosidically bound compounds in the berry.[34,35]

In addition, in Pinot Noir wines, it has been observed that some esters have a clear trend to decrease, whereas monoterpenoids and C_{13}-norisoprenoids tend to increase with grape maturation.[36] A study carried out on Cabernet Sauvignon wine concluded that C_6-alcohols, isobutyl methoxy pyrazine and hexyl acetate decrease as ripening proceeds.[32]

A great deal of knowledge has been made available over the past few years regarding grape berry aromatic composition. Some of that knowledge addresses the impact of water irrigation regimes, and other information addresses the ripening stage on the aromatic profile of berries and wines. However, very few studies have been carried out aiming to determine which of these factors is the most decisive in the aroma profile of the resulting wines. Therefore, the present study aimed to evaluate the effect of different irrigation regimes and two different harvest dates on the volatile compounds of Cabernet Sauvignon wines from the Maipo Valley in Chile. Insights from the present study will provide knowledge that may eventually be applied to issues such as harvest timing and may narrow the knowledge gap between water stress treatments and wine aroma for one of the most important varieties cultivated around the world.

MATERIALS AND METHODS

Plant material and experimental site

The assay was carried out in two seasons, 2014 and 2015, on 12-year-old own-rooted *Vitis vinifera* plants cv. Cabernet Sauvignon, in a commercial vineyard located in the Maipo Valley (Haras de Pirque) in central Chile (33°42′30′′S, 70°36′13′′W). Grapevines were trained to a vertical trellising system (Guyot double pruning method) with almost north–south oriented rows planted in a plot size of 2.5 × 1.5 m and irrigated by conventional drip irrigation. The historical average yield was 8 ton ha^{-1}. The maximum and minimum temperature in both seasons, from January to April, showed warmer conditions with an average maximum temperature of 28.9 °C in 2014 and 29.7 °C in 2015 and an average minimum temperature of 7.1 °C in 2014 and 8.4 °C in 2015. In the 2014 growing season, there was an average of 0.6 mm of rain, although no rain occurred during the assay in 2015.

Irrigation treatments and experimental design

The experimental design in the vineyard consisted of a randomized complete block with five biological replicates. Each replicate consisted of seven consecutive homogeneous vines. Three deficit irrigation treatments were established by means of a combination of drip emitters with different water volumes that began approximately 10 days before veraison to maintain midday stem water potentials: T1, $\Psi = -0.8$ MPa; T2, $\Psi = -0.9$ MPa and T3, $\Psi = -1.0$ MPa, throughout the season. The veraison date in both seasons was determined by visual observation and historical data from the winery. Plant water status was monitored weekly by measuring midday stem water potential determined by means of a pressure chamber. For wine making, three replicates per treatment were made by harvesting and mixing the fruit of the same treatment from five replicates. Therefore, there were three final wine replicates for each treatment. The harvest data in 2014 was 55 days after veraison (DAV).

In the case of 2015, the grapes were harvested on two different dates: 55 and 64 DAV. Wine was produced with these grapes of two different maturities. The characteristics of the grapes harvested on 55 DAV in 2015 were compared with the grapes harvested in 2014, in terms of grape ripening.

Winemaking procedure

The grapes were manually harvested, destemmed and crushed by a semiautomatic crusher machine and then disposed in 25-L plastic alimentary vats per triplicate. All vats were inoculated with a commercial yeast inoculum in accordance with the manufacturer's instructions (20 g hL^{-1}) (Lamothe-Abiet, Canéjan, France). The density and temperature were checked daily. The fermentation process was maintained at a temperature of 23–24 °C with punch-down twice per day. After 8–9 days, the wines were dry (< 2 g L^{-1} fermentable sugar). Then, the replicates were pressed using a single basket press. Free-run fractions were racked, cold-stabilized and then SO_2 free levels were adjusted to 30 mg L^{-1} preventing malolactic fermentation and finally bottled and stored at 15 °C for further analysis. The same winemaking procedure was utilized in all treatments and for both seasons.

Grape and wine chemical analysis

The analytical methods recommended by OIV (1990)[37] were used to determine the pH in the grape juice and wine, the soluble solid content in the grapes (°Brix), the sugar content in the wines (g glucose L^{-1}), the titratable acidity in the grape juice and wine (g tartaric acid L^{-1}) and the ethanol content (% v/v) in the wine.

Wine aroma analysis

Reagents and standards

All standard compounds employed in the present study were supplied by Sigma-Aldrich (Hamburg, Germany), except for acetic acid, ethyl acetate, sodium chloride and 4-methyl-2-pentanol, which were purchased from Merck (Darmstadt, Germany). MilliQ water was obtained from a Purelab Ultra MK2 purification system (Elga, St Albans, UK). Helium gas was supplied by Indura SA (Santiago, Chile).

Headspace solid phase microextraction (HS-SPME) and GC/MS conditions

Parameters of the HS-SPME method were optimized for the most suitable extraction and desorption of wine volatiles. Subsequently, the conditions employed were 7 mL of wine, 1.5 g of sodium chloride, 15 μL of internal standard, 4 methyl-2-pentanol (0.75 g L^{-1}), which were placed into a 20-mL glass vial and then in the autosampler tray for HS-SPME sampling.

Static headspace sampling was employed after the fibre was cleaned and conditioned. After 20 min of incubation at 45 °C and agitation at 500 rpm, volatiles from the headspace of the wine were extracted over a period of 40 min employing a 2-cm 50/30 μm Carboxen/DVB/PDMS SPME fibre (Agilent Technologies, Santa Clara, CA, USA). The fibre penetration in the vial during extraction was 30 mm. Following this, the fibre was desorbed over 180 s. The sample was injected using the splitless mode with a transfer line temperature of 280 °C.

Gas chromatography analysis was then carried out using a 7890B Agilent GC system coupled to a quadrupole mass spectrometer (Agilent 5977 inert; Agilent Technologies).

A DB Wax capillary column with dimensions 60 × 0.25 mm and a film thickness of 0.25 μm (Agilent Technologies) was used with helium carrier gas at a flow rate of 1 mL min^{-1}. The oven temperature programme comprised: temperature of 35 °C for 10 min, which was then raised to 100 °C at 5 °C min^{-1}, and then to 210 °C at 3 °C min^{-1} (holding for 40 min). The electron ionization mass spectra in the scan mode were recorded at 70 eV with the electron energy in the range 40–300 amu.

All data were recorded using ChemStation (Agilent Technologies). The samples were analyzed in triplicate, and blank runs using an empty glass tube were performed before and after each analysis. Compound identification was based on mass spectra matching using the 2.0 version of the standard NIST library and the retention index of authentic reference standards. Calibration curves were performed to calculate the concentration of the volatile compounds determined. These standard curves were constructed by graphing the relative area of each compound versus the concentration. The relative area was calculated by dividing the peak area of the target ion of each compound by the peak area of the target ion of the internal standard. Those compounds for which we do not have the standard were quantified as equivalents using the curve equation of another compound of the same chemical group with the same target ion, as indicated where appropriate.

Statistical analysis

The statistical method used for analysing the general parameters of the berry and wine, as well as the aroma composition in the wines (from the first harvest data in 2014 and 2015), was one-way analysis of variance (ANOVA).

The volatile composition of the wine in 2015 was analyzed for statistical significance by two-way [irrigation treatment (I) and harvest data (HD)] ANOVA.

Principal component analysis (PCA) was carried out to observe the distribution of different treatments depending on the aromatic composition of the wines.

The data were analyzed using XLstat-Pro (2011) (Addinsoft, Paris, France).

