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Influence of water regime on grape aromatic composition of Muscat of Alexandria in a semiarid climate

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ABSTRACT

Irrigation effects in relation to the environmental conditions on grape aromas are still unknown. This study aims to clarify the effects of water regime on the aromatic composition of “Muscat of Alexandria” grapes under the semiarid climate conditions of eastern Spain and over three seasons. The relationships between total volatile composition in free and glycosidically-bound fractions, vine performance, and grape composition were also assessed. The watering treatments studied were: sustained deficit irrigation (SDI) at 50% of the estimated crop evapotranspiration (ET_c); early deficit (EDI), where pre-veraison water deficit was imposed; late deficit (LDI), in which a water shortage was applied during post-veraison; and control (C), irrigated at 100% of ET_c during the entire season. The effects of water regimes on volatile and glycosidically-bound composition were different between seasons due to the predominant effect of the environmental conditions on aromatic composition. The seasons with the greater yield were associated to a lower grape aromatic composition, however, this effect at the crop level was less pronounced in the most irrigated treatment. In drier seasons, irrigation delayed ripening and increased the terpene concentration of “Muscat of Alexandria” grapes, showing a high treatment and season interaction. Specific water regimes have the potential to buffer the effects of environmental conditions on the aromatic composition of “Muscat of Alexandria” grapes in a semi-arid climate, as the result of the complex relationships between climatic factors, vine performance and grape maturity.

1. Introduction

Viticulture for wine making commonly deals with water deficit conditions that induce physiological responses to water stress, which modulate primary and secondary metabolism (Jackson and Lombard, 1993). For this reason, under semiarid climates, the effects of the vintage on grape composition are normally very pronounced (Vilanova et al., 2019a; Wang et al., 2019). The watering regimes have the potential to alleviate vine water stress, as they affect many interactive factors related to vine performance and grape ripening (Intrigliolo et al., 2012; Mirás-Avalos and Intrigliolo, 2017). Deficit irrigation strategies (DI), such as regulated deficit irrigation (RDI), have been explored for improving grape composition through the reduction of water availability at specific phenological periods (El-Ansary et al., 2005; Peyrot des Gachons et al., 2005; Intrigliolo et al., 2012), but this can also have a detrimental effect on yield (Mirás-Avalos and Intrigliolo, 2017). In this sense, two main periods in a season are normally considered when applying RDI: i) between flowering and veraison, and ii) after veraison during berry ripening. While most secondary berry metabolites are accumulated during the ripening period (Jackson and Lombard, 1993), it has also been demonstrated that important precursors can be synthesized before veraison (Castellarin et al., 2007) and, in addition, the pre-veraison period is also a determinant for the final berry size (Ojeda et al., 2001). Grape volatile compounds contribute to the wine aroma, thereby affecting its quality. The accumulation of volatiles (free) and bound forms (an important reserve of potential wine flavor) in grapes is affected by environmental factors, such as water availability (Song et al., 2012; Bouzas-Cid et al. (2018); Vilanova et al., 2019a; Wang et al., 2019). In general, water deficit can increase the concentration of C_{13}-norisoprenoids and terpenes (Song et al., 2012) by modulating structural and regulatory genes involved in volatile compounds biosynthesis (Deluc et al., 2009). In grapes, monoterpenes are

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predominantly found in their glycosylated forms (Williams et al., 1982). A possible detoxification function was associated to glycosylation, because the free volatiles may be toxic at high concentrations to the plant itself (Hjelmeland and Ebeler, 2015). However, the metabolic responses of grapes to water deficit varied according to the cultivar. In this sense, deficit irrigation may be applied to improve the concentration of volatiles by adjusting it to the specific cultivar.

Vitis vinifera cv. "Muscat of Alexandria" is cultivated since ancient times, and it is currently grown worldwide for table grape and raisin production, and crushed into wine. In some viticulture areas, it is also used for making white sparkling, dry, and fortified wines ("mistella" and "passito") (Corona et al., 2020) as well as concentrated musts (Peiró et al., 2018). The aromatic profile of this cultivar is highly appreciated, and it has been blended with other wines to improve the final aromatic composition of white wines. But the cultivar typicity is threatened by climate change (Jones et al., 2005), as the environmental conditions determine final grape composition (Jackson and Lombard, 1993). The main factors expected to affect grapevine cultivation are warm air temperature and water scarcity, which will affect fruit composition to a greater extent rather than yield (Sadras et al., 2017). Since aroma and flavor content are paramount for grape and wine quality, several studies have focused on the vine water status effects on grape and wine volatile compounds as well as aromatic precursors. For instance, the analysis of bound volatile components in "Agiorgitiko" wines suggested that a low water uptake had a positive effect on the aromatic potential of grapes (Koundouras et al., 2006). Deficit irrigation has been shown to be associated with increases in the fruity characteristics in *Vitis Vinifera* L. cv. "Cabernet Sauvignon" wines, as determined sensorially (Chapman et al., 2005). Also, C_{13}-norisoprenoids concentration increased in Cabernet Sauvignon grapes by the degradation of carotenoids, in response to water stress and the influence of sunlight in the fruit zone (Bindon et al., 2007). On the other hand, the analysis of volatile components in cv. "Tempranilo" suggested that no irrigation and cluster thinning led to an increase in the majority of wine aromatic compounds analyzed (Talaverano et al., 2016). However, the effects of water stress on the aromatic composition were not fully conclusive. For instance, Reynolds et al. (2005) and Vilanova et al. (2019a) reported that water stress promoted the aromatic composition in "Gewurztraminer" and "Verdejo" berries, respectively, whereas the opposite was found in cv. "Muscat Blanc" and cv. "Godello" by Giordano et al. (2013) and Bouzas-Cid et al. (2018), respectively. Very recently, Kovalenko et al. (2021), under a cool climate, found that only post-veraison deficit irrigation was able to improve the aroma composition of "Gewurztraminer" grapes. These different results, in terms of the effect of water stress on grape aroma composition, indicate that there are many other interactive factors that influence the accumulation of aroma compounds in grapes. The effects of DI and RDI strategies under different environmental conditions on grape aromas are still unknown, which may also be dependent on the variety. A study conducted by Lopez et al. (2009) concluded that non-irrigation improved the wine sensory quality for all the cultivars studied except for cv. "Muscat", while supplemental irrigation positively affected the wine sensory scores. In this sense, cv. "Muscat of Alexandria" is a peculiar variety with a high concentration of terpenes, which are not frequently found in other varieties (Marais, 1983). Previous works carried out in cv. "Muscat of Alexandria" were rather focused on the application of a specific deficit irrigation technique, such as the Partial Root Zone Drying (dos Santos et al., 2007) or were more focused on the berry technological maturity (El-Ansary et al., 2007).

In this context, our hypothesis is that DI can improve the aromatic composition of "Muscat of Alexandria" grapes grown under a Mediterranean and semiarid climate. To test this, RDI strategies (pre- and post-veraison water deficits) were compared to different irrigation regimes applied during the entire growing season. The present study complements previous research carried out in the same vineyard, which focused on quantifying the effects of deficit irrigation on vine performance and grape technological composition parameters (Buesa et al., 2017). Here, the impact of four watering regimes on vegetative and yield components, and grape composition, were considered in relation to the aromatic composition of "Muscat of Alexandria" grapes.

2. Materials and methods

2.1. Experimental design

The experiment was carried out over the 2012–2014 seasons in a commercial vineyard (*Vitis vinifera* L.) of cv. "Muscat of Alexandria", located in Pedralba (39°33' N, 0°42' W, elevation 197 m), Valencia (Spain). The vineyard was planted in 1996 with 161–49C rootstock at a spacing of 2.75 × 1.8 m (2020 vines/ha). Vines were trained to a vertical trellis system oriented north 23° west direction on a bilateral cordon leaving ten two-bud spurs per vine. The climate was typical Mediterranean and semi-arid, with an average annual rainfall of 422 mm. According to the climatic classification proposed by Tonietto and Carbonneau (2004) for grape-growing regions, the experimental conditions were warm with temperate nights and moderately dry.

