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From grape berries to wines: drought impacts on key secondary metabolites

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ABSTRACT

Aim: We aimed to study the impact of water deficit on the concentration of key flavour and phenolic secondary metabolites of wines.

Methods and results: A drought-stress field trial was conducted on *Vitis vinifera* cv. Merlot and Tocai Friulano for two seasons. Fully irrigated (C) and deficit irrigated (D) grapes were microvinified and the resulting wines were analysed to determine the concentrations of anthocyanins, tannins, and free and glycosidically-bound Volatile Organic Compounds (VOCs). A descriptive sensory test was undertaken on the same wines. Water stressed grapes produced wines with higher concentrations of anthocyanins in Merlot and of free and glycosidically-bound monoterpenes in Tocai Friulano. Both cultivars displayed higher amounts of glycosidically-bound C_{13}-norisoprenoids.

Conclusions: Previously observed drought-induced compositional changes to the grapes were transfered to the wines, with an increase in polyphenols and VOCs. However, the timing and the duration of the water stress in the field only heavily impacted the final wine composition with major metabolic modification when the severe water deficit started early (at approximately 40 days after anthesis) and lasted over the entire season until harvest.

Significance and impact of the study: This study highlights the positive role of a controlled water deficit on the composition of the wines in terms of secondary metabolites.

KEYWORDS

abiotic stress, anthocyanins, aroma, monoterpenes, volatile organic compounds

INTRODUCTION

Grape berry quality, and hence the value of the derived wines, is tightly linked to the accumulation of secondary metabolites, such as polyphenols and Volatile Organic Compounds (VOCs), which contribute to the final colour, taste, and aroma of a wine. Secondary metabolites play a pivotal role in the adaptation of plants to the environment, acting as a defence mechanism and aiding reproduction by attracting pollinators in the form of floral scent or colouration (Ferrandino and Lovisolo, 2014). Previous research has revealed that higher concentrations of several classes of secondary metabolites, such as anthocyanins and terpenes, will accumulate in grapevine experiencing water deficit (Castellarin *et al*., 2007; Deluc *et al*., 2009; Zarrouk *et al*., 2012; Savoi *et al*., 2016, 2017; Gambetta *et al*., 2020). Nevertheless, few reports have addressed how such modifications in grape metabolites can impact final wine composition and flavour, as most studies have mainly focussed on the sensorial analysis of wine (Balint and Reynolds, 2014; Chapman *et al*., 2005; Herrera *et al*., 2015; Intrigliolo and Castel, 2008; Ou *et al*., 2010). For example, in our previous work (Savoi *et al*., 2016) we showed, for the first time, a significant increase in terpene synthases in white grapes experiencing water deficit, and it would be interesting to know if this can be translated into more terpenes being present in wines.

Polyphenols biosynthesis and accumulation in grape has been widely studied, especially in terms of the environmental effects on these compounds (Downey *et al*., 2006; Teixeira *et al*., 2013). Two polyphenols classes are commonly detected in grape and wine and are of major importance: anthocyanins and tannins. Anthocyanins are present in red grape berry skins (except for the Teinturier varieties, of which anthocyanins also accumulate in the flesh). They start to accumulate at the onset of ripening, and they are responsible for the colour of the red grape varieties. Different levels of hydroxylation, methylation and acylation of the five most detected anthocyanins can impact the colour intensity and hue of red wines. It has been reported that water deficit can enhance the biosynthesis and accumulation of tri-hydroxylated anthocyanins (delphinidin, petunidin and malvidin), thus shifting the anthocyanin profile towards the enrichment of purple pigments (Castellarin *et al*., 2007; Savoi *et al*., 2017). Condensed tannins are complex polymers of flavan-3-ols which are primarily synthesised in grape berry skins and seeds at pre-veraison. They have a role in the taste of wines (e.g., astringency, bitterness, mouthfeel, and body) and they contribute to colour stability by forming several adducts with anthocyanins (Oliveira *et al*., 2017). Previous studies have reported little or no modulation of flavan-3-ols and condensed tannins resulting from a water deficit (Castellarin *et al*., 2007; Hochberg *et al*., 2015; Savoi *et al*., 2016, 2017).