RESULTS AND DISCUSSION

Water relationships

In both seasons, the xylem water potential of the vines was reduced by hastening irrigation from 25 days before veraison to 10 days before veraison. The use of different combinations of drip emitters per treatment yielded substantial differences in the xylem water potential throughout ripening. The average xylem water potential values in 2014 were $\Psi = -0.83 \pm 0.03$ MPa for T1, $\Psi = -0.90 \pm 0.03$ MPa for T2 and $\Psi = -1.00 \pm 0.02$ MPa for T3. For the 2015 season, stem water potential values were: $\Psi = -0.85 \pm 0.02$ MPa for T1, $\Psi = -0.96 \pm 0.02$ MPa for T2 and $\Psi = -1.04 \pm 0.01$ MPa for T3. In both years, significant differences in the potentials were observed among treatments, particularly when irrigation was resumed after veraison, although, in 2014, as a result of an unexpected rainfall event, differences between treatments were more evident only from 35 DAV until the end of the season (Fig. 1).

Grape and wine composition

Table 1 shows the analyses of the general parameters of the grape and wine samples. In 2014, there were no significant differences across the irrigation treatments in terms of pH, titratable acidity and soluble solids in the grapes, as well as pH, titratable acidity, residual sugar content and ethanol content in the wines. The climatic conditions of 2014 produced that the grape water potentials of the different treatments took longer to differentiate (advanced maturation stage) (Fig. 1) and could be related to the absence of differences in the general parameters analyzed. For 2015, there were no differences in terms of pH or titratable acidity, although a higher soluble solids value was observed in grapes from the most restrictive irrigation treatment harvested 64 DAV compared to T1 and T2 but not in grapes harvested at 55 DAV, suggesting that water stress affected the concentration of sugars, although only after a rather late harvesting time. Still, such differences in grapes were not observed as differences in ethanol concentrations in wines between irrigation regimes, although all of the wines from grapes harvested at 64 DAV reached higher ethanol concentrations compared to those harvested at 55 DAV (Table 1).

Volatile compound composition

Effects of different irrigation treatments

The results reported here correspond to the 2014 harvest and the 2015 early harvesting dates, considering that their maturity and vegetative condition were very similar.

Table 2 presents volatile compounds from the two vintages, 2014 and 2015, in red wines, together with the ANOVA results for the 'deficit irrigation' factor. Data are arranged into eight chemical families (ethyl, acetate and isoamyl esters, alcohols, terpenes, acids, aldehydes and C_{13}-norisoprenoids). In wines from both years, 47 compounds were found in 2014 and 46 compounds in 2015 (Table 2). In 2014, seven volatile compounds showed significant differences among irrigation treatments, as did twelve volatile compounds in 2015. Alcohols, acids, ethyl esters and total esters varied significantly in 2015, whereas only the group of

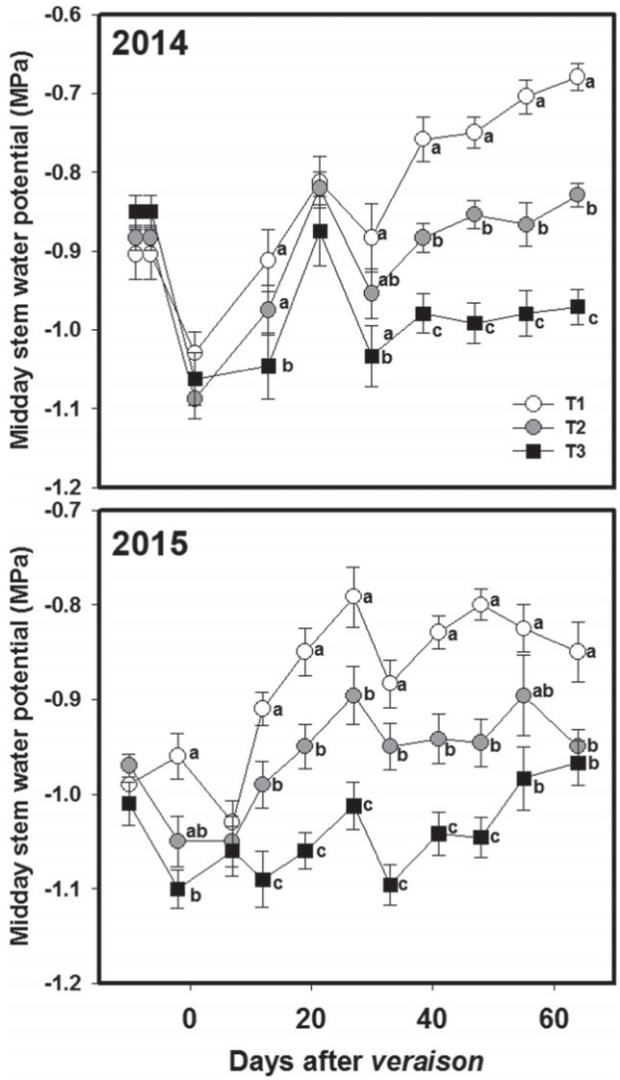

Figure 1. Midday stem water potential in 2014 and 2015 season. Different letters denote significant differences among treatments: $P < 0.05$, Tukey's honestly significant difference test (T1, $\Psi = -0.8$ MPa; T2, $\Psi = -0.9$ MPa and T3, $\Psi = -1.0$ MPa).

isoamyl esters changed in 2014 as a function of the irrigation treatment.

In terms of amounts, the alcohol group was the most abundant, mainly as a result of the high concentration of 3-methyl-1-butanol; however, the ester group presented a higher number/variety of compounds. Within esters, most were ethyl esters and the most abundant esters in both years were ethyl acetate, followed by ethyl hexanoate, isoamyl acetate and propyl acetate in 2014, and isoamyl acetate, ethyl hexanoate and ethyl butyrate in 2015. Their content depends on several factors, such as yeast strain, fermentation temperature, aeration and sugar content. Even though the same winemaking equipment, procedures and replications of fermentation lots were used in both years, the variability in ester concentrations suggests the existence of non-obvious enological factors influencing annual fermentations for each level of irrigation (e.g. the different weather conditions between vintages). According to the ANOVA, the ester group was the most modified by irrigation treatments. These correspond to six compounds in 2014 (ethyl succinate, ethyl 3 methylbutyl succinate, ethyl hexadecanoate, propyl acetate, isoamyl lactate and isoamyl hexanoate) and seven compounds in 2015 (ethyl octanoate, ethyl nonanoate, ethyl decanoate, ethyl hydrogen succinate, isobutyl acetate, isoamyl hexanoate and isoamyl decanoate). It is notable that the wines produced with grapes from the more restricted vines (T3), resulted in the lowest total amount of esters. By contrast, the wines from T1 and T2 (i.e. the least water-restricted treatment and the intermediate irrigation treatment, respectively) resulted in the highest number of esters. This could be partially explained by the fact that water deficit affects the fatty acid metabolism, as reported for Cabernet Sauvignon by Deluc *et al.*[17] The abundance of transcripts leading to the synthesis of the enzymes responsible for the synthesis of plant volatile esters derived from fatty acids, such as lipoxygenase and hydroperoxide lyase, was increased by water deficit conditions. Our results suggest that esters, which contribute to the typically fruity character of red wines, such as Cabernet Sauvignon wines, as characterized by black and red fruit aromas, are affected by the water status of vines.

Twelve alcohols were identified in the present study. The major alcohols were 3-methyl-1-butanol followed by 2-phenylethanol and isobutanol in wines from both years, although without a clear trend with respect to the irrigation regimes. In 2014, higher amounts were obtained in wines from T2, and in 2015 from T3. Therefore, it is expected that environmental conditions are more important with respect to the alcohol content in wines than the plant water status of the vines alone.