During the experiment, weather data were recorded at an automated meteorological station 1.4 km from the plot. Reference evapotranspiration (ET_o) was calculated with the Penman–Monteith equation (Allen et al., 1998). Cumulative growing degree days (GDD) were computed as the sum of the average daily temperature above a base temperature of 10°C (Winkler, 1962). The experimental treatments imposed were: sustained deficit irrigation (SDI), irrigated at 50% of estimated crop evapotranspiration (ET_c) for the entire season; early deficit (EDI), where pre-veraison irrigation was withheld until stem water potential (Ψ_{stem}) values reached −1.0 MPa, followed by 100% ET_c; late deficit (LDI), irrigated as for the control until veraison, and thereafter at 25% ET_c; and (C) control, irrigated at 100% of ET_c during the entire season. The experimental design was a completely randomized block layout with four replicates. Each treatment replication consisted of 24–48 vines, with the surrounding perimeter vines acting as borders. The amount of water applied with irrigation was measured with on-line water meters at each treatment replicate. Additional information about the vineyard site and the experimental design has been fully described by Buesa et al. (2017).

2.2. Water potential and stress integral

The plant water status was characterized by determining Ψ_{stem} at midday (1130–1230 solar time) on bag-covered leaves from four representative vines per treatment replication on a weekly basis from May to October with a pressure chamber (Model 600, PMS Instrument Company, Albany, OR, USA). The cumulative effect of water deficit duration and intensity was quantified by calculating the water stress integral (S_Ψ) as the sum of plant water potential measured every day during a given period (Myers, 1988). It was obtained from the Ψ_{stem} values, subtracting those with the least negative value registered during the season (−0.24 MPa) and multiplying it by the number of days between measurements. The values of Ψ_{stem} considered for the calculation of S_Ψ were obtained two weeks after anthesis (May 1st approximately).

2.3. Vine performance

Pruning fresh mass was weighed from samples from eight vines per treatment replicate. In those selected vines, total leaf area (LA) was estimated 2–3 weeks after veraison. The LA was estimated from allometric relationships between shoot length and LA per shoot, measured with a LI-3100 Area Meter (LICOR Biosciences, Lincoln, NE, USA) as fully described in Buesa et al. (2017).

Grape yield, number of clusters per vine and average cluster mass were determined at harvest on each experimental vine. Berry fresh mass was determined on random samples from 250 berries per treatment replicate. From these samples, five berries of different weights were peeled to weigh the skin, on the one hand, and the pulp on the other.

2.4. Must chemical composition

Must composition was determined at harvest in two subsamples weighing approximately 250 g, which came from berry sampling that was randomly collected from each experimental vine. Musts were physicochemically analyzed by determining total soluble solids (TSS), pH, titratable acidity (TA), and malic and tartaric acids, by using the official methods from the International Organisation of Vine and Wine 2015. Must TSS was determined by refractometry (PR-101, Series Palette, Atago, Tokyo, Japan) and pH and TA were measured with an automatic titrator (Metrohm, Herisau, Switzerland). Juice was titrated with a 0.1 N NaOH solution to an end point of pH 8.2, and the results were expressed as tartaric acid equivalents. Must potassium was determined by atomic absorption spectrophotometry by using an elemental analyzer (NC 2500 Thermo Finnigan; Bremen, Germany) coupled to a mass spectrometer (Delta Plus, Thermo Finnigan). All determinations per replication were performed in triplicate.

2.5. Volatile and composition of musts

The extraction of volatile compounds in free and glycosidically-bound fractions followed the method described by Oliveira et al. (2008) with some modifications (Vilanova et al., 2019b). About 300 mL of “Muscat” must was centrifuged (RCF=9660, 20 min, 4°C) and filtered through a glass wool bed. To 75 mL of juice, 3 µg of 4-nonanol (Merck, ref. 818,773, Darmstadt, Germany) were added as an internal standard and passed through a LiChrolut EN cartridge (Merck, 500 mg, 40–120 µm). The resin was previously pre-conditioned with 10 mL of dichloromethane (Merck ref. 1.06054), 5 mL of methanol, and 10 mL of an aqueous alcoholic solution (10%, v/v). Free and glycosidically-bound fractions were eluted successively with 5 mL of pentane–dichloromethane azeotrope and 7 mL of ethyl acetate, respectively. The pentane–dichloromethane elute was dried over anhydrous sodium sulphate and concentrated to 200 µL by solvent evaporation with N_2 prior to analysis. The ethyl acetate eluate was concentrated to dryness (40°C) in a Multivapor™ from Buchi (Flawil, Switzeland) and redissolved in 200 µL of 0.1M citrate–phosphate buffer (pH=5.0). Fourteen milligrams of the enzyme Rapidase Revel Aroma (Erbslöh, Germany) were added to the glycoside extract, and the mixture was incubated at 40°C, for 14 h The released aglycons were extracted with pentane-dichloromethane azeotrope, after addition of 3 µg of 4-nonanol as the internal standard. The organic phase was then concentrated to 200 µL with N_2.

The gas chromatography analysis of volatile compounds was performed using an Agilent GC 6890 N Chromatograph coupled to an Agilent 5975C mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). A 1 µL sample was injected into a capillary column, coated with CP-Wax 52 CB (50m × 0.25mm i.d., 0.2 µm film thickness, Chrompack (Agilent Technologies, Santa Clara, CA, USA). The temperature of the injector was programmed from ramp from 20°C to 250°C, at a rate of 180°C/min. The oven temperature was held at 40°C for 5 min, then programmed to rise from 40°C to 250°C, at a rate of 3°C/min, then held 20 min at 250°C, and finally programmed to go from 250°C to 255°C at a rate of 1°C/min. The carrier gas was helium N60 (Air Liquide, Paris, France) at 103 kPa, which corresponds to a linear speed of 180 cm/s at 150°C. The detector was set to electronic impact mode (70 eV), with an acquisition range from 29 to 360 *m/z*, and an acquisition rate of 610 ms. The semi-quantification of the volatiles such as 4-nonanol equivalents (µL) was performed using the WSearch free software (Wsearch32 v1.6.2005), by comparing the retention indices with those of the pure standard compounds from Sigma-Aldrich (Darmstadt, Germany) and confirming these by GC/MS.

2.6. Statistical analysis

Grape aromatic composition is influenced by multiple environmental factors. To account for this complexity, data were analyzed using three complementary approaches.

Firstly, single data were subjected to a two-way ANOVA to examine the effects of the treatments (Tr), season (Y), and treatment and season interaction (Tr*Y) using XLSTAT-Pro (Addinsoft SARL, Paris, France). From this first analysis, we detected that the season exerted a significant influence on most of the variables studied and showed significant interactions with the treatment in some of them. Therefore, data is shown seasonally, and the mean differences between treatments were calculated separately for each season according to the least significant difference from Tukey’s test with a confidence interval of 95% ($P < 0.05$). Secondly, the relationship between the water regime treatments and vintages, with the entire families of the volatile and glycosidycally-bound chemical compounds quantified in “Muscat” musts, was assessed with a multivariate approach using two principal component analyses (PCAs). Lastly, seeking to explore the main variables influencing grape aromatic composition, linear regressions between total free and bound fractions of grape aroma and the agronomic variables across seasons were obtained using “SigmaPlot”, Systat Software, Inc. (version 11.0). The differences between treatments were assessed by analyzing its residuals with respect to the general regression curve, following Bonada et al. (2018).