VOCs are commonly present in plants in a free and volatile form - contributing directly to odour - or in glycosidically-bound, non-volatile and odourless compounds (Hjelmeland and Ebeler, 2014). A higher concentration of the latter form is present in grape berries when conjugated to a sugar. In grape, these sugar moieties often comprise a disaccharide with a preponderance of α-L-arabinofuranosyl-β-D-glucopyranosides, α-L-rhamnopyranosyl-β-D-glucopyranosides, β-D-xylopyranosyl-β-D-glucopyranosides, and β-D-apiofuranosyl-β-D-glucopyranosides (Robinson *et al*., 2014). The glycosylation process occurs through the activity of specific glycosyltransferases (*VviGTs*). In the grapevine genome (Jaillon *et al*., 2007), more than one hundred putative *VviGTs* have been identified, but up to now only three of them (*VviGT7*, *VviGT14*, and *VviGT15*) have been functionally characterised as having a specificity for terpenes (Bönisch *et al*., 2014a, 2014b; Li *et al*., 2017). The hydrolysis of these glycoconjugates by acids or enzymes can release the odour-active aglycones. Glycosidically-bound VOCs can thus release free VOCs by means of either slow chemical acid hydrolysis, endogenous grape-derived glycosidases, or the action of exogenous yeast-derived or bacteria-derived glycosidases. The main cultivar-related VOCs are terpenes and C_{13}-norisoprenoids, which mainly accumulate in the grape berry skin during the ripening process. Terpenes largely contribute to the aroma of white wines in developing floral scents. Among the forty main monoterpenes found in grape, the most odoriferous are linalool and its oxides, α-terpineol, nerol, geraniol, citronellol, and hotrienol. While the detection threshold of some of these is quite low (a few µg/L), together they can show synergistic activity. C_{13}-norisoprenoids are the products of the oxidative degradation of carotenoids. They have very low olfactory perception thresholds and, therefore, a high sensorial impact on wine aroma (Mendes-Pinto, 2009). The main compounds found in grapes are β-damascenone (with fruity-floral aromas of apple-plum-raisin), β-ionone (with a violet scent), 3-oxo-α-ionol (with a tobacco flavour), 3-hydroxy-β-damascone (with a tea and tobacco scent), β-damascone (with a tobacco and fruity scent), TDN (1,1,6-trimethyl-1,2-dihydronaphthalene; with a petroleum scent), and vitispirane (with camphor/woody nuances (Winterhalter and Schreier, 1994). However, these compounds are found as non-volatile precursors; i.e., they are glycosidically-bound and sometimes have multiple precursors (Lin *et al*., 2019). The release and formation of their free form during wine ageing is often slow and depends on several factors (e.g., duration and temperature of storage) and the pH value. It has been reported that water deficit enhances the biosynthesis and accumulation of C_{13}-norisoprenoid compounds in grape (Deluc *et al*., 2009; Song *et al*., 2012).

In previous studies (Savoi *et al*., 2016, 2017), we evaluated the impact of water deficit on grape berry ripening and composition for two different wine grape varieties widely cultivated in north-eastern Italy (Tocai Friulano and Merlot) using a transcriptomics and metabolomics approach. In the present study, we aimed to determine if and to what extent the altered composition of grapes resulting from water deficit is transferred to wine, focussing on secondary metabolites, such as anthocyanins and terpenes, that largely contribute to the visual, olfactory and gustative components of flavour.

MATERIALS AND METHODS

1. Experimental design

The field experiment was conducted on Merlot and Tocai Friulano vines in the experimental vineyard of the University of Udine (Italy) in 2011 and 2012. Detailed vineyard descriptions are available in Herrera *et al*. (2015) and Savoi *et al*. (2016, 2017). Merlot vines were covered with an open-sided plastic film of ethylene-vinyl-acetate (EVA, Patilux, Italy) at the beginning of the season until harvest, while Tocai Friulano vines grew uncovered in the open field. Two different irrigation treatments, fully irrigated vines as the control (C) and deficit irrigated vines (D), were established at approximately 25 days after anthesis (DAA) and applied until harvest, as explained in Savoi *et al*. (2016, 2017). Each irrigation treatment was replicated on four plots (each consisting of 10 vines) arranged in a completely randomised design. Plant water status was monitored weekly by measuring Ψ_{Stem} using a Scholander pressure chamber. The accumulated water deficit integral (S_{Ψ}) was calculated for each variety and year separately from the weekly stem water potential (Ψ_{Stem}) measurements as reported by Fernández *et al*. (2009):

$$S_{\Psi} = \left| \sum_{i=0}^{i=t} (\Psi_{i,i+1} - c)n \right|$$

where $\Psi_{i,i+1}$ is the Ψ_{Stem} of each interval i, i+1; *c* is the Ψ_{Stem} of reference measured at the beginning of the seasons (T0); and *n* is the interval in number of days between the two measurements.

The grapes were harvested at 115 and 106 DAA for Merlot and at 105 and 93 DAA for Tocai Friulano in 2011 and 2012 respectively.

2. Microvinification and wine analysis

Wines were produced for each of the two growing seasons with a standard microvinification protocol developed by the Viticulture and Oenology Research Group at the University of Udine (Italy), as described in Herrera *et al*. (2015), with slight differences between red and white vinification.

For both Merlot and Tocai Friulano, an average of 18 kg grapes from each treatment replicate were harvested manually and transported to the experimental winery of the University of Udine. Grapes from the single replicate/plot were kept separately, except for Tocai Friulano in 2012 when the yield per plot was low, and the grapes of the fourth plot were distributed among the others within the same treatment to reach the desired mass for fermentation.

Overall, for Merlot and Tocai Friulano in the two seasons, thirty independent fermentations were carried out. Each lot was mechanically destemmed and crushed. For Tocai Friulano, the skins and seeds were separated using a manual press. The Merlot juice with skins and seeds and the Tocai Friulano clear juice were each transferred to a separate 20-L glass fermentation container. 35 mg/kg of sulfur dioxide (SO_2) were added to Merlot and 50 mg/L of SO_2 to Tocai Friulano, and then both were inoculated with 0.2 g/L of a commercial yeast strain (Lalvin EC-1118, Lallemand Inc.). Musts were fermented at 18 °C for 10 days. A punch-down was performed twice a day for Merlot. After alcoholic fermentation, Merlot wines were pressed, and 25 mg/L of SO_2 was added, while Tocai Friulano wines were racked and 50 mg/L of SO_2 was added. Both Merlot and Tocai Friulano wines were racked twice, at 10 and 30 days after the end of fermentation and then bottled in 0.5 L bottles closed with synthetic stoppers. Malolactic fermentation was not carried out. Alcohol content, titratable acidity, pH and dry extract were determined, as described in Herrera *et al.* (2015), for both Merlot and Tocai Friulano wines. Wine colour intensity (OD420 nm + OD520 nm), colour hue (OD420nm / OD520 nm), and the concentrations of anthocyanins, tannins and small and large polymeric pigments (SPP and LPP) were determined for Merlot wines by spectrophotometric analyses, as described in Herrera *et al.* (2015). The wines were stored in the dark at 4 °C. All the analyses were done when the wines were aged 9-10 months.