Terpenes identified in the present study include *o*-cymene, α-terpineol, citronellol and linalool (Table 2). In 2014, the major terpene was *o*-cymene and, in 2015, it was citronellol, with a similar trend in concentration based on irrigation treatment in both years. Vine water stress has been reported to increase the concentration of aroma glycosides of grapes.[23] It has also been reported that red wines produced from vines under water stress (compared to fully irrigated) have higher concentrations of terpene alcohols, depending on the individual compound and vintage year,[25] which is similar to our results. Indeed, we observed that the intermediate deficit irrigation treatment (T2) resulted in wines with a higher terpene content than in the most irrigated treatment (T1); however, the lowest content was obtained in wines from the most restrictive irrigation treatment (T3). This might be an indication that extreme water stress conditions could lead to wines with lower terpene content, meaning that the intermediate controlled water stress conditions are a more suitable strategy for increased terpenes. The concentrations of these terpenes are typically much lower in non-floral wines such as Cabernet Sauvignon compared to Muscat or Gewurztraminer, although their aroma contribution could have an important synergistic effect.[25]

As for the volatile acids composition, acetic acid, hexanoic acid, octanoic acid and decanoic acid were identified. The major volatile acid in 2014 was hexanoic acid and, in 2015, it was octanoic acid. In 2014, the T1 and T2 treatments resulted in wines with higher volatile acid contents, whereas, in 2015, T2 was significantly higher, although no clear trend was observed.

Effects of different harvest dates

Table 3 shows the results corresponding to the two harvest dates in 2015, under different deficit treatments. The results show that the factor harvest date produced more significant differences than the irrigation regimes on the aromatic profiles on the wines. Twenty-nine volatile compounds showed significant differences among ripening stages, whereas the irrigation regime caused eleven significant differences. From a global point of view, the total sum of the volatiles in the wine decreased from harvest

Table 1. Grape and wine chemical parameters from different irrigation treatments in Cabernet Sauvignon variety harvested in 2014 and 2015 (n = 3, mean ± SE)

		2014			2015		
Grape parameters	Harvest date	T1	T2	T3	T1	T2	T3
pH	55 DAV	3.5 ± 0.0	3.6 ± 0.0	3.6 ± 0.0	3.7 ± 0.0	3.7 ± 0.1	3.6 ± 0.0
	64 DAV				3.8 ± 0.0	3.7 ± 0.1	3.7 ± 0.0
Titratable acidity (g tartaric acid L^{-1})$^{-}$	55 DAV	3.4 ± 0.1	3.0 ± 0.1	3.1 ± 0.2	3.4 ± 0.1	3.6 ± 0.1	3.6 ± 0.1
	64 DAV				3.4 ± 0.1	3.3 ± 0.1	3.1 ± 0.0
Soluble solids (°Brix)	55 DAV	21.5 ± 0.4	21.6 ± 0.4	21.1 ± 0.3	21.1 ± 0.2	21.3 ± 0.3	21.3 ± 0.3 B
	64 DAV				21.9 ± 0.3 b	21.3 ± 0.2 b	24.0 ± 0.3 Aa
Wine parameters							
pH	55 DAV	3.8 ± 0.1	3.9 ± 0.2	3.8 ± 0.1	3.7 ± 0.1	3.7 ± 0.1	3.7 ± 0.1
	64 DAV				3.8 ± 0.1	3.7 ± 0.2	3.7 ± 0.1
Titratable acidity (g tartaric acid L^{-1})	55 DAV	3.2 ± 0.1	3.1 ± 0.2	3.1 ± 0.1	3.6 ± 0.1	3.4 ± 0.2	3.7 ± 0.1
	64 DAV				3.8 ± 0.1	3.6 ± 0.2	3.7 ± 0.1
Sugar content (g glucose L^{-1})	55 DAV	1.9 ± 0.1	2.2 ± 0.1	2.1 ± 0.2	1.2 ± 0.1	0.9 ± 0.2	1.3 ± 0.1
	64 DAV				1.4 ± 0.1	1.3 ± 0.1	1.6 ± 0.2
Ethanol content (% v/v)	55 DAV	13.4 ± 0.2	13.5 ± 0.1	13.0 ± 0.3	12.0 ± 0.2 B	11.0 ± 0.1 B	12.5 ± 0.3 B
	64 DAV				13.5 ± 0.2 A	13.6 ± 0.1 A	14.5 ± 0.3 A

Different lowercase letters in the same row within a vintage indicate significant differences ($P < 0.05$) between irrigation treatments (Tukey's honestly significant difference test).
Different uppercase letters indicate significant differences ($P < 0.05$) between harvest date (Tukey's honestly significant difference test).
Deficit irrigation treatments: T1, $\Psi = -0.8$ MPa; T2, $\Psi = -0.9$ MPa and T3, $\Psi = -1.0$ MPa. DAV, days after veraison

1 at 55 DAV (H1) to harvest 2 at 64 DAV (H2) when a mild water deficit was applied (T1); however, the total concentration of the compounds increased when medium and high water deficit treatments were applied to the grapes (T2 and T3). This is probably caused by the increase in the concentration of soluble solids in grapes. Medium and high water deficits combined with a late harvest date produced wines with a higher concentration of volatile compounds; however, a delayed harvest date on T1 treatments decreased the aromatic potential of wines as a result of possible degradation of volatile secondary metabolites in the grapes.

Among the 24 esters found, there were seven esters whose levels significantly increased when harvested later in the season, independent of the irrigation treatment: ethyl heptanoate, ethyl nonanoate, ethyl decanoate, ethyl dodecanoate, ethyl hexadecanoate, isoamyl lactate and isoamyl decanoate (Table 3). By contrast, seven other esters decreased when harvested later: ethyl butyrate, ethyl phenylacetate, ethyl hydrogen succinate, isobutyl acetate, isoamyl acetate, isoamyl butanoate and isoamyl hexanoate.

Additionally, there were nine esters in which a significant interaction between harvest date and deficit irrigation (HD × DI) was observed (Table 3). The esters group presented a heterogeneous trend, which is in agreement with the results of Fang and Qian.[36] In general, esters were reduced for the later harvesting in the mild and intermediate water stress conditions T1 and T2; however, esters increased at the second date of harvest in wines produced from the T3 vines under severe water stress. Bowen and Reynolds[30] suggested that the decrease in esters in later harvest dates is most likely related to the concomitant reductions in titratable acidity in the wine when harvested later in the season. These observations contrast with the results of the present study because the titratable acidity did not show significant differences between both harvests dates, nor between water status treatments (Table 1). However, other studies in Cabernet Sauvignon showed a ripening-related increase in volatile esters, caused by an increase of the sugar content,[32] which agrees with the significant increase in sugar levels in T3 between H1 and H2 (Table 1). In addition, some studies have reported that the formation of total esters is directly proportional to the quantity of amino acids.[38] Indeed, the up-regulation of the amino acid biosynthesis and their biomolecular solubilization occurs during the late ripening phase of grape berries.[39,40]

Similar to the esters group, some of the 12 alcohols determined were found in significantly higher concentrations in H2 (with respect to H1), regardless of the irrigation treatments, such as butanol, 3-methyl-1-butanol and 2,3-butanediol (Table 3). By contrast, some alcohols decreased: 4-methyl-1-pentanol, 1-octanol, 1-nonanol, 1-decanol and hexanol. Similar to our results, Bindon *et al.*[32] also showed that butanol increased and hexanol decreased in wines with advancing harvest dates for Cabernet Sauvignon. In addition, we found three interactions between HD × DI and the total alcohols group, caused by a decrease between harvest date in T1, and an increase in the other two treatments, except for 2-phenylethanol, which was found in small amounts in T3H2 with respect to T3H1 (Table 3). The presence of 2-phenylethanol contributes to flowery sweet and flowery/rose/honey notes.[41] The alcohol content in wines from T3 was almost the same for H1 and H2. Overripe grapes are known to have higher free amino acids, thus reducing the requirement of yeasts to produce higher alcohols.[42] In addition, alcohols possess a higher propensity to form fruity esters in the presence of carboxylic acids during vinification.[43] This could be an explanation for the behaviour observed in the T3 treatment, where there were no differences in alcohol content, although there was a higher ester content between H1 and H2.