3. Results and discussion

3.1. Meteorological conditions, irrigation and water relations

The meteorological conditions showed two dry and warm seasons (2012 and 2014), while the 2013 season was wetter and cooler. The values for rainfall, ET_o and GDD during the three growing seasons are shown in Table 1. The seasonal irrigation volumes applied resulted in 48%, 40% and 29% less water applied in SDI, EDI and LDI when compared with that of the control (Table 2). Regardless of the considerable differences between treatments in the irrigation volumes applied, the seasonal S_Ψ only differed between the control and the deficit irrigated (DI) treatments (Table 2). Nevertheless, when S_Ψ was calculated separately between pre- and post-veraison periods and in some seasons, S_Ψ differed significantly between SDI, EDI, and LDI. It should be noted that the relative S_Ψ differences between treatments were seasonally consistent.

3.2. Effect of water regime on vine performance and berry composition

Both treatments and seasons had significant effects on vegetative growth, yield and its components, and grape composition (Table 2), with the important yield variations between seasons in all water regimes worth noting, which were on average up to 70% between the 2013 and 2014 seasons. The yield results showed a tendency to decrease due to the DI treatments, as compared with that of the control, and this reduction appeared to be cumulative. In 2014, all the DI treatments had a significantly reduced yield as compared to that of the control. The yield losses were due to the reduction in the number of clusters per vine, as well as the mass of the cluster and the berry. The average yield in the SDI treatment was intermediate between that of the control and the other two DI treatments (EDI and LDI). This was despite the fact that seasonal S_Ψ was higher in SDI than in the latter (Table 2), which suggests that high water stress levels during specific periods were more detrimental for yield than moderate stresses sustained over time, as previously reported in a meta-analysis carried out by Mirás-Avalos and Intrigliolo (2017). Moreover, total leaf area and pruning mass were significantly lower in all the DI treatments as compared to that of the control. This is an expected response of vegetative growth to water shortage (Intrigliolo et al., 2012).

Table 1
Values of climate variables for different phenological periods in “Muscat of Alexandria” grapevines during the 3 years of the experiment in Vilamarxant (Valencia), Spain.

Phenological period	2012 Rainfall (mm)	ET_o (mm)	GDD (°C * day)	2013 Rainfall (mm)	ET_o (mm)	GDD (°C * day)	2014 Rainfall (mm)	ET_o (mm)	GDD (°C * day)
Budburst-veraison (April-July)	62,0	470,2	885,2	142,4	400,5	675,9	44,9	438,1	864,4
Veraison - harvest (August-September)	77,4	463,2	1325,7	42,6	429,0	1292,4	91,0	441,1	1314,0
Budburst-harvest (April-September)	139,4	933,4	2210,9	185,0	829,5	1968,3	135,9	879,2	2178,3

ET_o, reference evapotranspiration; GDD, growing degree days.

Table 2
Average values of irrigation, water stress integral S_Ψ, vegetative growth, yield components and must composition attributes over the 3 years of the experiment for “Muscat of Alexandria” wine grapes subjected to different irrigation strategies. Some of these agronomic data were previously published as a seasonal average in Buesa et al. (2017).

Parameter	2012 SDI	EDI	LDI	C	2013 SDI	EDI	LDI	C	2014 SDI	EDI	LDI	C	Sig. TR	Y	TR*Y
Irrigation (pre-veraison)	116b	98c	198a	202a	74b	29c	135a	137a	76b	27c	147a	151b	***	***	***
Irrigation (post-veraison)	40b	79a	20c	81a	46c	108a	25d	97b	55c	117a	30d	110b	***	***	***
Irrigation (season)	156d	179c	218b	281a	120d	137c	161b	234a	131d	145c	177b	261a	***	***	***
S_Ψ (pre-veraison)	35.4b	40.7a	32.4bc	31.6c	30.3ab	32.3b	30.4ab	27.8a	39.4bc	42.7c	35.7ab	31.8a	***	***	ns
S_Ψ (post-veraison)	37.1a	30.9b	39.8a	31.4c	35.9bc	30.3ab	39.6c	29.2a	31.6b	27.2a	37.6c	26.1a	***	***	ns
S_Ψ (season)	72.3a	72.6a	71.6a	63.0b	70.1a	66.1a	66.8a	56.0b	74.4a	71.0a	70.0a	57.9b	***	*	ns
Yield (kg/vine)	12.9a	9.4b	11.9ab	13.1a	15.9	15.8	16.1	19.4	5.0b	4.2c	5.2b	6.9a	**	***	ns
Clusters/vine	31.7	28.3	29.1	30.9	31.8ab	29.7b	33.4ab	35.1a	19.8b	19.8b	18.8b	23.0a	**	***	ns
Cluster mass	406a	326b	405a	425a	484	522	479	553	247ab	205b	266a	297a	***	***	ns
Berry mass (g)	5.3ab	4.8b	5.4a	5.6a	4.4	4.3	4.4	4.7	5.0	4.6	5.2	5.5	***	***	ns
Skin-to-pulp ratio (%)	4.8	4.9	4.9	4.3	5.5	4.8	5.1	5.3	6.2	5.3	5.5	6.3	ns	ns	ns
Total leaf area (m^2/vine)	6.9c	6.7c	8.1b	10.2a	7.1b	6.7b	6.5b	8.5a	5.4b	4.5b	5.1b	7.9a	***	**	*
Pruning mass (kg/vine)	0.68	0.57	0.63	0.91	0.73b	0.63b	0.70b	0.93a	0.55ab	0.45b	0.53ab	0.76a	***	*	ns
TSS (°Brix)	21.3b	22.4a	21.9ab	21.7ab	20.8	20.8	20.6	20.0	24.0a	24.1a	23.8a	22.4b	***	***	*
TA (g/L)	3.0	3.0	3.2	3.6	4.8	4.7	4.8	4.9	4.4a	4.1b	4.3ab	4.4a	*	***	ns
pH	3.79b	3.82ab	3.87a	3.86ab	3.6	3.58	3.6	3.55	3.94ab	3.9b	3.96a	3.92ab	ns	***	ns
Potassium (g/L)	2,0	2.1	2.2	1.9	2.3a	2.1b	2.2ab	2.2ab	2.7a	2.2b	2.8a	2.7a	**	***	ns
TSS-to-TA ratio	7.0	7.5	6.8	6.2	4.3ab	4.4a	4.3ab	4.1b	5.5ab	5.8a	5.5ab	5.1b	**	***	ns

S_Ψ, stress integral; TSS, total soluble solids; TA, titratable acidity. Different letters indicate significant differences for Tukey’s test at $P < 0.05$. *, **, ***, and ns indicate significance at $P < 0.05$, 0.01, 0.001, and not significant, respectively. Treatments (TR) were SDI, sustained deficit irrigation; EDI, early deficit irrigation; LDI, late deficit irrigation; and C, control.