3. Extraction and analysis of the wines' free and glycosidically-bound volatiles

Sample preparation and extraction were performed according to Boido *et al.* (2003) with some modifications, as reported in Vrhovsek *et al.* (2014). Briefly, solid-phase extraction was performed using Isolute ENV+ cartridges, 1 g (Biotage, Sweden). The cartridge was pre-conditioned with 15 mL methanol, followed by 20 mL of Milli-Q water. Fifty mL of wine was diluted with 50 mL of Milli-Q water and 0.1 mL of internal standard n-heptanol (250 mg/L) was added. This was then loaded onto the cartridge and washed with 15 mL of Milli-Q water to remove possible impurities. The free volatile compounds were eluted from the cartridge with 30 mL of dichloromethane; the solution was added with 60 mL of pentane, dried with sodium sulfate anhydrous (Na_2SO_4) and concentrated to 1 mL on a Vigreux column. The glycosidically-bound volatile precursors were eluted with 30 mL of methanol and evaporated until dry by using a rotary vacuum evaporator. The flasks were washed with a solution of pentane-dichloromethane (2:1 v/v) to remove any residual traces of free VOCs. The bound fraction was then re-dissolved in 4 mL of citrate buffer at pH 5; 200 µL of enzyme AR2000 (70 mg/mL) (DSM Food Specialties B.V., Netherlands) (Ghaste *et al.*, 2015) was added and tubes were kept in a 40 °C bath at dark for 24 h. After the addition of 10 µL of internal standard (n-heptanol), the compounds were extracted three times with 2 mL of pentane/dichloromethane (2:1 v/v). The organic phase containing the free released volatiles was collected, dried with Na_2SO_4 anhydrous, and then concentrated to 0.5 mL on the Vigreux column. GC analysis was performed using Trace GC Ultra gas chromatography coupled with a TSQ Quantum Tandem mass spectrometer (Thermo Scientific, Italy) equipped with a CTC Combi-PAL autosampler (Zwingen, Switzerland). GC separation was performed on a 30 m VF-WAXms capillary column (J&W Scientific, Inc. CA) injecting a volume of 1 µL. Run conditions are reported in Vrhovsek *et al.* (2014). Data acquisition and analyses were performed using the XCalibur software (Thermo Scientific, Italy). Compounds were identified by comparing the retention times of individual peaks with the retention times of their reference standards when available or by identifying the mass spectra using the NIST library, in combination with the retention index. The response of the internal standard n-heptanol was used for area normalisation. Compounds were then expressed as µg/L of n-heptanol equivalents.

4. Sensory analysis

The wines were subjected to a descriptive sensory test in duplicate, as described in Herrera *et al.* (2015). Briefly, the panel was composed of 10 to 12 trained tasters. In the first session, a subset of experimental wines was tasted and described according to a series of attributes, which were scored on a scale of one-to-ten on scorecards.

The Merlot scorecard comprised the following attributes: visual (colour intensity and hue); aroma (intensity, fruitiness, spiciness, and jam and vegetal aroma); taste (body, sapidity, acidity, astringency and bitterness); retronasal (intensity, persistence, fruitiness and vegetal flavour).

The Tocai Friulano scorecard comprised the following attributes: visual (colour intensity and hue); aroma (intensity, fruitiness, and floral and vegetal aroma); taste (body, sapidity, acidity, and bitterness); retronasal (intensity, persistence, fruitiness and vegetal flavour).

These scorecards were used in the second session. Wine samples of 50 mL were randomly served to each member of the panel. The participants were asked to taste the wines, scoring each attribute on a 1 (low) to 10 (high) intensity scale, except for the attribute 'colour hue', for which 1 represented red-orange hues and 10 red-violet hues for Merlot, and 1 represented yellow hues and 10 green hues for Tocai Friulano.

5. Statistical analyses

A one-way ANOVA test was performed using JMP 7 (SAS Institute Inc.) to detect significant differences ($P<0.05$) in wine composition between C and D treatments. For the sensory analysis, we applied a mixed model ANOVA, keeping the two years separated and using the treatment (C and D) as a fixed factor and the judges as a random factor (Herrera *et al*., 2015).

RESULTS AND DISCUSSION

1. Vine water status and wine composition

Two water treatments were applied to the vines during two consecutive seasons which mainly differed in rainfall distribution (Figure S1). It is important to highlight that the Merlot vines were sheltered from rainfall under an open-sided transparent cover during the experimental seasons, while uncovered Tocai Friulano vines were exposed to natural precipitation. Seasonal differences are therefore reflected in the accumulated water deficit integral (S_Ψ), calculated for both cultivars and years from the weekly stem water potential (Ψ_{Stem}) measurements performed during the experiments (Figure 1). Thus, for Merlot, water stress intensity and length are comparable between seasons as they underwent a similar level of water deficit, which occurred early (at about 50-60 DAA) and lasted until harvest with increasing differences between treatments (Figure 1). In contrast, seasonal differences in water availability were evident for Tocai Friulano, with a very late deficit (starting only at 90 DAA) recorded in 2011, and an early deficit (at about 40 DAA) in 2012 which lasted until harvest, when temperatures fell and humidity rose following a few days of rainfall (Figure S1).