Previous studies have reported that isobutyl methoxypyrazine is one of the main compounds responsible for the typical aroma of Cabernet Sauvignon wines.[44] A harvest delay of 15 days can significantly diminish the concentration of isobutyl methoxypyrazine.[45] This aroma, described as 'green bell pepper', is produced by C_6

Table 2. Concentration ($\mu g\ L^{-1}$) of volatile compounds in the headspace of the wines obtained from different irrigation treatments in Cabernet Sauvignon variety at early harvesting date in 2014 and 2015

		2014				2015			
Compound	Linear retention index	T1	T2	T3	ANOVA	T1	T2	T3	ANOVA
Ethyl isobutyrate	1010	33.7 ± 1.9	28.9 ± 2.0	29.0 ± 3.8	NS	12.8 ± 0.7	9.39 ± 2.01	12.4 ± 3.4	NS
Ethyl butyrate	1055	83.2 ± 3.6	76.8 ± 3.9	77.4 ± 1.2	NS	197 ± 15	190 ± 14	187 ± 7	NS
Ethyl-2-methylbutyrate	1084	57.0 ± 3.2	46.6 ± 0.3	48.5 ± 6.3	NS	10.6 ± 0.7	9.75 ± 0.61	11.3 ± 0.0	NS
Ethyl isovalerate	1089	10.8 ± 1.3	7.78 ± 0.54	8.60 ± 1.63	NS	ND	ND	ND	NS
Ethyl hexanoate	1245	1258 ± 21	1146 ± 101	1047 ± 14	NS	1287 ± 49	1314 ± 71	1114 ± 15	NS
Ethyl heptanoate [Θ]	1348	4.48 ± 0.49	3.85 ± 0.03	4.05 ± 0.32	NS	2.15 ± 0.00	1.74 ± 0.17	2.26 ± 0.54	NS
Ethyl octanoate	1437	51.7 ±0.8	48.9 ± 2.4	47.6 ± 0.6	NS	92.8 ± 1.0 ab	105 ± 3 a	83.2 ± 4.9 b	*
Ethyl nonanoate [Θ]	1534	3.41 ± 0.06	3.47 ± 0.10	3.36 ± 0.45	NS	1.94 ± 0.06 a	1.69 ± 0.04 ab	1.50 ± 0.15 b	*
Ethyl decanoate	1647	2.97 ± 0.78	2.84 ± 0.51	2.03 ± 0.17	NS	8.54 ± 0.66 b	10.3 ± 0.0 a	6.84 ± 0.26 b	**
Ethyl succinate	1679	50.4 ± 3.8 a	55.3 ± 1.3 a	37.2 ± 0.7 b	*	2.94 ± 0.38	2.50 ± 0.11	2.40 ± 0.09	NS
Ethyl phenylacetate	1799	5.93 ± 0.16	5.23 ± 0.27	5.26 ± 0.26	NS	1.73 ± 0.05	1.61 ± 0.02	1.69 ± 0.06	NS
Ethyl dodecanoate [Θ]	1864	7.34 ± 1.52	4.24 ± 0.15	5.23 ± 0.21	NS	11.4 ± 0.2	15.2 ± 1.0	12.0 ± 2.0	NS
Ethyl tetradecanoate [Θ]	2041	3.08 ± 0.57	4.63 ± 0.20	4.26 ± 0.71	NS	2.07 ± 0.32	1.68 ± 0.37	1.61 ± 0.16	NS
Ethyl hexadecanoate	2233	9.09 ± 0.64 b	20.1 ± 2.5 a	21.3 ± 2.1 a	*	12.4 ± 1.3	10.1 ± 1.4	8.71 ± 0.36	NS
Ethyl hydrogen succinate [α]	2292	17.0 ± 3.8	19.8 ± 1.1	15.1 ± 0.7	NS	1.25 ± 0.22 a	0.62 ± 0.04 b	0.53 ± 0.05 b	*
Ethyl esters		*1596 ± 38*	*1474 ± 116*	*1356 ± 4*	*NS*	*1646 ± 63 ab*	*1674 ± 90 a*	*1445 ± 15 b*	*
Ethyl acetate	879	19905 ± 476	19443 ± 1012	19266 ± 142	NS	22696 ± 911	22013 ± 245	21515 ± 1701	NS
Propyl acetate	985	409 ± 31 a	123 ± 5 b	113 ± 2 b	***	ND	ND	ND	NS
Isobutyl acetate	1015	9.05 ± 0.01	10.7 ± 1.8	10.3 ± 0.2	NS	46.6 ± 0.5 b	58.3 ± 1.1 a	49.2 ± 1.9 b	**
Isoamyl acetate	1122	208 ± 31	272 ± 9	296 ± 34	NS	2149 ± 116	2341 ± 168	1942 ± 82	NS
Acetate esters		*20532 ± 538*	*18849 ± 1027*	*19685 ± 110*	*NS*	*24891 ± 1027*	*24413 ± 76*	*23506 ± 1785*	*NS*
Isoamyl butanoate	1306	0.60 ± 0.01	0.55 ± 0.04	0.49 ± 0.03	NS	0.82 ± 0.10	0.76 ± 0.09	0.74 ± 0.02	NS
Isoamyl hexanoate	1479	1.06 ± 0.00 a	0.99 ± 0.02 b	0.96 ± 0.00 b	**	0.74 ± 0.02 ab	0.83 ± 0.05 a	0.65 ± 0.02 b	*
Isoamyl lactate	1524	30.2 ± 5.9 ab	39.1 ± 0.5 a	17.6 ± 1.3 b	NS	28.6 ± 0.4	23.0 ± 3.9	25.3 ± 6.1	NS
Isoamyl octanoate	1680	33.3 ± 1.0	35.4 ± 0.4	35.4 ± 0.8	*	145 ± 27	156 ± 28	87.9 ± 16.4	NS
Isoamyl decanoate	1888	14.5 ± 3.4	9.38 ± 3.06	8.97 ± 0.13	NS	34.8 ± 1.1 b	47.3 ± 2.9 a	31.2 ± 3.0 b	*
Isoamyl esters		*79.7 ± 3.5 a*	*85.4 ± 3.1 a*	*63.5 ± 0.4 b*	**	*210 ± 26*	*228 ± 27*	*146 ± 26*	*NS*
Total esters		*22208 ± 579*	*21408 ± 1146*	*21104 ± 114*	*NS*	*26747 ± 990 a*	*26315 ± 193 a*	*25097 ± 1774 b*	*
Isobutanol	1074	13414 ± 341	13449 ± 1626	11871 ± 516	NS	16767 ± 43 b	17289 ± 93 b	18050 ± 281 a	*
Butanol	1172	1631 ± 97	1657 ± 54	1728 ± 46	NS	1078 ± 34	850 ± 74	1005 ± 52	NS
3-Methyl-1-butanol	1200	156397 ± 4269	157473 ± 3833	150546 ± 1619	NS	178171 ± 2404 a	168561 ± 93 b	179583 ± 949 a	*
4-Methyl-1-pentanol [β]	1315	45.9 ± 1.9	48.1 ± 1.6	44.3 ± 1.3	NS	141 ± 13	143 ± 7	127 ± 2	NS
3-Methyl-1-pentanol	1332	402 ± 12	425 ± 13	411 ± 26	NS	1542 ± 95	1445 ± 78	1431 ± 18	NS
3-Hexen-1-ol	1410	60.2 ± 3.3	61.5 ± 0.8	55.2 ± 3.7	NS	70.9 ± 3.4	67.2 ± 0.5	62.5 ± 8.4	NS