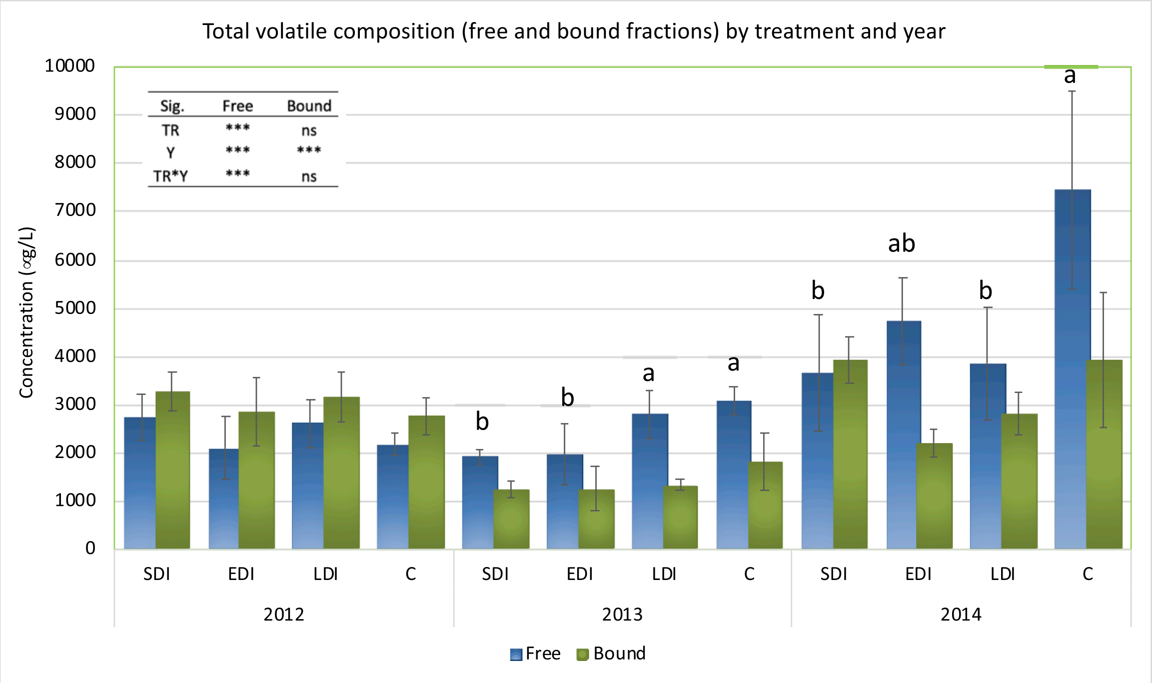


Fig. 1. Total volatile composition (μg/L) of “Muscat of Alexandria” grapes from the different irrigation treatments (2012–2014).

Just as vine performance, grape composition at harvest was significantly affected by treatments and seasons, but in some attributes, also by treatment and season interaction (Table 2). For instance, in 2012 and 2014, control berries had a lower TSS as compared to all the DI treatments. The opposite trend was observed regarding TA. Malic acid showed a slight tendency to increase its concentration as the amount of irrigation increased, whereas tartaric acid showed an inconsistent response between seasons. Must potassium content tended to be reduced in the EDI as compared to the SDI and control treatments. Remarkably, there were no effects of the irrigation treatment in pH (Table 2). Overall, the control treatment seemed to delay the so-called technological ripeness (sugars, acids and pH), as compared to DI treatments, as shown by the TSS-to-TA ratio.

3.3. *Effect of water regime on volatile and glycosidycally-bound composition*

Figure 1 shows the total volatile composition in free and glycosidycally-bound fractions of "Muscat" musts from different water regimes over three vintages (2012–2014). In the 2013 and 2014 seasons, significant differences between irrigation treatments were observed in the total free fraction of volatiles, where the LDI and control water regimes for 2013, and the EDI and control regimes for 2014 reached the highest values. A similar tendency was observed in the glycosidically-bound fraction for both vintages, which increased in concentration in control grapes. In contrast, in the 2012 season, non-significant effects of the treatments on the total concentration of both fractions, free and glycosylated, were detected. The ANOVA results showed a significantly

Table 3
Free aroma volatile composition (µg/L) of "Muscat of Alexandria" musts from different water regimes over three vintages (2012–2014).

Free compounds (µg/L)	2012				2013				2014				Sig.		
	SDI	EDI	LDI	C	SDI	EDI	LDI	C	SDI	EDI	LDI	C	TR	Y	TR*Y
Hexanal	151	117	171	150	99	78	97	119	341	490	370	150	ns	***	*
(E)−2-hexenal	45	32	44	32	67	49	72	66	248	356	325	142	ns	***	*
1-hexanol	193	190	206	171	158	197	188	290	722b	580b	546b	2922a	*****	***	***
(E)−3-hexen-1-ol	nd	nd	nd	nd	94	98	104	138	15b	16b	16b	86a	***	***	ns
(Z)−3-hexen-1-ol	130	121	107	106	106	132	131	182	60b	79b	68b	182a	**	*	*
(E)−2-hexen-1-ol	93	70	99	88	nd	nd	nd	nd	264	315	321	453	ns	***	ns
(Z)−2-hexen-1-ol	nd	nd	nd	nd	3	2	8	5	14b	21b	22b	38a	***	***	***
C6 compounds (%)	***22.4***	***22.0***	***23.8***	***25.0***	***27.5***	***28.2***	***21.3***	***25.9***	***45.2***	***39.1***	***43.1***	***53.3***			
2-methyl-1-propanol	5	4	4	4	nd	nd	nd	nd	nd	nd	nd	nd	ns	–	–
2 + 3 methyl-1-butanol	6	4	7	5	9	9	14	15	39	40	25	72	ns	***	ns
3-methyl-3-buten-1-ol+1-pentanol	11	7	11	9	14	14	19	19	19	25	25	26	ns	***	ns
3-methyl-2-buten-1-ol+*Z*-2-penten-1-ol+2-heptanol	18	13	17	13	34	37	35	50	nd	nd	nd	nd	ns	ns	ns
3-methyl-1-pentanol	nd	nd	nd	nd	nd	nd	nd	nd	21	28	23	32	ns	–	–
Benzyl alcohol	14	11	15	9	13c	15bc	24ab	28a	23	36	31	36	ns	***	ns
2-phenylethanol	28	22	30	31	30c	35bc	53ab	61a	37	51	37	62	*	***	ns
1-butanol	nd	nd	nd	nd	22a	20a	7b	9b	21	28	23	25	*	***	*
2-ethyl-1-hexanol	2	3	2	2	2b	2b	2b	3a	nd	nd	nd	nd	ns	ns	ns
Alcohols (%)	***3.1***	***2.6***	***3.2***	***3.3***	***6.5***	***6.7***	***5.5***	***6.0***	***4.3***	***4.4***	***4.3***	***3.4***			
Ethyl octanoate	nd	nd	nd	nd	67	54	51	77	nd	nd	nd	nd	ns	–	ns
Isoamylacetate	nd	nd	nd	nd	3	2	4	4	nd	nd	nd	nd	ns	–	ns
Ethyl esters (%)	–	–	–	–	***69.9***	***56.5***	***55.2***	***81.3***	–	–	–	–			
trans-furan linalool oxide	17a	8b	15a	14ab	nd	nd	nd	nd	25b	43a	32ab	39ab	ns	***	**
cis-furan linalool oxide	31	25	28	13	16	15	22	20	31b	46a	42ab	44ab	ns	***	**
Linalool	1095	752	1033	866	494c	517bc	775ab	961a	618b	884ab	633b	1416a	***	**	***
α-terpineol	10	8	12	10	10b	11ab	18a	17ab	32	44	36	50	*ns	***	**
trans-pyran linalool oxide	190	151	168	144	100b	101b	195a	165ab	158	218	176	205	ns	**	**
cis-pyran linalool oxide	116	99	101	82	32b	36b	62a	55ab	59	98	64	71	ns	***	**
Nerol	65ab	45b	75a	64ab	48b	53b	75ab	90a	73b	107ab	98b	137a	***	***	*
B-citronelol	nd	nd	nd	nd	nd	nd	nd	nd	20	32	21	39	**	–	–
Geraniol	267	195	254	235	254	261	364	382	385b	543ab	444ab	612a	*	***	**
Diendiol I	67	73	73	39	49b	44b	144a	57b	99	139	96	124	ns	**	**
Diendiol II	65	70	66	39	55b	51b	164a	76b	79	111	67	103	ns	*	***
E-8-hydroxylinalool	9	10	9	4	5b	4b	12a	7b	7	9	7	12	ns	ns	*
Z-8-hydroxylinalool	12	7	7	4	5	6	10	9	11	14	12	17	ns	***	*
Hotrienol	nd	nd	nd	nd	nd	nd	nd	nd	12b	19ab	16ab	21a	ns	–	–
Terpenes (%)	***71.2***	***59.8***	***70.0***	***69.3***	***55.7***	***55.9***	***65.5***	***59.5***	***43.6***	***48.6***	***45.1***	***38.8***			
Butanoic acid	nd	nd	nd	nd	nd	nd	nd	nd	64	80	64	33	ns	–	–
Hexanoic acid	5	8	6	4	9	7	7	11	nd	nd	nd	nd	ns	ns	ns
Geranic acid	45	26	43	25	61	60	45	83	70b	161ab	125ab	173a	*	***	**
(E)−2-hexanoic acid	nd	nd	nd	nd	nd	nd	nd	nd	44	37	19	11	ns	–	–
Hexadecanoic acid	nd	313	nd	nd	13b	14b	25ab	32a	18b	37ab	39ab	56a	***	***	ns
Propanoic acid	nd	nd	nd	nd	9	7	28	8	17	18	12	3	*	ns	ns
Volatile acids (%)	***1.8***	***14.4***	***1.9***	***1.3***	***4.8***	***4.4***	***3.7***	***4.4***	***5.8***	***7.0***	***6.7***	***3.7***			
Benzaldehyde	nd	nd	nd	1	8	10	14	9	nd	nd	nd	nd	ns	ns	ns
Aldehydes (%)	–	–	–	***0.1***	***0.4***	***0.5***	***0.5***	***0.3***	–	–	–	–			
Vanillin	22	12	15	12	10b	13b	16b	25a	nd	nd	nd	nd	ns	ns	ns
4-vinylguaiacol	nd	nd	nd	nd	3b	4b	10a	4b	nd	nd	nd	nd	ns	–	ns
Phenol volatiles (%)	***0.8***	***0.5***	***0.6***	***0.6***	***0.7***	***0.8***	***0.9***	***1.0***	–	–	–	–			
Butyrolactone	7	8	nd	nd	4	3	4	4	17	18	15	21	**	***	ns
Lactones (%)	***0.3***	***0.3***	–	–	***0.2***	***0.2***	***0.2***	***0.1***	***0.4***	***0.4***	***0.4***	***0.3***			
Acetoine	11	9	13	9	9	7	12	6	20b	26ab	20b	39a	ns	ns	**
Carbonyl compounds (%)	***0.4***	***0.4***	***0.5***	***0.4***	***0.5***	***0.3***	***0.4***	***0.2***	***0.5***	***0.5***	***0.5***	***0.5***			