As highlighted in the following sections, the response of grape berry secondary metabolism to water deficit changed between the two seasons, possibly in relation to the grape berry phenological stage when the drought was established and the length of the drought. The composition at harvest of the grape berries in this experiment has previously been published by Savoi *et al*. (2017) for Merlot 2011 and 2012, and by Savoi *et al*. (2016) for

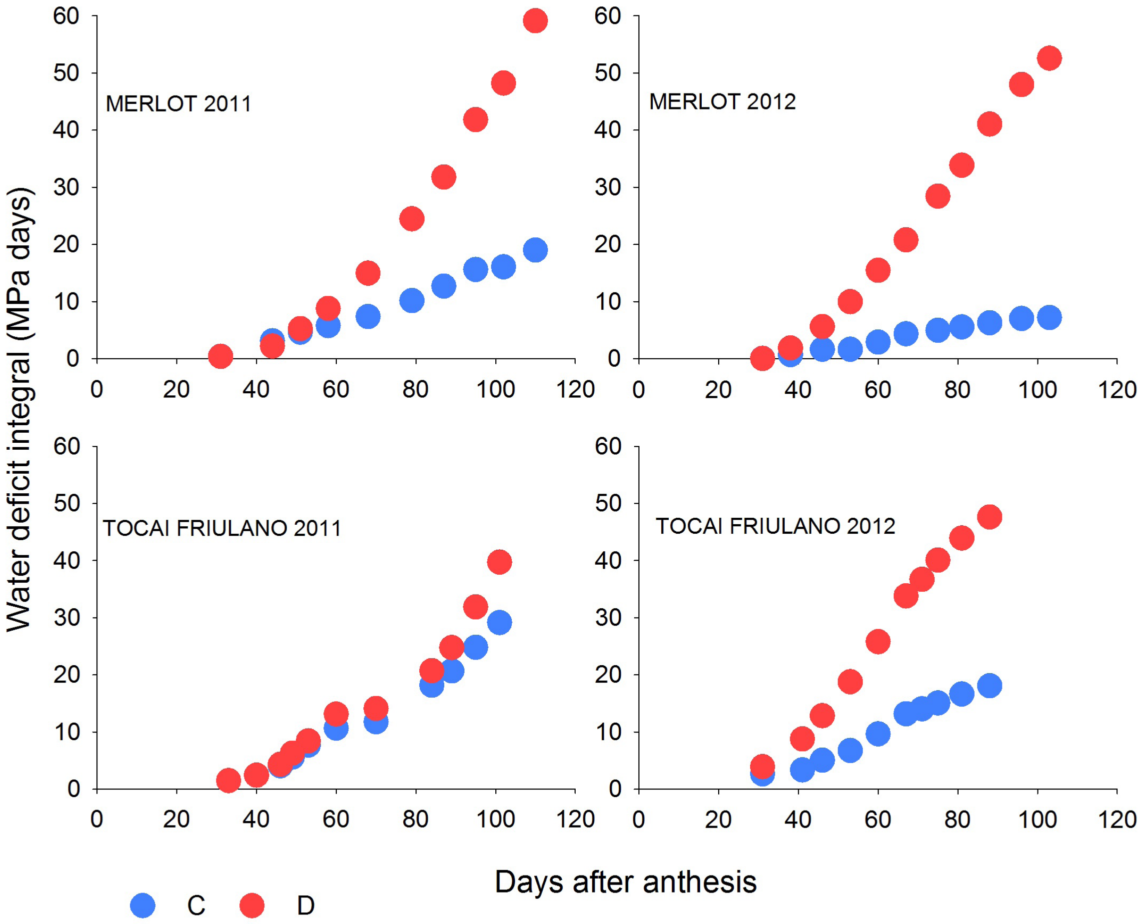


FIGURE 1. Seasonal water stress integral (MPa·days) in Merlot and Tocai Friulano in 2011 and 2012 for fully irrigated control (C) and deficit irrigated (D) vine plots.

TABLE 1. Effect of water deficit on Merlot and Tocai Friulano wine composition from fully irrigated (C) and deficit irrigated (D) grape berry in 2011 and 2012.

	Merlot				Tocai Friulano			
Year	2011		2012		2011		2012	
Treatment	C	D	C	D	C	D	C	D
Alcohol (%)	12.8 ± 0.15	13.1 ± 0.46	13.4 ± 0.02	13.8 ± 0.21	13.0 ± 0.46	12.2 ± 0.05	13.4 ± 0.08	14.4 ± 0.29
	ns		ns		ns		*	
Titratable Acidity (g/L)	5.7 ± 0.11	6.1 ± 0.15	6.8 ± 0.10	6.7 ± 0.05	5.3 ± 0.28	4.7 ± 0.04	5.3 ± 0.28	4.7 ± 0.03
	ns		ns		ns		ns	
pH	3.5 ± 0.02	3.4 ± 0.03	3.6 ± 0.02	3.5 ± 0.01	3.5 ± 0.02	3.6 ± 0.02	3.7 ± 0.06	3.7 ± 0.04
	**		**		ns		ns	
Dry extract (g/L)	27.1 ± 0.53	28.9 ± 0.73	32.6 ± 0.71	36.0 ± 1.01	20.1 ± 0.19	20.0 ± 0.30	23.1 ± 0.38	26.6 ± 1.19
	ns		*		ns		*	

Values are averaged ± SE (n = 4, except in Tocai 2012 where n = 3).Differences between treatments were assessed with a one-way ANOVA. The level of significance is reported within the columns: *, **, *** or ns, P < 0.05, P < 0.01, P < 0.001 or not significant, respectively.

