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Volatile Composition of Merlot Wine from Different Vine Water Status

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The impact of deficit irrigation during berry development on Merlot wine volatile composition was investigated in this study. Own-rooted Merlot vines grown in a commercial vineyard in Idaho were supplied with 100 or 35% of their estimated crop evapotranspiration needs throughout the berry development. Wines were produced from those grapes from the 2002, 2003, and 2004 growing seasons. Volatile compounds in the wines were analyzed using the stir bar sorptive extraction−gas chromatography−mass spectrometry technique. The results demonstrated that despite vintage differences in volatile composition, in each of 3 years of this study, deficit irrigation during berry development had a consistent effect on wine volatile composition. Wine produced from deficit-irrigated vines had increased amounts of vitispiranes, β-damascenone, guaiacol, 4-methylguaiacol, 4-ethylguaiacol, and 4-vinylguaiacol relative to wine produced from well-watered vines. Deficit irrigation had no effect on the concentrations of other measured volatiles such as esters and terpenes.

KEYWORDS: Wine volatile; water stress; irrigation; norisoprenoids; β-damascenone; stir bar sorption extraction

INTRODUCTION

Manupulating vine water status through deficit irrigation is a common commercial practice for controlling grape canopy size and density (*1*). It is well-known that water status during berry development will affect berry size and therefore crop yield, but what is less well understood is whether the economic loss incurred from yield reduction can be offset by a beneficial increase in levels of aroma compounds and result in enhanced product quality.

Water is required for normal vine growth and berry development. Water status during berry development directly affects the vine physiology and secondary metabolism of the plant. It has been reported that water stress during the growth period accelerates sugar accumulation, increases the levels of skin and total anthocyanins and phenolics in the grapes, and alters the sensory profile of the wine (*2*, *3*).

Aroma is one of the most important attributes for wine quality. Although many aroma compounds in wine are formed through fermentation, grape-derived aroma and aroma precursors are most important to wine varietal aroma and wine quality. Since grape-derived aroma compounds and their glycosides are the secondary metabolites of the grapevine, their formation in the grapes could be affected by vine water status. The volatile compositional differences in the grapes induced by water status could directly affect the aroma composition of the wines.

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Under nondrought conditions, slight water stress seems to improve wine quality. Wine produced from water-stressed vines of Cabernet Sauvignon have significantly higher blackberry, jam, cooked berry, dried fruit, raisin, and fruity aroma and less vegetal, bell pepper, and black pepper aromas than wines from well-watered treatments (*4*). Vine water stress has been reported to increase the concentration of aroma glycosides of grapes (*2*, *5*); these glycoside bound aroma compounds can be released during fermentation or aging and contribute to varietal aroma and wine quality.

Under drought conditions, vines may experience poor shoot growth and poor fruit composition development. Irrigation is necessary to improve the water status of the vine. It was reported that irrigation in arid climates resulted in wines with greater intensities of apple, citrus, and floral aromas and reduced earthy aroma in Chardonnay wines (*6*).

On the other hand, too much irrigation, especially during the later stages of ripening, had negative effects on wine aroma. Too much irrigation results in wine with more vegetal, bell pepper, and herbaceous aroma (*3*, *4*, *7*), and this aroma defect could be related to a higher content of of 3-isobutyl-2-methoxypyrazine in highly irrigated and high-plantation density grapes (*8*). In general, moderate water stress improves wine quality. However, the detailed chemical compounds responsible for the flavor attribute difference have not been well studied.

The red wine grape (*Vitis vinifera* L.) cultivar Merlot, used traditionally for blending and more recently as a varietal, is the seventh most widely cultivated vine worldwide (155000 ha worldwide) (*9*). The exact origin of Merlot is unknown, but

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Table 1. Calibration Curves for Volatile Compounds in Wine ($n = 6$)