Nd: no detected; Different letters indicate significant differences for Tuckey's test at $P < 0.05$. *, **, ***, and ns indicate significance at $P < 0.05$, 0.01, 0.001, and not significant, respectively. Treatments (TR) were SDI, sustained deficit irrigation; EDI, early deficit irrigation; LDI, late deficit irrigation; and C, control.

effect of the treatment and vintage on free volatiles, however in bound fraction only the effect of the vintage was significantly. A significant interaction between treatment and year (TR*Y) was also observed in free fraction (Figure 1).

The volatile compositional differences in grapes induced by water status are known to directly affect aroma composition of wines (Mattews et al., 1990). Positive and negative effects of water stress on free and glycosylated compounds have been reported in previous works, in which most of the studies were conducted in wines. Thus, the concentration of "Verdejo" wine volatiles was affected by irrigation treatments (Vilanova et al., 2019b), and in general, lower levels of water availability induced a higher concentration of volatiles. However, the effect of the irrigation treatment varied depending on the season. In another study, deficit irrigation had little effect on aroma from cv. "Muscat of Alexandria" (El-Ansary et al., 2005). Several factors, such as grape cultivar, amount and timing of water applied, training systems, leaf

Table 4

Glycosidycally-bound composition (μg/L) of "Muscat of Alexandria" musts from different water regimes over three vintages (2012–2014).

Bound compounds (μg/L)	2012				2013				2014				Sig.		
	SDI	EDI	LDI	C	SDI	EDI	LDI	C	SDI	EDI	LDI	C	TR	Y	TR*Y
Hexanal	27a	16b	17b	18b	4	6	2	5	17	8	11	13	ns	***	***
(E)−2-hexenal	5	5	6	6	nd	nd	nd	nd	68	25	19	nd	ns	ns	ns
1-hexanol	6	10	10	8	2	3	4	4	268	12	25	101	ns	ns	ns
(E)−3-hexen-1-ol	12	21	11	11	2	3	3	4	18	nd	9	20	ns	***	ns
(Z)−3-hexen-1-ol	7	8	6	6	1	3	2	2	6	8	9	14	ns	***	ns
(E)−2-hexen-1-ol	10	8	7	7	6	9	9	11	24	nd	17	nd	ns	*	ns
(Z)−2-hexen-1-ol	2	2	3	4	nd	2	2	3	8	77	12	22	ns	ns	ns
C6 compounds (%)	***2.1***	***2.3***	***1.9***	***2.2***	***1.3***	***2.0***	***1.7***	***1.6***	***9.0***	***5.4***	***3.5***	***2.2***			
1-butanol	2	4	4	3	8a	7a	3b	2b	17	61	nd	nd	ns	**	ns
1-pentanol	nd	nd	nd	nd	nd	nd	nd	nd	19	23	9	23	*	–	–
3-methyl-1-butanol	4	6	4	5	3	3	3	3	15	9	nd	nd	ns	**	ns
3-methyl-1-pentanol	nd	nd	nd	nd	3	5	5	6	nd	nd	nd	nd	ns	–	ns
1-octen-3-ol	nd	nd	nd	nd	1b	1ab	2ab	2a	nd	nd	nd	nd	ns	–	ns
1-propanol	7	6	6	7	nd	nd	nd	nd	nd	nd	nd	nd	ns	–	ns
3-methyl-3-buten-1-ol+1-pentanol	8	10	11	10	nd	nd	nd	nd	41	12	5	40	ns	*	*
3-methyl-2-buten-1-ol+*Z*-2-penten-1-ol+2-heptanol	9	7	11	9	nd	nd	nd	nd	nd	25	9	23	ns	**	ns
Benzyl alcohol	28	26	36	34	11	19	19	24	50	28	34	105	**	***	*
2-phenylethanol	43	45	57	49	16	21	31	36	39	26	44	153	ns	ns	**
Alcohols (%)	***3.1***	***3.6***	***4.0***	***4.2***	***3.4***	***4.5***	***4.6***	***3.9***	***4.0***	***7.7***	***3.5***	***4.5***			
2-ethyl-1-hexanol	9	10	7	8	37	nd	35	nd	nd	nd	nd	nd	ns	ns	ns
Ethyl butirate	18	19	18	21	nd	nd	3	nd	nd	nd	nd	nd	ns	ns	ns
Isoamylacetate	1	4	5	5	nd	nd	nd	nd	nd	nd	nd	nd	ns	–	–
Ethyl decanoate	9	10	11	10	nd	nd	nd	nd	nd	nd	nd	nd	ns	–	–
Ethyl esters (%)	***1.1***	***1.4***	***1.3***	***1.6***	***3.0***	–	***2.9***	–	–	–	–	–			
trans-furan linalool oxide	26	22	31	26	nd	nd	nd	nd	15	49	11	42	ns	ns	ns
cis-furan linalool oxide	5	5	6	5	nd	1	nd	nd	nd	33	12	nd	*	***	**
Linalool	407	354	511	432	100	156	124	246	252	81	172	529	ns	ns	ns
Ho-trienol	nd	nd	nd	nd	nd	nd	nd	nd	152	109	46	131	ns	–	–
B-pinene	nd	nd	nd	nd	nd	nd	nd	2	196a	57b	17b	65b	ns	ns	–
D-Limonene	nd	nd	nd	nd	nd	nd	nd	nd	84	42	11	48	ns	–	–
Terpinen-4-ol	8	nd	nd	nd	nd	nd	nd	nd	34	27	nd	nd	–	**	–
α-terpineol	18ab	12b	22a	18ab	5	8	9	11	27	15	19	63	ns	ns	**
trans-pyran linalool oxide	40	42	53	40	8b	6b	13b	32a	74	39	69	165	ns	*	*
cis-pyran linalool oxide	27	29	38	29	3	3	8	8	281	70	32	267	ns	**	ns
β-citronenol	30	39	nd	nd	nd	nd	nd	nd	50	nd	nd	nd	–	ns	ns
Nerol	276	248	314	267	84	68	146	131	279	115	266	855	ns	ns	*
β-damascenone	14	15	11	12	nd	nd	nd	nd	nd	nd	nd	nd	ns	–	–
Geraniol	451	430	469	289	180	288	193	280	675	197	455	1357	ns	ns	*
Diendiol I	321	304	313	263	119	131	146	226	406	288	297	583	–	ns	*
Diendiol II	239	217	216	191	129	67	128	97	301	197	224	638	ns	*	**
(E)−8-hydroxylinalool	116	100	114	100	37	31	40	65	41	nd	nd	nd	ns	***	ns
(Z)−8-hydroxylinalool	59	41	40	49	22	29	24	42	75	nd	nd	nd	ns	*	ns
Terpenes (%)	***61.3***	***63.1***	***66.1***	***61.9***	***55.7***	***62.6***	***61.8***	***61.1***	***66.1***	***55.0***	***56.8***	***62.0***			
Butanoic acid	5	4	4	5	2b	1b	4a	1b	nd	nd	nd	nd	ns	ns	ns
Geranic acid	699a	530b	625ab	614ab	279	171	251	367	500	298	647	1406	ns	ns	*
Dodecanoic acid	39	36	nd	nd	8	6	5	nd	nd	nd	nd	nd	ns	–	ns
Hexadecanoic acid	212	172	132	117	100	148	70	152	123	184	301	686	–	–	**
Hexanoic acid	35	31	31	34	7	4	15	20	nd	nd	nd	nd	ns	***	ns
Octanoic acid	13	25	nd	nd	4b	5ab	7ab	14a	nd	nd	nd	nd	**	***	**
Volatile acids (%)	***30.2***	***27.1***	***24.5***	***27.7***	***32.3***	***26.6***	***26.1***	***29.5***	***13.8***	***20.1***	***33.0***	***27.3***			
Benzaldehyde	nd	nd	nd	nd	1	1	1	nd	nd	nd	nd	nd	ns	–	ns
Aldehydes (%)	–	–	–	–	***0.1***	***0.1***	***0.1***	–	–	–	–	–			
4-vinylguaiacol	45	42	49	43	13	18	12	27	180	154	39	181	–	–	*
Vanillin	17	23	14	16	34	33	24	41	40	nd	13	nd	ns	**	ns
Eugenol	nd	nd	nd	nd	nd	nd	nd	nd	nd	47	18	61	ns	–	–
Volatile Phenols (%)	***1.8***	***2.2***	***1.9***	***2.1***	***3.8***	***4.1***	***2.7***	***3.6***	***4.9***	***8.4***	***2.4***	***3.2***			
Butyrolactone	8a	2b	3b	2b	3	nd	nd	6	104a	79a	18b	60a	ns	ns	ns
Lactones (%)	***0.2***	***0.1***	***0.1***	***0.1***	***0.3***			***0.3***	***2.3***	***3.3***	***0.6***	***0.8***			
Acetoine	7	7	7	6	2	1	1	nd	nd	nd	nd	nd	ns	***	ns
Carbonyl compounds (%)	***0.2***	***0.2***	***0.2***	***0.2***	***0.2***	***0.1***	***0.1***	–	–	–	–	–			