Tocai Friulano 2012, while that of Tocai Friulano 2011 is presented as Supplementary Material here (Figure S2).

The wines of both cultivars in the two seasons showed no differences in alcohol content, except for Tocai Friulano 2012 with slightly higher alcohol content in D wines. Water deficit decreased wine pH in both seasons in Merlot, while no significant differences were found for Tocai Friulano. No effects due to water deficit were recorded in terms of titratable acidity. For both cultivars, the dry extract was only higher in D wines in 2012 (Table 1).

2. Impact of water deficit on wine phenolics in Merlot

In Merlot, the results of the metabolite analyses described in Savoi *et al.* (2017) showed that the water deficit strongly increased the concentration of most anthocyanins in the entire grape berry in both seasons; specifically, an increase in delphinidin, petunidin, and malvidin was observed (+22, +22, +48 % and +36, +36, +107 % in 2011 and 2012 respectively). Meanwhile, with water deficit, flavan-3ols showed alternative behaviour: some compounds increased (e.g., gallocatechin), others decreased (e.g., catechin, epicatechin gallate), and others were not affected at all (e.g., epigallocatechin, epicatechin). However, in a previous study (Herrera *et al.*, 2015), it was reported that water deficit in Merlot increased the tannins in the skins of the grape berry.

In the present study, the derived wines were analysed to determine total amount of anthocyanins and tannins, content of small and large polymeric pigments, and colour and hue. The concentration of anthocyanins in the wines was higher for D than for C by 92 % and 151 % in 2011 and 2012 respectively. In contrast, wine tannins were not significantly affected by water deficit (Figure 2). As shown by the results of Herrera *et al.* (2015), more precipitable tannins in the grape berry did not result in more tannins in the wines, either because they complexed to other molecules, such as the anthocyanins, or because their extractability during maceration was difficult.

However, tannins from grape skins and seeds during winemaking, as well as during ageing, can combine with anthocyanins to form polymeric pigments. According to Harbertson *et al.* (2003), the amount of small polymeric pigments (SPP) is mostly due to grape berry content, and hence refers to the concentration of the anthocyanins and tannins present in the grape skins. We found that SPP increased in D wines by 139 % and 126 % in the 2011 and 2012 seasons respectively, mainly as a result of an overproduction of anthocyanins (Figure 2). Large polymeric pigments (LPP), however, are formed during fermentation and ageing, and have a significant role in stabilising the colour of a finished wine. We found that there was a 61 % and 44 % increase in amount of LPP in the D wines in the two seasons (Figure 2). Lastly, the analysis of colour intensity and hue of red wines reflected this contribution. The spectrum of red wines has a maximum at 520 nm and a minimum at 420 nm (Ribéreau-Gayon *et al.*, 2006). Merlot

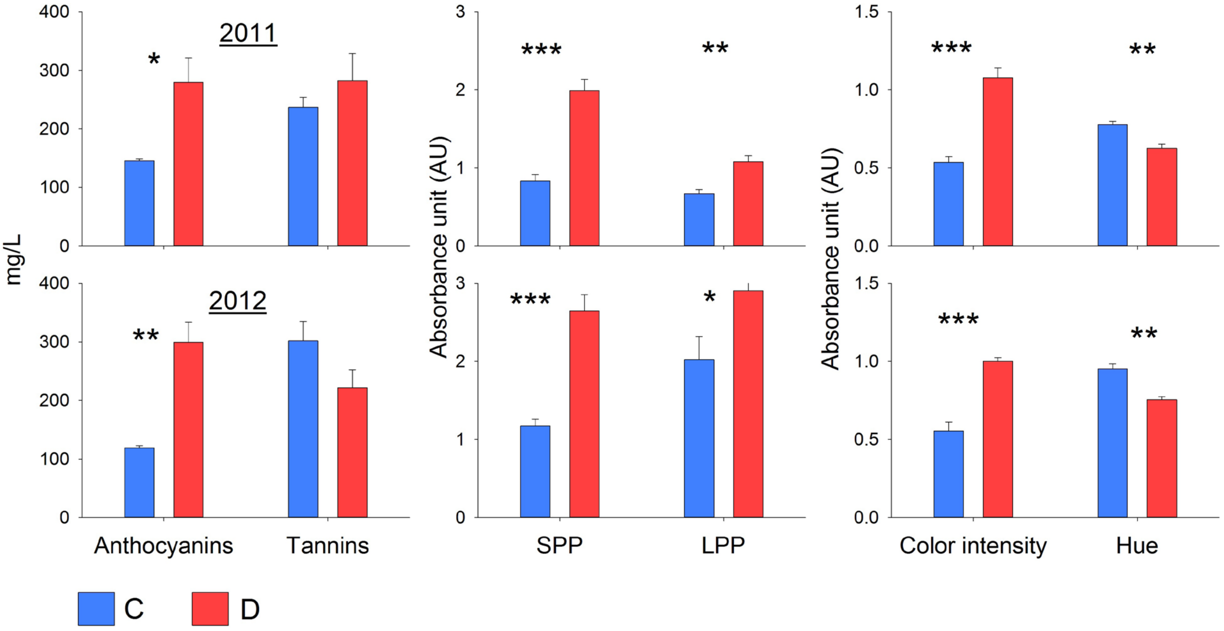


FIGURE 2. Effect of water deficit on Merlot wine anthocyanins, tannins, short polymeric pigments (SPP), large polymeric pigments (LPP), colour intensity and hue in 2011 and 2012.