	quantify ion	equation	regression correlation coefficient	relative standard error (%)
trans-carveol (IS)	109			
guaiacol	109	resp. ratio = 0.026 × amt. ratio	0.968	7.49
linalool	71	resp. ratio = 0.736 × amt. ratio	0.997	3.60
geraniol	69	resp. ratio = 0.689 × amt. ratio	0.998	2.87
eugenol	164	resp. ratio = 0.366 × amt. ratio	0.996	4.07
phenylethanol	122	resp. ratio = 0.008 × amt. ratio	0.999	2.51
citronellol	69	resp. ratio = 0.552 × amt. ratio	0.999	9.70
2-phenoxyethanol[a]	138	resp. ratio = 0.550 × amt. ratio	0.999	9.73
4-ethylguaiacol	152	resp. ratio = 0.026 × amt. ratio	0.972	5.65
hexyl formate (IS)	56			
ethyl 2-methylpropanoate	71	resp. ratio = 0.019 × amt. ratio	0.999	7.21
ethyl butanoate	71	resp. ratio = 0.018 × amt. ratio	0.999	5.42
3-methylbutyl acetate	70	resp. ratio = 0.129 × amt. ratio	0.999	3.95
2-methylbutyl acetate[b]	70	resp. ratio = 0.129 × amt. ratio	0.999	3.22
ethyl 3-methylbutanoate	88	resp. ratio = 0.179 × amt. ratio	0.998	4.73
octyl propanoate (IS)	112			
methyl hexanoate	74	resp. ratio = 0.988 × amt. ratio	0.992	8.34
ethyl hexanoate	88	resp. ratio = 0.659 × amt. ratio	0.990	6.56
methyl octanoate	74	resp. ratio = 1.789 × amt. ratio	0.981	7.40
ethyl octanoate	88	resp. ratio = 1.287 × amt. ratio	0.983	4.68
ethyl decanoate	88	resp. ratio = 1.105 × amt. ratio	0.981	7.05
2-nonenal (IS)	70			
β-damascenone	121	resp. ratio = 1.102 × amt. ratio	0.997	3.26
β-ionone	177	resp. ratio = 0.002 × amt. ratio	0.987	3.68
γ-nonalactone	85	resp. ratio = 0.230 × amt. ratio	0.997	3.98
linalyl isobutyrate (IS)	93			
ethyl phenylacetate	164	resp. ratio = 0.192 × amt. ratio	0.998	7.99
ethyl dihydrocinnamate	178	resp. ratio = 0.272 × amt. ratio	0.996	8.98
ethyl anthranilate	165	resp. ratio = 0.224 × amt. ratio	0.983	9.94
ethyl cinnamate[c]	131	resp. ratio = 0.272 × amt. ratio	0.996	5.70
methyl vanillate	151	resp. ratio = 0.013 × amt. ratio	0.987	4.70
ethyl vanillate	196	resp. ratio = 0.224 × amt. ratio	0.983	5.88
ethyl 3-methyl thiopropionate[d]	148	resp. ratio = 0.698 × amt. ratio	0.998	8.16
phenylethyl acetate	104	resp. ratio = 0.853 × amt. ratio	0.999	5.57
ethyl 3-phenylpropanoate	104	resp. ratio = 0.698 × amt. ratio	0.998	8.46

[a] The phenol standard curve was used for calculation. [b] The standard curve of 3-methylbutyl acetate was used for calculation. [c] The standard curve of ethyl dihydroxycinnamate was used for calculation. [d] The standard curve of ethyl 3-phenylpropanoate was used for calculation.

DNA analysis suggests genetic similarity to Cabernet Franc and Carmenere (*10*). Merlot is well suited to cultivation in eastern Washington, a semiarid region with warm days, cool nights, and mild, dry weather during bloom (*11*).

The viticultural production region of the Western Snake River Plain of Idaho is similar to that of eastern Washington (*12*), where vine water status can be manipulated through irrigation management to enhance fruit attributes for wine production (*13*). The impact of irrigation on grape composition and wine quality has been investigated over the past 30 years and is still of interest today because of the complexity of factors that impact grape and wine quality. The objective of this research was to use the stir bar sorption extraction−gas chromatography−mass spectrometry technique to investigate the impact of vine water status during berry development on the aroma profile of Merlot wine.

MATERIALS AND METHODS

Chemicals. β-Damascenone was from Firmenich (Princeton, NJ). All other volatile standards listed in **Table 1** were purchased from Sigma-Aldrich (St. Louis, MO) unless specified. Ethanol was purchased from Aaper Alcohol and Chemical Co. (Shelbyville, KY), and tartaric acid was from Mallinckrodt Inc. (Paris, KY).