Table 2. Continued

Compound	Linear retention index	2014				2015			
		T1	T2	T3	ANOVA	T1	T2	T3	ANOVA
1-octanol [δ]	1577	1.40 ± 0.15	1.54 ± 0.15	1.22 ± 0.16	NS	2.90 ± 0.32	3.53 ± 0.49	3.03 ± 0.11	NS
2,3-Butanediol	1534	670 ± 134	667 ± 14	677 ± 2	NS	378 ± 11	281 ± 29	310 ± 74	NS
1-Nonanol	1681	3.21 ± 0.12	3.32 ± 0.36	2.74 ± 0.40	NS	3.90 ± 0.10	4.49 ± 0.12	3.99 ± 0.55	NS
1-Decanol	1793	2.07 ± 0.00	2.05 ± 0.04	2.04 ± 0.02	NS	2.45 ± 0.11	2.57 ± 0.06	2.44 ± 0.00	NS
2-Phenylethanol	1940	38715 ± 1930	38815 ± 1375	37384 ± 160	NS	31911 ± 413	29517 ± 40	31311 ± 1203	NS
Hexanol	1375	1387 ± 38	1380 ± 33	1357 ± 86	NS	51.5 ± 2.8	39.1 ± 4.7	46.9 ± 5.9	NS
Total alcohols		*212729 ± 6820*	*213983 ± 6923*	*204080 ± 1873*	*NS*	*230120 ± 2621 a*	*218202 ± 1078 b*	*231937 ± 1997 ab*	*
o-Cymene	1242	5.15 ± 0.82	4.95 ± 0.64	4.31 ± 0.33	NS	1.59 ± 0.08	1.61 ± 0.15	1.61 ± 0.16	NS
Linalool	1555	2.95 ± 0.09	3.04 ± 0.03	2.44 ± 0.11	NS	2.40 ± 0.02	2.53 ± 0.33	2.18 ± 0.00	NS
α-Terpineol	1693	3.04 ± 0.05	3.10 ± 0.06	2.83 ± 0.10	NS	1.33 ± 0.03	1.32 ± 0.06	1.30 ± 0.05	NS
Citronellol	1785	3.10 ± 0.25	3.48 ± 0.32	3.00 ± 0.15	NS	6.01 ± 0.69	7.26 ± 1.05	6.50 ± 0.74	NS
Total terpenes		*14.2 ± 0.5*	*14.6 ± 1.1*	*12.6 ± 0.4*	*NS*	*11.3 ± 0.8*	*12.7 ± 1.6*	*11.6 ± 1.1*	*NS*
Acetic acid	1484	76901 ± 325	85458 ± 6542	82277 ± 3979	NS	47381 ± 2265 b	64056 ± 3352 a	72072 ± 3465 a	**
Hexanoic acid	1880	802 ± 25	763 ± 38	729 ± 5	NS	1006 ± 5	1167 ± 87	959 ± 52	NS
Octanoic acid	2076	13.2 ± 0.8	12.0 ± 12.0	2.8 ± 0.4	NS	221 ± 28 b	386 ± 51 a	199 ± 19 b	*
Decanoic acid	2329	27.2 ± 0.0 a	24.7 ± 0.9 b	22.9 ± 0.3 b	*	28.7 ± 1.5 b	36.4 ± 2.6 a	28.9 ± 0.5 b	*
Total acids		*77743 ± 351*	*86257 ± 6593*	*83031 ± 3984*	*NS*	*48636 ± 2289 b*	*65645 ± 3211 a*	*73259 ± 3394 b*	*
Furfural	1438	73.8 ± 1.4	70.1 ± 9.2	76.1 ± 2.2	NS	ND	ND	ND	NS
Benzaldehyde	1565	3.16 ± 0.21	2.88 ± 0.04	2.96 ± 0.13	NS	1.93 ± 0.24	1.56 ± 0.01	1.90 ± 0.03	NS
Total aldehydes		*76.9 ± 1.6*	*73.1 ± 9.1*	*79.1 ± 2.3*	*NS*	*1.93 ± 0.24*	*1.56 ± 0.01*	*1.90 ± 0.03*	*NS*
β-Damascenone	1849	10.8 ± 1.4	15.7 ± 4.0	13.1 ± 2.4	NS	54.1 ± 11.8	53.9 ± 12.2	58.6 ± 8.7	NS
Total		*236304 ± 7426*	*236758 ± 8165*	*226494 ± 2017*	*NS*	*258472 ± 1678*	*246537 ± 746*	*258693 ± 3707*	*NS*

Values followed by different lowercase letters in the same row within a vintage, indicate the existence of significant differences between irrigation treatments ($P < 0.05$).
Level of significance: *$P < 0.05$, **$P < 0.01$ and **$P < 0.001$ respectively. NS, no significant difference. ND, not detected. Italic letters mean summatory.
Deficit irrigation treatments: T1, Ψ = −0.8 MPa; T2, Ψ = −0.9 MPa and T3, Ψ = −1.0 MPa.
[Θ]Equivalents of ethyl undecanoate.
[α]Equivalents of ethyl succinate.
[β]Equivalents of 3-methyl-1-pentanol.
[δ]Equivalents of nonanol.

Table 3. Concentration ($\mu g\ L^{-1}$) of volatile compounds in the headspace of the wines obtained from different irrigation treatments in Cabernet Sauvignon variety harvested at 55 DAV (H1) and 64 DAV (H2) in 2015