Bars represent the metabolites concentration averages ± SE (n = 4, except in Tocai 2012 where n = 3) in fully irrigated control (C) and deficit irrigated (D) wines. Asterisks indicate significant differences (*, ** or ***: $P < 0.05$, $P < 0.01$ or $P < 0.001$) between treatments in a given year.

wines had significant higher colour intensity (OD420 + OD520) (+100 % and +79 % in 2011 and 2012) and lower hue (OD420 / OD520) (-24 % and -27 % in 2011 and 2012) (Figure 2). Bearing in mind the young age of the wines analysed, this means that there was a high accumulation of tri-hydroxylated anthocyanins in the grapes (leading to an enrichment of purple pigments, as reported by Savoi *et al.* (2017), since the concentration of delphinidin, petunidin and malvidin increased in the grape with the water deficit. Similar results related to the effect of water deficit on polyphenol concentration have already been reported for several red grape varieties (Castellarin *et al.*, 2007; Deluc *et al.*, 2009; Castellarin *et al.*, 2007; Herrera *et al.*, 2015; Bucchetti *et al.*, 2011; Koundouras *et al.*, 2009; Ollé *et al.*, 2011; Fernandes *et al.*, 2015).

3. Impact of water deficit on wine VOCs in Tocai Friulano and Merlot

Because the goal of this trial was to identify any secondary compounds affected by a water deficit, and to correlate those changes to the previous data given for grapes (Savoi *et al.*, 2016, 2017), we mainly focussed our attention on grape-derived aroma compounds, such as monoterpenes and C_{13}-norisoprenoids. As a reference, the concentrations of all VOCs identified by the analyses are shown in Table S1 for the wines of both cultivars.

As described above for the white cultivar Tocai Friulano, the water deficit occurred late in the 2011 season and was short in length; it therefore did not affect grape berry metabolism (Figure S2). Consequently, no significant effect of water deficit was found in 2011 for wine volatiles, except for citronellol, which increased in D wines. On the other hand, the results of grape berry metabolite analyses carried out in 2012 (fully described in Savoi *et al.* (2016) highlighted that a water deficit can significantly increase the concentration of VOCs, such as the monoterpenes, linalool, α-terpineol, nerol, and hotrienol in grapes. Accordingly, in 2012, D wines showed higher concentrations of linalool, *cis* and *trans* linalool oxide, α-terpineol and diendiol (I) in the free form, and higher concentrations of linalool, *cis* and *trans* linalool oxide, *cis* and *trans* 8-hydroxylinalool, 7-hydroxygeraniol and geranic acid in the glycosidically-bound form (Figure 3). Among the free monoterpenes with a significantly higher concentration in D wines, we observed that only linalool was above its sensory threshold of 25 µg/L (Supplementary Table S1, Ferreira *et al.*, 2000; Francis, 2013). The sensory threshold of linalool is one of the lowest among monoterpenes, and therefore linalool may contribute to enhancing the floral wine bouquet of Tocai Friulano D wines. We cannot exclude that compounds under their sensory threshold may undergo an additive synergistic interaction which contributes to the overall wine sensory perception, because several monoterpenes can interact with each other and enhance the aroma profile. This was previously observed in a study on the aroma of Muscat, in which the authors showed that a monoterpene mixture can significantly lower the sensory threshold of each singular compound when taken separately (Ribéreau-Gayon *et al*., 1975). Moreover, a recent study on Pinot gris showed that the non-volatile wine composition strongly increased the volatility of the free monoterpenes (Tomasino *et al*., 2020). Finally, diendiol (I) was the most concentrated compound that we observed in the D wine of Tocai Friulano 2012. Notably, diendiol (I) - also known as terpendiol (I) - is odourless, but it is a potential source of hotrienol and nerol oxides (Rocha *et al*., 2007) under favourable circumstances.

The concentration of terpenes in the berries of Merlot, however, was not affected by water deficit (Savoi *et al*., 2017). In the wine (Figure 3) we found that citronellol and *p*-cymene in the free form were significantly lower in D than C wines in 2011, while geranic acid was lower in D than C wines in 2011, but higher in 2012. This shows contrasting behaviour in the two seasons, despite similar intensity and timing of the water stress, indicating that a more complex interaction with other environmental variables is occuring (Herrera *et al*., 2017). It is interesting to note that the concentration of free α-terpineol in both Merlot D wines increased, even though no significance was detected (while in the case of Tocai friulano 2012 D its concentration was significantly higher). This compound has been highlighted as one of the key precursors of the potent balsamic odorant, 1,8-cineole, during the ageing of red wines (Slaghenaufi and Ugliano, 2018). Regarding the glycosidically-bound fraction, the water deficit increased the concentration of *trans* 8-hydroxylinalool, 7-hydroxygeraniol and geranic acid in both seasons, while *cis* 8-hydroxylinalool decreased in D wines in 2011, but it increased in 2012.