Plant Material and Field Trial Site. Ungrafted vines of Merlot were subjected to well-watered or deficit-irrigated conditions over three consecutive growing seasons (2002, 2003, and 2004), and the harvested fruit was used to produce replicated lots of wine. The irrigation trial was situated in a commercial vineyard near Nampa, ID (latitude 43°28′N, longitude 116°42′W, elevation 841 m), and had a randomized block design with irrigation amount as the main effect and four field replications. Each plot contained four rows of 14 vines per row (56 vines). Vines were irrigated weekly with 35 or 100% of their estimated evapotranspiration requirement (ET_c) beginning just after fruit set until at least 2 weeks after harvest as described previously (*13*). Vines were cordon trained, spur pruned, and vertically shoot positioned in north to south oriented rows with 2.4 m × 1.8 m row by vine spacing (~2242 vines/ha). Vines were managed according to standard commercial practice with the exception of irrigation amount.

Wine Production. Wines were produced from each of three growing seasons (2002, 2003, and 2004) using 67 kg of fruit per fermentation and three replicate fermentations per irrigation level. An equal amount of fruit was harvested from the interior vines of each of four replicated field plots when average juice soluble solid concentration, pH, and titratable acidity suggested commercial maturity (23% Brix, pH 3.5, titratable acidity of 5.0 g/L). Variability among fermentation replications was assumed to be greater than variability among treatment level field replications, so fruit harvested from each irrigation treatment level replication was combined and randomly allocated into three lots each weighing 67 kg. Each lot of fruit was fermented independently, providing triplicate fermentations for each irrigation treatment level.

The grapes were crushed and stems removed (Mori Crusher-Destemmer E20, model 1502SA06, The Compleat Winemaker, St. Helena, CA) on the day of harvest, and potassium metabisulfite was added to provide a calculated amount of 40 ppm total sulfur dioxide. After 24 h, the must was inoculated with 0.26 g of Premier Cuvée yeast (Davis 796) per liter of must. The must was fermented in 100 L stainless steel tanks at 23 °C for 7 days, and then skin and seeds were removed using a 160 L, stainless steel bladder

press (model 2448, GW Kent, Ann Arbor, MI) with the pressure increased gradually to 0.05 kPa. The wine was allowed to settle for 3 days before being racked off the yeast lees, and potassium metabisulfite was added. The wine was transferred to 750 mL glass bottles, and the bottles were corked and stored horizontally at 22 °C until the wine was analyzed.

Analysis of Volatile Compounds in Wine. The volatiles in the wine were analyzed using a stir bar sorptive extraction−gas chromatography−mass spectrometry technique reported previously with some modifications (*14*). We made a synthetic wine by dissolving 3.5 g of L-tartaric acid in 1 L of a 12% ethanol solution and adjusting the pH to 3.5 with 1 M NaOH (*15*). We prepared stock solutions of volatile compounds by dissolving 10000 mg of each compound individually in 1 L of ethanol. We prepared standard solutions by mixing the individual stock solution in synthetic wine and then diluting to give a range of concentrations as described previously (*14*). We made an internal standard stock solution by dissolving 46 mg of hexyl formate, 48 mg of octyl propanoate, 7 mg of *trans*-carveol, 9 mg of *trans*-2-nonenal, and 9 mg of linanyl 3-methylbutanoate per liter of ethanol and stored it at −15 °C.

Each sample (10 mL) was diluted with 10 mL of water in a 40 mL vial, to which 6 g of sodium chloride and 20 μL of internal standard solution had been added. A stir bar (Twister) coated with poly(dimethylsiloxane) (PDMS) phase (1 cm length, 0.5 mm thickness, Gerstel, Inc., Baltimore, MD) was used to extract the volatile compounds from the sample. The Twister bar was constantly stirred for 12 h at a speed of 1000 rpm. After samples had been taken, the Twister bar was rinsed with distilled water, dried with tissue paper, and placed into the sample holder.

The volatiles were thermally desorbed at a thermal desorption unit (TDU) (Gerstel, Inc.) mounted on a gas chromatography−mass spectrometry apparatus (Agilent 5973 GC-MS, Agilent Technologies, Little Falls, DE). The TDU was set in splitless mode, ramping from 35 to 300 °C at a rate of 700 °C/min, and held at the final temperature for 3 min. The desorbed analytes were cryofocused (−60 °C) in a programmed temperature vaporizing (PTV) injector (CIS 4, Gerstel, Inc.) with liquid nitrogen. After desorption, the PTV was heated from −60 to 250 °C at a rate of 10 °C/s and held at 250 °C for 3 min. The solvent vent injection mode was employed. A Rtx-1 capillary GC column (60 m, 0.25 mm inside diameter, 0.5 μm film thickness, Restek Inc., Bellefonte, PA) was employed to separate the volatile compounds. Carrier gas (helium) was set at a constant flow rate of 1.8 mL/min. The oven temperature was initially set at 50 °C for 2 min, increased to 210 °C at a rate of 2 °C/min and then to 250 °C at a rate of 10 °C/min, and held at 250 °C for 15 min. MSD was used in scan mode (35−350 mu). The electron impact (EI) energy was 70 eV, and the ion source temperature was set to 230 °C.