Nd: no detected; Different letters indicate significant differences for Tukey's test at $P < 0.05$. *, **, ***, and ns indicate significance at $P < 0.05$, 0.01, 0.001, and not significant, respectively. Treatments (TR) were SDI, sustained deficit irrigation; EDI, early deficit irrigation; LDI, late deficit irrigation; and C, control.

area-to-yield ratio, and climatic conditions could determine the effects of water regime on grape composition (Bucchetti et al., 2011; Intrigliolo et al., 2012; Mirás-Avalos and Intrigliolo, 2017; Vilanova et al., 2019a).

Tables 3 and 4 show the results of volatile composition as individual compounds of cv. "Muscat" according to water regime in the free and glycosidycally-bound fractions, respectively. Both fractions were grouped into nine chemical families: C_6-compounds, alcohols, ethyl esters, terpenes, volatile acids, aldehydes, phenol volatiles, lactones, and carbonyl compounds. The contribution (%) of each chemical group to the total concentration in musts is also shown.

The free fraction (Table 3) was represented by 47 volatile compounds. The ANOVA results showed a significantly stronger effect of the vintage on free volatiles, than the water regime. Thus, twenty-eight volatile compounds were affected by vintage vs fifteen compounds affected by the irrigation treatments imposed. However, a significant interaction between treatment and year (TR*Y) was also observed for twenty volatile compounds quantified, mainly free terpenes.

Free terpenes (represented by fourteen compounds) highly contributed to the total free concentration in all the treatments for the 2012 season (more than 59.8%). However, this result was only observed in LDI for 2013 (65.5%), and EDI and LDI for 2014 (more than 48.6%). Monoterpenes are associated to aromatic cultivars because of their low olfactory perception threshold. In "Muscat" cultivars, some monoterpenes (linalool, geraniol, nerol, citronelol, and α-terpineol) have been described as the major determinants of flavor because of their concentrations in musts (Ribéreau-Gayon et al., 1975; Gunata et al., 1985; Mateo and Jiménez, 2000; Corona et al., 2020). In our study, these five free monoterpenes showed the highest concentrations in all "Muscat" musts, and all of them were affected by the treatment and vintage (Table 3). In general, terpenes increased their concentration in LDI and control water regimes, the treatments which received the highest water volumes (Table 2). In contrast, in "Gwürztraminer" wine, water stress has been reported to enhance the formation of terpenes, mainly when irrigation deficit was applied at veraison as compared with deficit at pre-veraison (Reynolds et al., 2005). Other authors have also shown that the application of deficit irrigation before veraison increased the concentration of some terpenes, such as free linalool and α-terpineol (Wang et al., 2019). However, in cv. "Muscat Blanc", free linalool and geraniol reached higher concentrations under standard irrigation (50% ET_o before veraison) than in the drought regime, increasing free linalool by about 78%, and free geraniol by about 73%, with respect to the rain-fed treatment (Giordano et al., 2013). The high interaction TR*Y effect on the terpene composition of musts could be highlighting the important effects that meteorological conditions, which are very variable from year-to-year, have on aromatic free fraction across semiarid climates. In fact, in "Godello" and "Albariño" cultivars from Galicia (NW Spain) the weather conditions affected the must and wine composition to a greater extent than the in-season effects caused by irrigation (Mirás-Avalos et al., 2019a,b).

Ethyl esters and C_6-compounds (represented by two aldehydes and five alcohols) also highly contributed to the free volatile composition of "Muscat" musts. Thus, total ethyl esters in the SDI, EDI and control treatments for 2013, and total C_6-compounds in the SDI and control treatments in 2014, exhibited the highest contributions to free volatiles. In the same manner as terpenes, a major effect of the vintage on free C_6-compounds concentrations, as compared to the treatment, was observed, as well as a high TR*Y interaction.