	2015						Significance		
	T1		T2		T3		GLM		
Compound	H1	H2	H1	H2	H1	H2	HD	DI	HD × DI
Ethyl isobutyrate	12.8 ± 0.7	6 ± 3	9.39 ± 2.01	15.8 ± 5.3	12.4 ± 3.4	14.6 ± 2.2	NS	NS	NS
Ethyl butyrate	197 ± 15	133 ± 10	190 ± 14	131 ± 2	187 ± 7	132 ± 5	***	NS	NS
Ethyl-2-methylbutyrate	10.6 ± 0.7	10.7 ± 0.7	9.75 ± 0.61	12.4 ± 1.6	11.3 ± 0.0	10.2 ± 0.2	NS	NS	NS
Ethyl hexanoate	1287 ± 49	1302 ± 133	1314 ± 71	1306 ± 18	1114 ± 15	1219 ± 59	NS	NS	NS
Ethyl heptanoate [Θ]	2.15 ± 0.00	3.12 ± 0.49	1.74 ± 0.17	4.11 ± 0.15	2.26 ± 0.54	2.95 ± 0.44	***	NS	*
Ethyl octanoate	92.8 ± 1.0	85.7 ± 8.3	105 ± 3	78.3 ± 18.6	83.2 ± 4.9	84.8 ± 7.5	NS	NS	NS
Ethyl nonanoate	1.94 ± 0.06	2.63 ± 0.15	1.69 ± 0.04	3.94 ± 0.04	1.50 ± 0.15	4.33 ± 0.01	***	***	***
Ethyl decanoate	8.54 ± 0.66	12.5 ± 0.9	10.3 ± 0.0	13.0 ± 2.0	6.84 ± 0.26	17.8 ± 1.4	***	NS	**
Ethyl dodecanoate [Θ]	11.4 ± 0.2	18.5 ± 3.2	15.2 ± 1.0	18.6 ± 0.0	12.0 ± 2.0	29.5 ± 6.0	***	NS	*
Ethyl succinate	2.94 ± 0.38	2.46 ± 0.13	2.50 ± 0.11	2.49 ± 0.26	2.40 ± 0.09	2.31 ± 0.08	NS	NS	NS
Ethyl phenylacetate	1.73 ± 0.05	1.54 ± 0.04	1.61 ± 0.02	1.63 ± 0.12	1.69 ± 0.06	1.52 ± 0.03	*	NS	NS
Ethyl tetradecanoate [Θ]	2.07 ± 0.32	1.33 ± 0.02	1.68 ± 0.37	1.91 ± 0.17	1.61 ± 0.16	2.56 ± 0.17	NS	NS	**
Ethyl hexadecanoate [Θ]	12.4 ± 1.3	8.19 ± 1.01	10.1 ± 1.4	11.9 ± 0.1	8.71 ± 0.36	16.3 ± 0.2	*	*	***
Ethyl hydrogen succinate [α]	1.25 ± 0.22	0.33 ± 0.02	0.62 ± 0.04	0.39 ± 0.10	0.53 ± 0.05	0.36 ± 0.04	***	**	**
Ethyl esters	*1646 ± 63 ab*	*1588 ± 158*	*1674 ± 90 a*	*1601 ± 44*	*1445 ± 15 a*	*1538 ± 57*	*NS*	*NS*	*NS*
Ethyl acetate	22696 ± 911	22511 ± 103	22013 ± 245	22946 ± 450	21515 ± 1701	23515 ± 276	NS	NS	NS
Isobutyl acetate	46.6 ± 0.5	23.7 ± 1.8	58.3 ± 1.1	26.0 ± 2.2	49.2 ± 1.9	22.8 ± 5.7	***	*	NS
Isoamyl acetate	2149 ± 116	1111 ± 10	2341 ± 168	1014 ± 71	1942 ± 82	1185 ± 17	***	NS	*
Acetate esters	*24891 ± 1027*	*23645 ± 95*	*24413 ± 76*	*23986 ± 523*	*23506 ± 1785*	*24723 ± 298*	***	*NS*	*NS*
Isoamyl butanoate	0.82 ± 0.10	0.68 ± 0.03	0.76 ± 0.09	0.62 ± 0.08	0.74 ± 0.02	0.67 ± 0.05	*	NS	NS
Isoamyl lactate	145 ± 27	94.3 ± 11.0	156 ± 28	91.3 ± 1.8	87.9 ± 16.4	98.7 ± 8.0	**	NS	*
Isoamyl hexanoate	0.74 ± 0.02	0.76 ± 0.12	0.83 ± 0.05	0.66 ± 0.01	0.65 ± 0.02	0.65 ± 0.03	NS	NS	NS
Isoamyl octanoate	28.6 ± 0.4	29.2 ± 3.0	23.0 ± 3.9	53.2 ± 8.0	25.3 ± 6.1	52.9 ± 10.8	*	NS	NS
Isoamyl decanoate	34.8 ± 1.1	51.8 ± 0.8	47.3 ± 2.9	49.1 ± 3.9	31.2 ± 3.0	76.4 ± 10.0	***	NS	**
Isoamyl esters	*210 ± 26*	*177 ± 9*	*228 ± 27*	*195 ± 6*	*146 ± 26*	*229 ± 13*	**	*NS*	*
Total esters	*26747 ± 990 a*	*25410 ± 72*	*26315 ± 193 a*	*25782 ± 561*	*25097 ± 1774 b*	*26490 ± 254*	*NS*	*NS*	*NS*
Isobutanol	16767 ± 43	16559 ± 2851	17289 ± 93	17136 ± 437	18050 ± 281	16512 ± 3023	NS	NS	NS
Butanol	1078 ± 34	965 ± 80	850 ± 74	1233 ± 92	1005 ± 52	1277 ± 71	**	NS	**
3-Methyl-1-butanol	178171 ± 2404	180472 ± 3730	168561 ± 938	175073 ± 5015	179583 ± 949	186354 ± 2162	*	**	NS
4-Methyl-1-pentanol [β]	141 ± 13	97.6 ± 1.7	143 ± 7	84.3 ± 2.5	127 ± 2	89.2 ± 4.4	***	NS	NS
3-Methyl-1-pentanol	1542 ± 95	1540 ± 14	1445 ± 78	1366 ± 27	1431 ± 18	1299 ± 26	NS	**	NS
3-Hexen-1-ol	70.9 ± 3.4	67.3 ± 1.9	67.2 ± 0.5	66.6 ± 0.9	62.5 ± 8.4	68.0 ± 3.2	NS	NS	NS
1-Octanol [δ]	2.90 ± 0.32	2.25 ± 0.54	3.53 ± 0.49	1.76 ± 0.21	3.03 ± 0.11	1.62 ± 0.51	**	NS	NS
2,3-Butanediol	378 ± 11	356 ± 6	281 ± 29	626 ± 115	310 ± 74	606 ± 49	**	NS	*
1-Nonanol	3.90 ± 0.10	3.15 ± 0.37	4.49 ± 0.12	2.92 ± 0.34	3.99 ± 0.55	3.38 ± 0.88	**	NS	NS
1-Decanol	2.45 ± 0.11	2.34 ± 0.03	2.57 ± 0.06	2.20 ± 0.05	2.44 ± 0.00	2.23 ± 0.08	***	NS	NS

Table 3. Continued

	2015						Significance		
	T1		T2		T3		GLM		
Compound	H1	H2	H1	H2	H1	H2	HD	DI	HD × DI
2-Phenylethanol	31911 ± 413	28250 ± 164	29517 ± 40	30833 ± 383	31311 ± 1203	31259 ± 1372	NS	NS	*
Hexanol	51.5 ± 2.8	36.5 ± 2.7	39.1 ± 4.7	38.7 ± 2.0	46.9 ± 5.9	41.4 ± 3.3	*	NS	NS
Total alcohols	*230120 ± 2621 a*	*228350 ± 982*	*218202 ± 1078 b*	*226463 ± 5607*	*231937 ± 1997 ab*	*237513 ± 3946*	*NS*	**	**
o-Cymene	1.59 ± 0.08	1.30 ± 0.05	1.61 ± 0.15	1.53 ± 0.04	1.61 ± 0.16	1.81 ± 0.02	NS	*	*
α-Terpineol	1.33 ± 0.03	1.09 ± 0.05	1.32 ± 0.06	1.11 ± 0.09	1.30 ± 0.05	1.21 ± 0.01	**	NS	NS
Citronellol	6.01 ± 0.69	6.66 ± 0.87	7.26 ± 1.05	5.59 ± 1.15	6.50 ± 0.74	4.79 ± 0.08	NS	NS	NS
Linalool	2.40 ± 0.02	1.41 ± 0.15	2.53 ± 0.33	1.46 ± 0.11	2.18 ± 0.00	1.38 ± 0.04	***	NS	NS
Total terpenes	*11.3 ± 0.8*	*10.5 ± 1.1*	*12.7 ± 1.6*	*9.68 ± 0.91*	*11.6 ± 1.1*	*9.18 ± 0.10*	**	*NS*	*NS*
Acetic acid	47381 ± 2265	96894 ± 17033	64056 ± 3352	224154 ± 47489	72072 ± 3465	247002 ± 4900	***	**	*
Hexanoic acid	1006 ± 5	992 ± 89	1167 ± 87	1004 ± 97	959 ± 52	864 ± 47	NS	*	NS
Octanoic acid	221 ± 28	174 ± 43	386 ± 51	118 ± 2	199 ± 19	85.5 ± 24.5	**	**	**
Decanoic acid	28.7 ± 1.5	36.5 ± 2.4	36.4 ± 2.6	32.0 ± 1.4	28.9 ± 0.5	39.2 ± 4.1	*	NS	**
Total acids	*48636 ± 2289*	*98097 ± 16899*	*65645 ± 3211*	*225308 ± 47583*	*73259 ± 3394*	*247991 ± 4968*	*NS*	*	*NS*
Benzaldehyde	1.93 ± 0.24	1.45 ± 0.06	1.56 ± 0.01	1.48 ± 0.01	1.90 ± 0.03	1.81 ± 0.12	*	*	NS
β-Damascenone	54.1 ± 11.8	32.1 ± 1.3	53.9 ± 12.2	37.1 ± 4.0	58.6 ± 8.7	43.2 ± 0.7	**	NS	NS
Total	*258472 ± 1678*	*255530 ± 857*	*246537 ± 746*	*257578 ± 5841*	*258693 ± 3707*	*266286 ± 4109*	*NS*	*NS*	*NS*