Selective mass ion (**Table 1**) was used to quantify the aroma-active compounds. Data were analyzed using ChemStation, and relative standard errors (RSD) were calculated on the basis of triplicate analysis of the wine samples. Triplicate analysis was performed on all samples.

Statistical Analysis. The volatile compounds were grouped into five categories: esters, terpenoids, C_{13} isoprenoids, phenols, and others. The concentrations of all compounds in each category except others were analyzed by multivariate analysis of variance (MANOVA). Year and irrigation and two-way interaction (year × irrigation) were included in the MANOVA model. The level of significance (α) was 0.05. To understand the paired mean differences, mean concentrations of volatile compounds in different wines were compared by multiple comparisons adjusted by the Tukey-HSD method. All statistical analyses were performed using SPSS 13.0 for Windows (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Reliable quantitative analysis of volatile compounds in wines is challenging because of the highly complex matrix, high alcohol content, and low concentration of aroma-active compounds. Interpretation is further complicated by a marked interaction between relative amounts of aroma-active compounds and their sensory perception. It is difficult to select a matrix that assembles the complete composition of wine. In this study, a synthetic wine matrix was used to build the standard calibration curves; the wine was diluted to reduce the impact of matrix variation. In addition, multiple internal standards were used to further minimize the effect of the matrix on the recovery of volatile compounds. Of the 30 compounds quantified, 21 had regression correlation coefficients of > 0.99, and the RSD was less than 10% for most of the compounds quantified (**Table 1**). This method was used to analyze volatile composition in the wines under different water status.

Viticultural factors can have a major impact on the physiology of grape vines and the final volatile composition of the wine. As reported previously (*13*), the 2004 vintage received 2-fold greater precipitation during the growing season (125 mm) than the 2002 (43 mm) vintage and ∼80% more precipitation than the 2003 (68 mm) vintage. Berry size and cluster weight in 2004 were higher than in 2002 and 2003. Berry weight at harvest in 2004 under 35% ET_c was 1.22 g, which is significantly higher than the values of 0.67 g in 2002 and 0.92 g in 2003. Similarly, the yield per vine of deficit-irrigated vines in 2004 (7.8 kg/vine) was higher than in 2002 (3.2 kg/vine) or 2003 (4.1 kg/vine) (*13*). Those differences can result in variations in volatile concentration in different vintages.

As shown in **Table 2**, esters were the major volatile compounds in the wine both quantitatively and qualitatively. Ethyl esters of butanoate, hexanoate, and octanoate were at concentrations of 200−800 μg/L. Most esters are derived from fermentation, and their concentrations reflect yeast and fermentation conditions. As shown in **Table 2**, the ester concentrations varied widely among the three years. Despite the same use of winemaking equipment and procedures and replicated fermentation lots, the large variability in ester concentration suggests unknown enological factors influenced annual fermentations, which also was seen in MANOVA analysis (year is the only significant factor; $p \leq 0.01$). Within the same year, the concentrations of ethyl butanoate, ethyl hexanoate, and ethyl octanoate were very similar for each level of irrigation.

Branch-chained esters, including ethyl 2-methylpropanoate, ethyl 3-methylbutanoate, 3-methylbutyl acetate, and 2-methylbutyl acetate, also had high concentrations. We observed that the concentrations of ethyl 2-methylpropanoate in 2002 and ethyl 3-methylbutanoate in 2002 and 2003 were increased under 35% irrigation. However, this was not consistent for all vintage years. It is likely that deficit irrigation will not affect the concentration of branched chain fatty acid esters.