When C_6-compounds are present at a concentration above the sensory threshold, they can be considered undesirable, because of their contribution to green aromas in grapes and wines (Roujou de Boubee, 2003; Cullere et al., 2007; Mendez-Costabel et al., 2014). During ripening, the concentration of C_6-compounds decreases, and thus, a high concentration of C_6-compounds is associated with lack of grape maturity. In this sense, in our study, the higher water availability in the control treatment increased the free C_6-compounds concentration in "Muscat" musts in agreement with a lower ripening (TSS-to-TA ratio) (Table 2). The higher irrigation level promoted canopy growth, and thus likely decreased fruit exposure, resulting in an increased concentration of some compounds during fruit maturation (Belancic et al., 1997; Mendes-Costabel et al., 2014). In contrast, several studies performed in grapes and wines have suggested that a low water availability increased the concentration of some C_6-compounds (Talaverano et al., 2016; Vilanova et al., 2019b; Wang et al., 2019). This result was not, however, confirmed in other studies on the "Merlot" cultivar, where free C_6-compounds decreased under water deficit (Song et al., 2012), or they were not affected by water status (Mendes-Costabel et al., 2014). In general, the concentration of some significant free volatile acids, alcohols and acetoine concentration, also increased in the control water regime treatment (Table 3).

The glycosidically-bound fraction (Table 4) was represented by 54 compounds. In the bound fraction, a larger effect of the vintage with respect to the treatment was observed, where twenty-two bound compounds were affected by vintage vs four compounds affected by treatment. Nevertheless, a significant TR*Y interaction was observed in sixteen glycosidically-bound compounds quantified.

In the same manner as in the free faction, the terpenes showed the highest contribution to the total glycosidically-bound concentration in all the treatments (more than 55.0%) for all vintages, followed by the volatile acids chemical group. Linalool, nerol, geraniol, diendiol I, and diendiol II, showed the highest concentrations in all water regimes studied and for all the vintages; however, these compounds were not affected by the treatments. The high concentration of terpenes, regardless of the vintage and treatment, revealed the importance of this group of compounds in this cultivar.

Grapevine water stress has been reported to increase the concentration of grape aroma glycosides (Bravdo and Shoseyov, 2000). In our study, a minor effect of vine water status on the glycosidically-bound fraction, rather than on the free fraction, was observed, with only four compounds affected by the treatment, although the TR*Y interactive effect was found in several compounds. Peyrot des Gachons et al. (2005) reported that severe water deficit stress limited aroma potential in "Sauvignon blanc" grape, where the higher levels of glycosylated compounds were found in vines under a mild water deficit. Limited water availability also had a positive effect on the aromatic potential of "Agiorgitiko" grapes (Koundouras et al., 2006). However, water deficit only had a limited effect on glycosylated compounds from the "Viognier" cultivar (Wang et al., 2019).

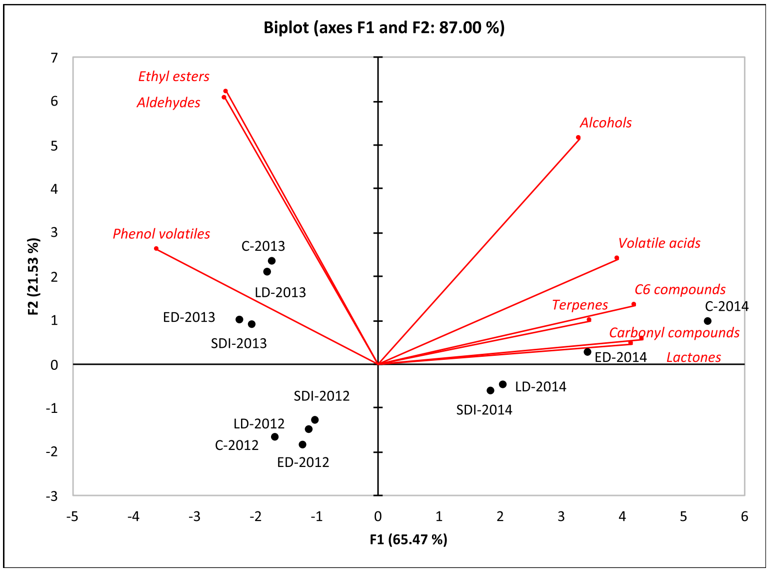
Among the glycosylated terpenes in "Muscat" grapes, only two compounds, *trans*-pyran linalool in 2013, and β-pinene in 2014, had a significant increase in concentration in the control and SDI treatments, respectively. In this sense, the EDI treatment likely had a detrimental effect on these terpene compounds, because important precursors were synthesized before veraison (Castellarin et al., 2007), and for this reason, the limited water availability in this period could be affecting the glycosidically bound compounds. On the other hand, post-veraison deficit irrigation, namely LDI, has been reported to improve the aroma composition of "Gewurztraminer" grapes (Kovalenko et al., 2021). However, this was not the case in "Muscat" grape.

Likewise, bound compounds such as hexanal, butyrolactone, 1-butanol geranic, and octanoic acids increased their concentration when the SDI treatment was applied, but this effect was not reported in all vintages. In general, a tendency of the SDI and EDI treatments to increase the most chemical families of glycosidycally-bound compounds, mainly for 2013 and 2014 seasons, was observed.

3.4. Principal components analysis (PCA)

A principal component analysis (PCA) was performed on the data of volatile and glycosidically-bound compounds identified and quantified in "Muscat" musts, grouped by chemical families (Figure 2). The application of PCA allowed establishing groups of water regime treatments and vintages on the basis of the chemical groups analyzed.

a) Free compounds



b) Bound compounds

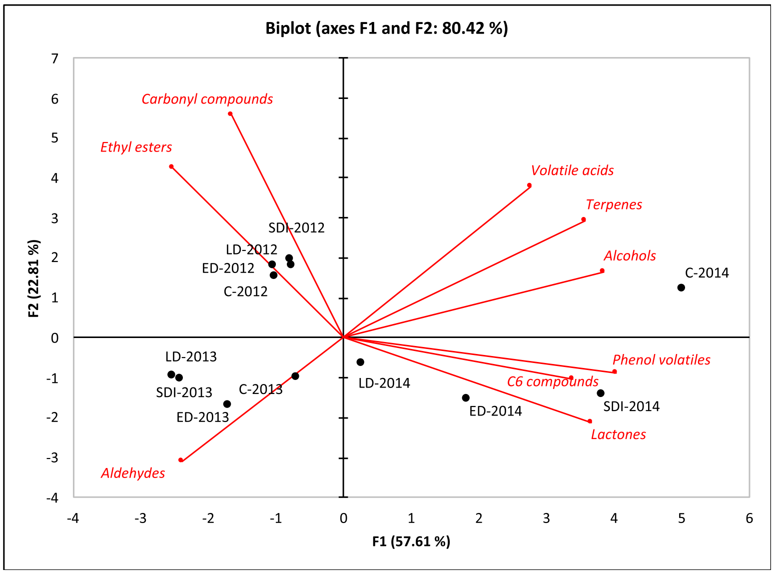


Fig. 2. Principal Component Analysis (PCA) based on chemical families of compounds in free (a) and glycosylated (b) fractions determined in "Muscat of Alexandria" musts under different water regimes from three vintages.

Figure 2a shows the PCA applied to the free fraction of volatiles, where 87.00% of the total variance was explained by the first two principal components. Principal component 1 (F1) explained 65.47% of the variance, and the second principal component (F2) explained 21.53%. The PCA showed three groups, where the water regime treatments were clearly grouped by season. The group corresponding to the 2014 season was positioned on the positive side of F1, and was characterized for high concentrations of lactones, carbonyl compounds, terpenes, C_6-compounds, and volatile acids. The group corresponding to all the treatments from the 2013 season was characterized by phenol volatiles, ethyl esters, and aldehydes. Lastly, all the treatments from the 2012 season, were positioned on negative side of both principal components (F1 and F2).