Level of significance: *$P < 0.05$, **$P < 0.01$ and **$P < 0.001$ respectively. NS, no significant difference. ND, not detected. Italic letters mean summatory.
GLM, general lineal model; HD, harvest date; DI, deficit irrigation; HD × DI: harvest date × deficit irrigation.
Deficit Irrigation Treatments: T1, Ψ = −0.8 MPa; T2, Ψ = −0.9 MPa and T3, Ψ = −1.0 MPa. Harvest Date: H1, 55 DAV; H2: 64 DAV.
$^{\Theta}$Equivalents of ethyl undecanoate.
$^{\alpha}$Equivalents of ethyl succinate.
$^{\beta}$Equivalents of 3-methyl-1-pentanol.
$^{\delta}$Equivalents of nonanol.

alcohols and their derivatives and is also related to attributes such as 'herbaceous' or 'green'.[43,46] Bindon *et al.*[32] observed that these compounds decrease in Cabernet Sauvignon wines with advancing harvest date. In the present study, hexanol decreased between harvest dates, and the most significant reduction was observed in the less restricted treatment (T1). In addition, T1 harvested earlier showed the highest values, similar to other studies reporting higher contents at commercial harvest when full irrigation was applied compared to deficit irrigation.[24] Recently, a direct relationship between hexanol concentration during fermentation and the action of yeast alcohol acetyl transferase has been demonstrated.[47] This is the likely explanation for the observed decline in hexanol in wines produced from grapes harvested later in the season. In addition, no difference was found in 3-hexen-1-ol content in wines between harvest dates. This may suggest that the characteristic aromas of Cabernet Sauvignon wine are better expressed when harvesting early in T1. For T2 and T3, the slight differences found between harvesting dates suggest that, in intermediate and severe water limitation conditions, the characteristic aromas of Cabernet Sauvignon are maintained regardless of the harvest date.

With respect to the terpenes, it was found that the total terpenes decreased when harvested later regardless of the irrigation treatment (Table 3), in accordance with other studies carried out on Cabernet Sauvignon.[43] In addition, α-terpineol and linalool content significantly decreased in the later harvesting date, with no effect from the irrigation treatments (Table 3). Fang and Qian[36] also reported reductions in linalool during grape ripening in wines. It has also been reported that linalool could be synthesized *de novo* by yeast metabolism, independently of the grape-derived precursors available during fermentation of Cabernet Sauvignon grapes.[48]

With respect to citronellol, this has been reported to increase in Pinot Noir wines along with the ripening of the grape berries.[36] However, in the present study, this effect occurred only in plants under mild water deficit (T1), decreasing along with ripening in wine from the medium and high water deficit treatments (T2 and T3) and without significant differences between those two. In addition, the total number of assessed terpenes was lower in H2 compared to the previous H1 harvest date.

With respect to the volatile acids, the total amounts were higher in wines from H2 grapes compared with H1, mainly as a result of the increase of acetic acid. Acetic acid can play a significant role in wine aroma and excessive concentrations of these fermentation by-products are highly detrimental to wine quality.[49] In addition, the decanoic acid content increased in the second harvest date compared to the earlier date, whereas the opposite occurred with octanoic acid, with no clear trend appearing from irrigation treatments.

Some studies have reported that more ripe grape berries are usually accompanied by higher levels of C_{13}-norisoprenoids in Muscat grapes[50] or Pinot Noir wines.[51] These non-volatile compounds are expected to be released during fermentation, and the resulting wines may in turn have more C_{13}-norisoprenoid in their composition. Nevertheless, we observed a decrease of β-damascenone, a C_{13}-norisoprenoid that arises from carotenoid degradation during ripening.[20] Our results are in agreement with other studies in Riesling wines that showed higher free norisoprenoid content from earlier rather than later harvest dates.[52] Additionally, and in agreement with previous studies,[25] we observed that for both harvest dates the higher β-damascenone content corresponded to the most restricted irrigation treatment (T3).

PCA

The PCA performed on the wine volatile compounds is illustrated in Figs 2 and 3. PCA provides a visual representation of the relationship between different irrigation treatments in both years (Fig. 2) and between different irrigation treatments in different harvest dates in 2015 (Fig. 3) based on their volatile compositions.

Regarding the first PCA (Fig. 2), all variables (47 aroma compounds) were selected for PCA and, from the scores obtained, it is suggested that the deficit irrigation treatments in both years were well separated. The first two principal components accounted for 92.30% of the variance. The first component explains 85.88% of the variance and the second explains 6.43% of the variance. PC1 was mainly linked to esters and terpenes, as well as all alcohols, aldehydes and C13-norisoprenoids (Fig. 2), whereas PC2 was related to ethyl isovalerates and linalool. Almost 44 variables show a loading higher than 0.8 (in absolute value). A good separation among years and irrigation treatment groups was observed in the first PCA. The wine scores for wines made from the 2014 harvest, located in the negative site of PC1, are different from those made from the 2015 harvest, situated in the positive site of PC1, because they are grouped separately along the PC1 axis. The 2014 wines were characterized mainly by ethyl ester, terpenes and aldehydes, whereas the 2015 wines were described mainly by acetate ester, alcohols and C13-norisoprenoids components. The T1 and T2 scores were located more closely between them, and separated from T3, for both years. In addition, the T1 and T2 were more related to PC1, which shows that the aroma contents were higher in those treatments than in T3.

Forty-seven variables were preselected for the second PCA analysis, and the wine scores obtained are shown in Fig. 3. PC1 explains 64.38% of the variance and PC2 explains 22.12% of the variance (the aggregated explained variance by the two first components is 86.50%). Almost all variables have a remarkable influence in PC1 because all loading values are roughly distributed between 0.7 and 1 (in absolute values), with the only exceptions being 2-phenylethanol (AL11), hexanol (AL12), *o*-cymene (T1) and benzaldehyde (ALD1), which are related to PC2. For PC1, it appears that the wine scores for wines made from H1 are different from those made from the H2 harvest date because they are separately grouped along the component 1 axis. In addition, wines made from T1 and T3 elaborated from H1 appear to be quite similar because they are grouped in the same range of scores. T1H1 and T3H1 wines are characterized by ethyl butyrate (E2), isobutyl acetate (E18), isoamyl acetate (E19), isoamyl butanoate (E20), 4 methyl 1 pentanol (AL4), 1-nonanol (AL5), α-terpineol (TE2) and linalool (TE4). By contrast, ethyl octanoate (E7), 1-octanol (AL7), 1-decanol (AL10), citronellol (TE3) and octanoic acid (AC3) are related to T2H1 wines. On the other hand, the wines produced from grapes harvested later, under T3, are characterized by ethyl nonanoate (E8), ethyl decanoate (E9), ethyl dodecanoate (E10), ethyl acetate (E16), isoamyl lactate (E21), butanol (AL2), 2,3-butanediol (AL8) and acetic acid (AC1). In addition, those aroma compounds were also related to T2H2 wines, although ethyl heptanoate (E6) was strongly associated with T2H2 wines. T1H2 was described by low concentrations of ethyl isobutyrate (E1), 2-phenylethanol (AL11), hexanol (AL12), benzaldehyde (ALD1) and *o*-cymene (TE1). The second PCA results strengthen the idea that wines made from different harvest dates are quite different. In addition, the higher number of aroma compounds associated with T3H2 was related to an increase of the sugar content from H1 to H2. However, the wines produced from H1 were associated with terpenes compounds, especially in T1 and T3.