To our knowledge, there are no studies that connect the terpenes content of grapes and wines to white and red varieties. In general, a few studies have analysed the terpenes content of white wine, focussing on the Muscat varieties, which accumulate a higher amount of monoterpenes, and a few other white varieties (Bordiga *et al*., 2013; Dziadas and Jeleń, 2010; Girard *et al*., 2002; Palomo *et al*., 2006; Vilanova and Sieiro, 2006).

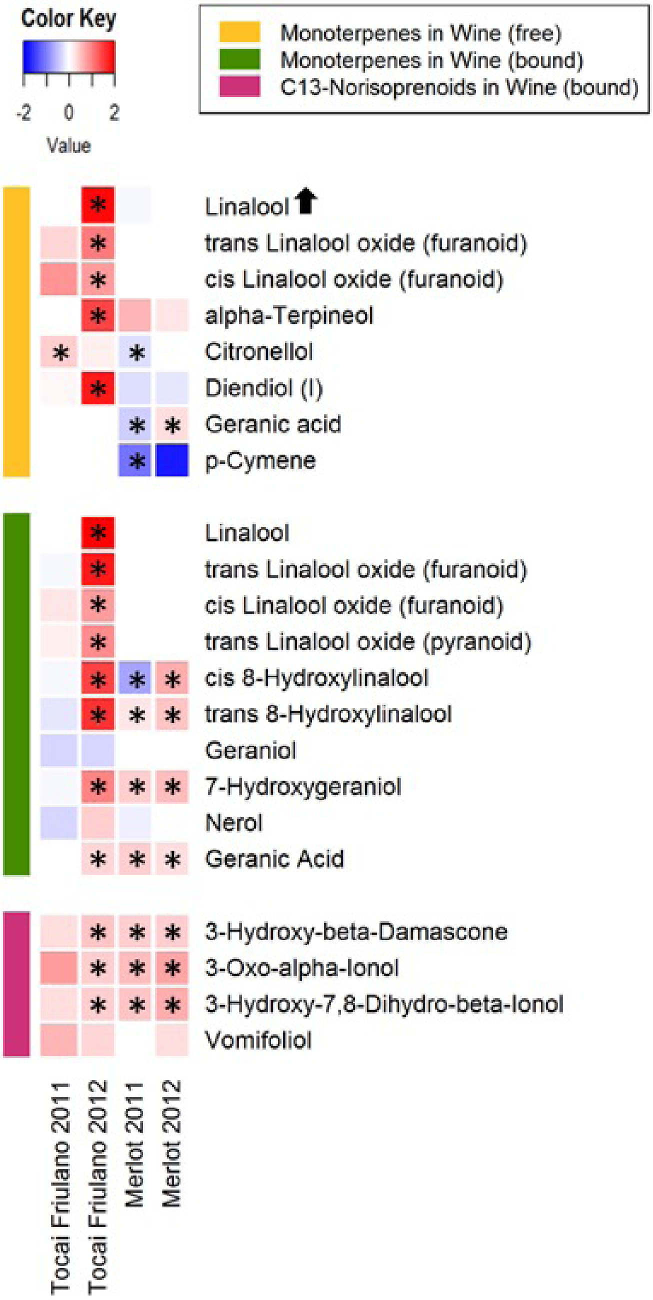


FIGURE 3. Effect of water deficit on free and glycosidically-bound VOCs in Tocai Friulano and Merlot wines in 2011 and 2012.

Heatmaps represent log2 fold change (D/C) of the grape-derived aroma (monoterpenes and C_{13}-norisoprenoids) under water deficit conditions. Blue and red boxes indicate lower and higher concentration in D respectively. Asterisks indicate significant differences (P<0.05) between treatments. The black arrow next to a compound name indicates that the compound concentration was above the sensory threshold.

One study (Ou *et al*., 2010) reported that Merlot wines produced from vines experiencing water deficit contained higher amounts of citronellol, nerol and geraniol compared to the control, but linalool was not affected. In the present study, we found that linalool was unaffected by water deficit in the free fraction, and we did not detect linalool in the glycosidically-bound fraction. Nevertheless, in the glycosidically-bound fraction, we found a higher concentration of *trans* 8-hydroxylinalool in both seasons, but *cis* 8-hydroxylinalool only in 2012 in D wines. In another study on Merlot (Qian *et al*., 2009), no significant differences were found in monoterpene concentration as a result of water deficit. The different results regarding Merlot terpene composition found in literature in comparison to our data could be due to the low concentration in Merlot grapes and wines, and/or the fact that different environmental interaction might modify the terpene profiles of Merlot wines.