Several aromatic esters were also investigated in this study, but low concentrations were found. Ethyl phenylacetate, 2-phenylethyl acetate, and ethyl 3-phenylpropanoate all had low concentrations; they have also been identified as being important aroma-active compounds in wines (*16*, *17*). Ethyl dihydrocinnamate, ethyl cinnamate, ethyl anthranilate, and ethyl vanillate all had low concentrations. Although some studies reported they could be important to wine aroma (*18*), their aroma contribution to wine is still controversial (*14*, *19*) and is probably related to wine variety. Overall, as significant contributions to the fruity aroma of wine, esters were not affected by irrigation.

Grape-derived C_{13} norisoprenoids (**Table 2**) are very important to the aroma of both white and red wines (*20*−*22*). C_{13} norisoprenoids arise from carotenoid degradation and are present in grapes in the free or glycoside form. Although the glycoside precursors cannot be hydrolyzed by grape and yeast glycosidases, they could be hydrolyzed under acidic conditions, directly incorporated or converted into other more powerful aroma-active compounds, and contribute to the wine aroma (*23*). C_{13} norisoprenoids contribute to complex aromas, including berry, honey, and fruity in many red wines.

The total concentration of all measured C_{13} norisoprenoids in wines was highly related to vine irrigation condition and vintage year. MANOVA analysis showed that vintage year, irrigation,

Table 2. Volatile Concentrations (micrograms per liter) in Merlot Wines from Different Water Status[a]