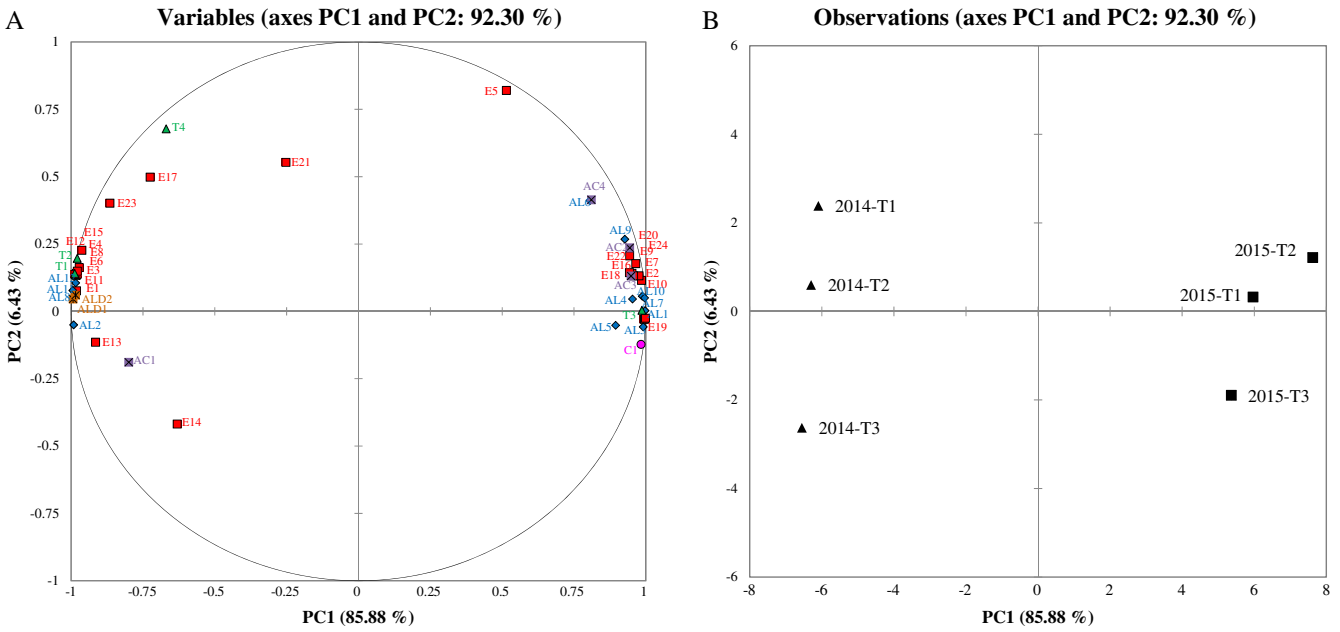

Figure 2. PCA of 47 volatile compound (a) and score plot of wines made from deficit irrigation treatments (T1, Ψ = −0.8 MPa; T2, Ψ = −0.9 MPa and T3, Ψ = −1.0 MPa) in 2014 and 2015 (b). E1, ethyl isobutyrate; E2, ethyl butyrate; E3, ethyl-2-methylbutyrate; E4, ethyl isovalerate; E5, ethyl hexanoate; E6, ethyl heptanoate; E7, ethyl octanoate; E8, ethyl nonanoate; E9, ethyl decanoate; E10, ethyl dodecanoate; E11, ethyl succinate; E12, ethyl phenylacetate; E13, ethyl tetradecanoate; E14, ethyl hexadecanoate; E15, ethyl hydrogen succinate; E16, ethyl acetate; E17, propyl acetate; E18, isobutyl acetate; E19, isoamyl acetate; E20, isoamyl butanoate; E21, isoamyl lactate; E22, isoamyl octanoate; E23, isopentyl hexanoate; E24, isopentyl decanoate; AL1, isobutanol; AL2, butanol; AL3, 3 methyl 1 butanol; AL4, 4 methyl 1 pentanol; AL5, 3-methyl-1-pentanol; AL6, 3-hexen-1-ol; AL7, 1-octanol; AL8, 2,3 butanediol; AL9, 1-nonanol; AL10, 1 decanol; AL11, 2-phenylethanol; AL12, hexanol; TE1, *o*-cymene; TE2, α-terpineol; TE3, citronellol; TE4, linalool; AC1, acetic acid; AC2, hexanoic acid; AC3, octanoic acid; AC4, decanoic acid; ALD1, furfural; ALD2, benzaldehyde; C1, β-damascenone.

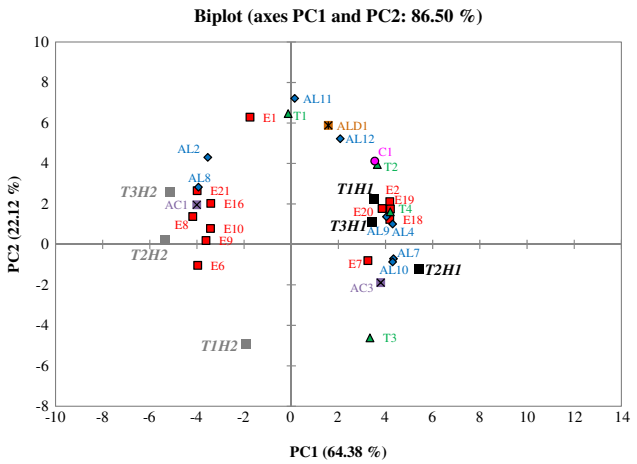

Figure 3. PCA biplot of the aroma compound variables selected from scores wines obtained from different irrigation treatments (T1, Ψ = −0.8 MPa; T2, Ψ = −0.9 MPa and T3, Ψ = −1.0 MPa) harvested at 55 DAV (H1) and 64 DAV (H2) in 2015. E1, Ethyl isobutyrate; E2, Ethyl butyrate; E6, Ethyl heptanoate; E7, Ethyl octanoate; E8, Ethyl nonanoate; E9, Ethyl decanoate; E10, Ethyl dodecanoate; E16, Ethyl acetate; E18, Isobutyl acetate; E19, Isoamyl acetate; E20, Isoamyl butanoate; E21, isoamyl lactate; AL2, butanol; AL4, 4 methyl1-pentanol; AL7, 1-octanol; AL8, 2,3-butanediol; AL9, 1-nonanol; AL10, 1-decanol; AL11, 2-phenylethanol; AL12, hexanol; T1, *o*-cymene; T2, α-terpineol; T3, citronellol; T4, linalool; AC1, acetic acid; AC3, octanoic acid; ALD1, benzaldehyde; C1, β-damascenone

CONCLUSIONS

The results of the 2-year experiment clearly demonstrate that deficit irrigation treatment was linked to wine aroma compounds for Cabernet Sauvignon grapes, although their composition was strongly influenced by year as a result of different weather conditions. Analysis of volatile aroma compounds in wines suggested that low water supply (T3) had a negative effect on the aromatic potential of wines at a similar ripening stage. However, this negative effect could be improved by increasing the physiological maturity at the second harvest time. Accordingly, although the results of T1 and T2 improved at the first harvest, they had worsened by the second harvest. Therefore, it is important to choose the correct moment for harvest because grape composition will determine the quality of the resulting wine, and this factor appears to have stronger influence in the aromatic composition of the wines than the irrigation treatment. For this reason, it is of great importance to know the volatile compounds of the wine and the mechanisms that influence their formation, such as the deficit irrigation treatments and the harvest date, and such data are essential for developing strategies to produce wines with specific sensory attributes that appeal to targeted markets.

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