Figure 2b shows the PCA applied to the glycosidically-bound fraction of compounds. The first principal component (F1) explained 57.61% of the variance, and the second principal component (F2) explained 22.81% of the variance, with their total accounting for 80.42% of total variance. F1 was positively correlated with volatile acids, terpenes+C_{13}-norisoprenoids, alcohols, C_6-compounds, phenol volatiles, and lactones, and negatively correlated with aldehydes, ethyl esters, and carbonyl compounds. In the same manner as the free fraction, the bound fraction grouped the samples by vintage. The treatments from the 2014 season, positioned on the positive side of F1, were characterized by high concentrations of lactones, C_6-compounds, and phenol volatiles, mainly in the EDI and SDI treatments. All the treatments from the 2013 vintage showed high concentrations of aldehydes, whereas in 2012, high concentrations of carbonyl compounds and ethyl esters were found.

The PCAs results highlighted the relevance of the vintage effect on the free and bound fractions of the aromatic composition of cv. "Muscat of Alexandria", as compared to the water regimes. It can be noted that for both free and glycosidically-bound fractions, the water regime had greater effects in the 2014 season than in the two previous ones.

Moreover, the 2014 season had higher concentrations of compounds from both free and bound fractions (Figure 1). The water regime effects, added to the environmental conditions in the 2014 season, increased the terpene composition in both free and glycosidycally-bound fractions, mainly in the control treatment. The control treatment showed a tendency to increase the concentration of volatile acids, C_6-compounds, terpenes, carbonyl compounds, and lactones, mainly in the free fractions.

3.5. Relationships of grape aroma with vine performance and grape composition

Both free and glycosidically-bound fractions were negatively correlated in all the treatments with grape yield, although this effect was stronger for the free fraction than for the bound fraction (Figure 3a, b). Nevertheless, the control treatment significantly buffered the detrimental effects of yield in both aromatic fractions.

Conversely, the opposite relationship was found between the total volatile composition and the total leaf area-to-yield ratio (Figure 3c, d) highlighting the importance of the grapevine sink-source balance and canopy microclimate on the increase in the synthesis of some volatile compounds (Marais et al., 1999; Deluc et al., 2009). In this sense, it should be noted that in addition to irrigation, vine balance is determined by several agronomic practices, such as canopy management and cluster thinning (Talaverano et al., 2016).

In general, cluster sunlight exposure may lead to an increased monoterpene content (Zhang et al., 2017). However, excessive exposure can also negatively affect the terpenol content by an increase in berry temperature (Belancic et al., 1997). In this sense, the content of one of the most important terpenols from the aromatic perspective, linalool, appeared to be the most sensitive to sun exposure. In our study, the treatment with the higher canopies (control) showed a significant tendency to increase linalool concentration in both free and glycosidically-bound fractions. However, these effects were only significant for the free fraction. Likewise, the relationship between the aromatic free fraction and the total LA-to-yield ratio was significant, whereas it was not significant with the bound fractions (Figure 4c, d). Previous results obtained in cv. “Albariño” also found that the volatile concentration of grapes aroma were associated to water supply, while the non-volatile compounds were not affected by the watering regime (Vilanova et al., 2019a).

Lastly, it is interesting to remark that the potassium content in the must was associated to the free fraction (r^2=0.57; p<0.0001), but not to the glycosidically-bound fraction (r^2=0.02; p<0.45). As vine potassium absorption is intimately linked to vine water status (Intrigliolo and Castel, 2011), the treatments with a higher irrigation had a greater positive influence on the concentration of free compounds (C>LDI>EDI>SDI) (Table 3). These results suggest that the potassium fertilization regime, in combination with the soil chemical composition, could be additional factors to explore to manipulate the Muscat of Alexandria grape aroma potential.

4. Conclusions

The environmental conditions were the predominant factors which affected the aromatic composition of “Muscat of Alexandria” grapes in a semi-arid climate, but the irrigation regime had the potential to modulate the weather effects. Furthermore, the effects of both factors interacted on the composition of free terpenes, the major determinants of Muscat’s flavor. Many agronomic factors affected by the water regime,

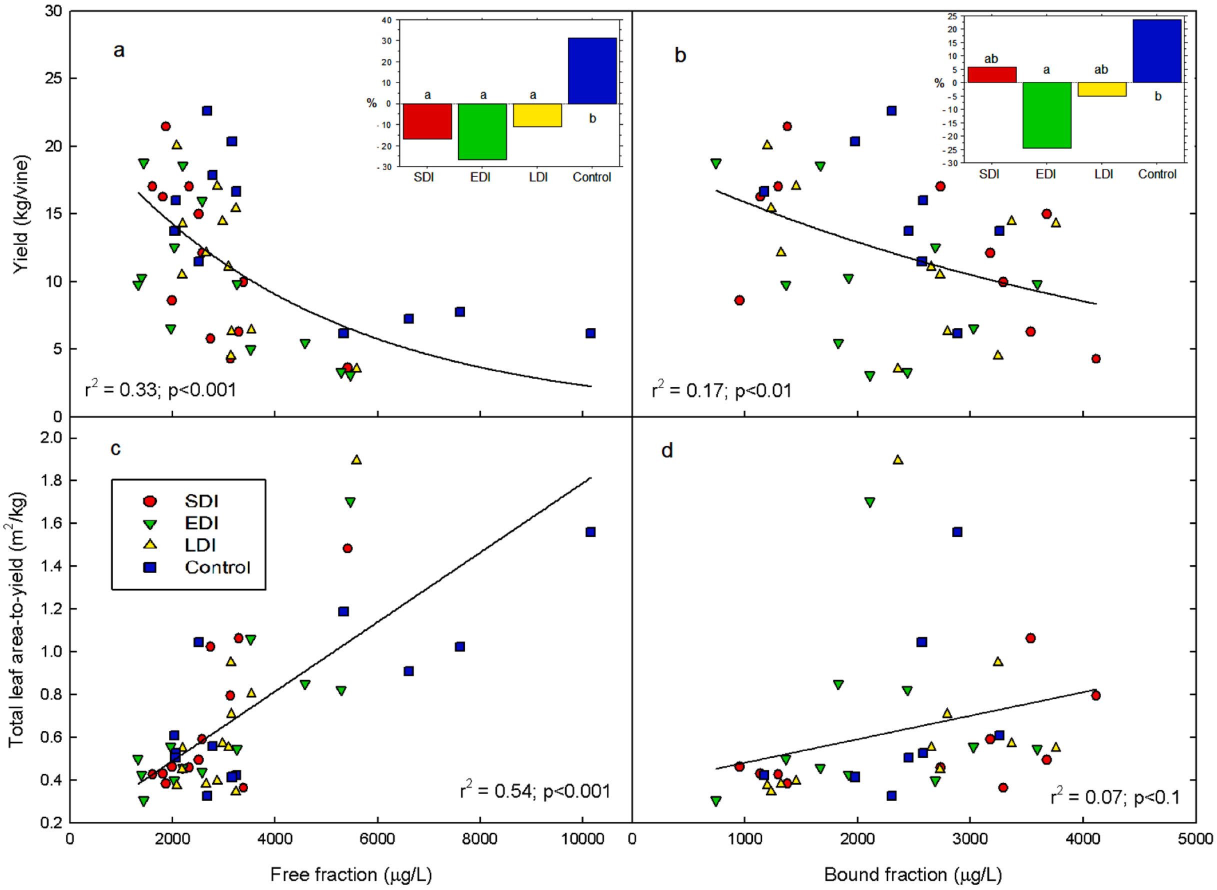


Fig. 3. Correlations between total free (a and c) and bound (b and d) fractions of “Muscat of Alexandria” grape aroma and (a and b) yield and (c and d) total leaf area-to-yield ratio subjected to different water regimes over three seasons.

such as vegetative growth, vine performance, and grape ripening, had a significant influence on both free and glycosidically-bound fractions. The seasons with higher yields were related to those with a lower grape aromatic content, more markedly in the free fraction. Nevertheless, the water regimes allowed mitigating these negative effects by balancing vines in terms of leaf area-to-yield ratio.

Declaration of Competing Interest

None.

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