	2002		2003		2004	
compound	100% irrigation	35% irrigation	100% irrigation	35% irrigation	100% irrigation	35% irrigation
esters						
ethyl 2-methylpropanoate	414 ± 20 a	582 ± 58 b	619 ± 34 b	653 ± 12 b	343 ± 3 a	413 ± 23 a
ethyl butanoate	860 ± 73 b	882 ± 69 b	659 ± 40 a	726 ± 18 a	698 ± 3 a	780 ± 22 ab
3-methylbutyl acetate	221 ± 13 a	232 ± 5 ab	329 ± 17 d	268 ± 17 bc	288 ± 11 c	297 ± 19 cd
2-methylbutyl acetate	68 ± 6 a	86 ± 1 bc	122 ± 6 d	96 ± 8 c	79 ± 5 ab	80 ± 3 ab
ethyl 3-methylbutanoate	151 ± 14 c	195 ± 10 d	110 ± 6 b	136 ± 2 c	61 ± 2 a	71 ± 3 a
methyl hexanoate	2.76 ± 0.28 cd	2.55 ± 0.53 bc	2.06 ± 0.05 abc	3.44 ± 0.36 d	1.55 ± 0.03 a	1.78 ± 0.10 ab
ethyl hexanoate	493 ± 43 b	391 ± 15 a	420 ± 14 ab	465 ± 38 ab	384 ± 21 a	430 ± 49 ab
methyl octanoate	2.84 ± 0.26 b	1.81 ± 0.01 a	1.81 ± 0.02 a	2.84 ± 0.26 b	1.89 ± 0.15 a	2.15 ± 0.30 a
ethyl octanoate	279 ± 29 c	176 ± 5 a	205 ± 3 ab	248 ± 27 bc	261 ± 24 bc	285 ± 33 c
ethyl decanoate	40.8 ± 3.5 a	32.5 ± 3.6 a	31.9 ± 3.1 a	38.5 ± 2.1 a	54.1 ± 6.3 b	58.0 ± 7.8 b
ethyl phenylacetate[b]	11.0 ± 0.6 b	14.0 ± 0.5 c	11.7 ± 1.4 bc	10.4 ± 0.5 c	5.6 ± 0.5 a	6.9 ± 1.6 a
ethyl dihydrocinnamate	0.43 ± 0.03 ab	0.18 ± 0.02 a	0.36 ± 0.04 ab	0.54 ± 0.09 b	0.90 ± 0.08 c	1.00 ± 0.23 c
ethyl anthranilate	0.15 ± 0.02 a	0.21 ± 0.01 a	0.22 ± 0.02 a	0.41 ± 0.11 b	0.08 ± 0.01 a	0.11 ± 0.05 a
ethyl cinnamate	1.57 ± 0.08 b	2.37 ± 0.03 c	1.32 ± 0.21 ab	2.50 ± 0.48 c	0.70 ± 0.07 a	1.02 ± 0.18 ab
methyl vanillate	29.0 ± 2.7 b	20.5 ± 0.9 a	23.4 ± 4.8 a	21.3 ± 2.2 a	26.7 ± 2.1 ab	26.0 ± 7.0 ab
ethyl vanillate	5.16 ± 0.54 ab	4.74 ± 0.37 ab	4.83 ± 0.69 ab	6.15 ± 0.92 b	3.56 ± 0.42 a	4.55 ± 1.07 ab
ethyl 3-methylthiopropionate	0.41 ± 0.03 bc	0.43 ± 0.05 bc	0.48 ± 0.05 bc	0.57 ± 0.08 c	0.23 ± 0.03 a	0.36 ± 0.08 ab
phenylethyl acetate	19.5 ± 1.1 a	24.6 ± 1.0 ab	47.7 ± 5.5 d	36.7 ± 3.9 cd	23.3 ± 2.2 ab	32.2 ± 7.7 bc
ethyl 3-phenylpropanoate	0.53 ± 0.05 ab	0.55 ± 0.03 ab	0.64 ± 0.09 b	0.50 ± 0.06 ab	0.41 ± 0.03 a	0.44 ± 0.11 a
terpenoids						
linalool	5.12 ± 0.64 a	6.10 ± 0.03 ab	6.39 ± 0.39 b	6.03 ± 0.54 ab	6.08 ± 0.40 ab	8.17 ± 0.37 c
geraniol	1.99 ± 0.18 a	2.13 ± 0.18 a	2.63 ± 0.06 a	1.96 ± 0.22 a	3.52 ± 0.33 b	4.12 ± 0.53 b
citronellol	9.62 ± 0.81 a	9.88 ± 1.32 a	14.58 ± 0.24 b	10.00 ± 0.10 a	19.64 ± 1.22 c	21.37 ± 0.83 c
C_{13} isoprenoids						
vitispirane[b]	5.69 ± 0.43 c	7.85 ± 0.17 d	7.20 ± 0.19 d	7.35 ± 0.08 d	2.20 ± 0.19 a	3.83 ± 0.23 b
β-damascenone	7.75 ± 0.40 b	10.41 ± 0.25 c	10.35 ± 0.47 c	12.33 ± 0.81 d	5.88 ± 0.41 a	7.36 ± 0.33 b
β-ionone	0.34 ± 0.01 bc	0.23 ± 0.02 a	0.52 ± 0.02 d	0.42 ± 0.02 c	0.40 ± 0.03 c	0.18 ± 0.02 a
phenols						
guaiacol	29.1 ± 4.4 b	36.8 ± 3.7 c	22.6 ± 0.8 a	27.5 ± 1.4 b	25.2 ± 2.3 b	35.0 ± 4.6 c
eugenol	2.30 ± 0.18 d	1.92 ± 0.06 c	2.18 ± 0.08 d	1.46 ± 0.03 b	0.88 ± 0.05 a	1.22 ± 0.06 b
4-ethylguaiacol	2.44 ± 0.34 cd	3.39 ± 0.19 e	2.33 ± 0.09 c	2.65 ± 0.25 d	1.19 ± 0.12 a	1.48 ± 0.14 b
4-vinylguaiacol[b]	2.89 ± 0.23 b	4.25 ± 0.35 c	2.36 ± 0.05 a	2.67 ± 0.09 b	2.94 ± 0.25 b	3.53 ± 0.44 c
4-methylguaiacol[b]	28.1 ± 3.5 bc	49.6 ± 7.0 d	21.8 ± 0.5 b	32.7 ± 1.9 c	14.4 ± 1.0 a	17.3 ± 1.0 b
others						
γ-nonalactone	13.0 ± 1.2 ab	19.9 ± 1.2 c	12.1 ± 1.2 ab	13.3 ± 1.5 b	10.2 ± 0.4 a	11.1 ± 0.7 ab
phenylethanol (mg/L)	99.3 ± 3.1 b	107.4 ± 7.0 b	72.6 ± 2.8 a	61.5 ± 5.5 a	61.6 ± 2.6 a	71.1 ± 1.4 a

[a] Values followed by different lowercase letters are significantly different ($p < 0.05$) as determined by Tukey's HSD test. [b] Compounds that are estimated on the basis of the standard curve from total ions of other similar compounds. 4-Vinylguaiacol and homoguaiacol estimated on the basis of 4-ethylguaiacol. Vitispirane estimated on the basis of β-damascenone. Ethyl phenylacetate estimated on the basis of phenylethyl acetate.

and their interaction were significantly different depending upon the levels of water deficit treatments ($p < 0.001$).

Among the C_{13} norisoprenoids, TDN (1,1,6-trimethyl-1, 2-dihydronaphthalene), vitispirane, β-damascenone, and β-ionone are probably the most important compounds. TDN was not investigated in this study because it may only appear during bottle aging. Vitispirane was identified as an important odorant, imparting a camphor odor to the wine. The concentrations of total vitispiranes in the experimental wines were between 2 and 8 μg/L, which is within the range reported for other wines (*24*). Wines produced from vines under deficit irrigation contained a significantly higher concentration of vitispiranes in 2002 and 2004, but the same in 2003. The content of vitispiranes in wines from 35% ET_c irrigation was 37 and 74% higher than wines from 100% ET_c in 2002 and 2004, respectively.

The concentration of vitispiranes was lower in the 2004 vintage than in the 2002 and 2003 vintages possibly because of higher precipitation and higher berry weight and yield in 2004 as discussed previously. Vitispirane has a high sensory threshold of 80 μg/L (*25*); however, it may still play a very important role in wine aroma because of the synergistic effect. Vitispirane is particularly important in bottle-aged wines (*26*, *27*), possibly because of enhanced formation under acidic conditions during aging (*20*).

β-Damascenone has a complex smell of flowers, tropical fruit, and stewed apple and a very low olfactory perception threshold of 0.05 μg/L in ethanol (*28*). Wines from 35% ET_c had significantly increased concentrations of β-damascenone in all vintage years, which were 34, 19, and 25% higher in the 2002, 2003, and 2004 vintages, respectively, than wines from 100% ET_c.

β-Ionone has an aroma of raspberry and violets. It has a very low perception threshold of 0.09 μg/L in synthetic wine (*29*). Because of its low sensory threshold, it could be important to wine aroma. The concentration of β-ionone was very low in the experimental wines in this study. Surprisingly, a significant decrease in β-ionone concentration was observed for the water deficit wines.

The MANOVA also showed a significant effect of irrigation and vintage years as well as two-way interaction on volatile phenolic compounds. The contents of guaiacol, 4-ethylguaiacol, 4-vinylguaiacol, and 4-methylguaiacol in wines produced from vines under water stress were significantly increased compared to those of wines from 100% ET_c. Guaiacol imparts a "smokey" character and can be generated through decarboxylation of vanillic acid. 4-Ethylguaiacol, 4-vinylguaiacol, and 4-methylguaiacol are associated with a spicy, clove character and can be generated from the breakdown of lignin.

Terpene alcohols, including linalool, α-terpineol, citronellol, nerol, geraniol, and ho-trienol, are important to the aroma of many wines, especially in the Muscat family. These compounds have aroma reminiscent of flower, rose, and geranium and typically have very low sensory thresholds. Quantitative analysis of linalool, geraniol, and citronellol did not yield a significant difference in these compounds under different water deficit treatments. This result contradicts the results reported by Reynolds et al. (*30*) that water stress enhances the formation of terpenes in Gewürztraminer wine. This discrepancy is probably caused by the low concentration of terpene alcohols in Merlot wine.

Phenylethanol was present at a very high concentration in the wine (**Table 2**). Phenylethanol can be present in the grapes, but it is largely produced during fermentation by wine yeast, and its concentration varied year to year from 60 to 100 mg/L, reflecting variability in the winemaking process. The level of irrigation had no influence on the amount of this compound based on the multiple comparisons.

γ-Nonalactone was also quantified, and a low concentration was found in these experimental wines. Statistical analysis did not find any significant difference among the wines subjected to different water management techniques. Alcohols and fatty acids were not analyzed in this study because of their high sensory thresholds. In addition, the nonpolar nature of the PDMS phase is not suitable for analysis of polar compounds such as alcohols and free fatty acids.

The formation of volatile compounds in grapes is complex, and light intensity, temperature, irrigation, and leaf removal have all been reported to affect vine physiology and thus the final concentrations of volatile compounds (*31*). Deficit irrigation may induce an increased level of synthesis of volatile and volatile precursors in the grapes; limited water availability also reduces vine vigor and thus increases berry sun exposure and berry temperature, which can accelerate degradation of carotenoids and enhance the formation of some volatile compounds. In addition, deficit irrigation may affect grape maturity, resulting in the difference in volatile composition. Long-term studies are needed to improve our understanding of the true effect of individual viticultural factor on wine quality.

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