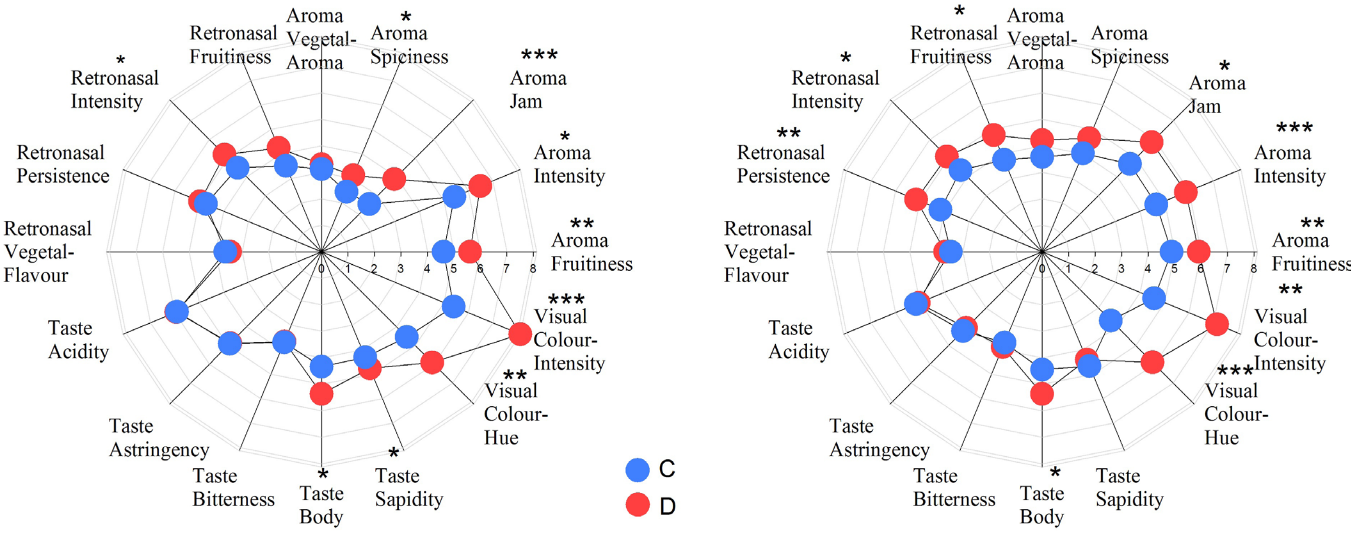
4. Impact of water deficit on wine glycosidically-bound C_{13}-norisoprenoids in Tocai Friulano and Merlot

The Merlot D wines from both seasons and the Tocai Friulano D wines from 2012 had a consistently high concentration of C_{13}-norisoprenoids in the glycosidically-bound form, such as 3-hydroxy-β-damascone, 3-oxo-α-ionol, and 3-hydroxy-7,8-dihydro-β-ionol (Figure 3). The analysis did not detect any C_{13}-norisoprenoids in the free form. C_{13}-norisoprenoids are derived from the degradation of carotenoids through the activity of specific carotenoid cleavage dioxygenases, and they have a strong impact on wine aroma. A higher accumulation of C_{13}-norisoprenoids in grape berries under water deficit conditions has already been reported in the literature (Bindon *et al*., 2007; Koundouras *et al*., 2006), possibly resulting from a water deficit-induced overproduction of carotenoid precursors; this may be due to either an increase in their synthesis in response to higher sunlight bunch exposure (an indirect effect resulting from leaf abscission in response to drought), affecting the xanthophylls cycle (Young *et al*., 2015), or to the fact that the carotenoid pathway is also the route of synthesis of the water stress-related hormone, abscisic acid, whose synthesis increases under water deficit conditions. In our grape analysis (Savoi *et al*., 2016, 2017), the concentration of β-ionone and β-damascenone in both varieties was not affected by the deficit, but we observed a higher concentration of the carotenoid, zeaxanthin. The degradation of zeaxanthin by the carotenoid cleavage dioxygenases genes (*VviCCDs*) in grape (Lin *et al*., 2019) (which has also been reported as up-regulated by water deficit (Deluc *et al*., 2009) leads to the synthesis of C_{13}-norisoprenoids (Mendes-Pinto, 2009).

5. Wine sensorial analysis in Tocai Friulano and Merlot

Merlot and Tocai Friulano are non-aromatic cultivars, hence containing minor levels of monoterpenes and C_{13}-norisoprenoids compared to other aromatic cultivars, such as Muscat or Gewurztraminer. The results of the sensory evaluation carried out by trained tasters on the 2011 and 2012 Tocai Friulano wines did not show a statistically higher aroma for D white wines when compared to the controls (Figure 4), and some trends related to increased aroma intensity and fruity scents were masked by panellist variability. However, a higher concentration of the aromatic compounds analysed here were found as a glycosidically-bound fraction, and therefore in an odour-inactive form, confirming results obtained for the berries of Tocai Friulano (Savoi *et al*., 2016) and other white cultivars (Peyrot des Gachons *et al*., 2005). When released, this reservoir of compounds can potentially improve the aromatic properties of wines. Only colour intensity and body were increased in 2012 D wines. The increase in colour intensity is probably related to the trend of increased flavonol concentration observed in grape berries under water deficit conditions (Savoi *et al*, 2016), as well as to the increased concentration of zeaxanthin in the grape berries (Sollazzo *et al*., 2018). Furthermore, a wine described as being more intense in 'body' agrees with the increased dry extract in D wines (Table 1).

The distribution of free and glycosidically-bound monoterpenes among skin, juice and pulp fractions in white grapes has been studied in the past (Wilson *et al*., 1986); it was found that the majority of these aromatic compounds had accumulated in the skin, rather than in the pulp (flesh). Moreover, other studies on fleshy fruits highlighted that a preponderance of aromatic compounds accumulate in the skin (Aubert and Milhet, 2007; Lalel *et al*., 2003; Radford *et al*., 1974). This can explain the low olfactive scores received in the Tocai Friulano sensory test, because (i) the free aromatic compounds of the skins were probably lost during the separation of solids before the must fermentation, and (ii) the majority of aromatic compounds we measured were in the odourless form (glycosidically-bound). Therefore, in order to thoroughly benefit from the aromatic properties of white grape grown under water deficit conditions, it would be useful to extend skin contact time or perform limited skin maceration during grape berry crushing, and/



TOCAI FRIULANO 2011

TOCAI FRIULANO 2012

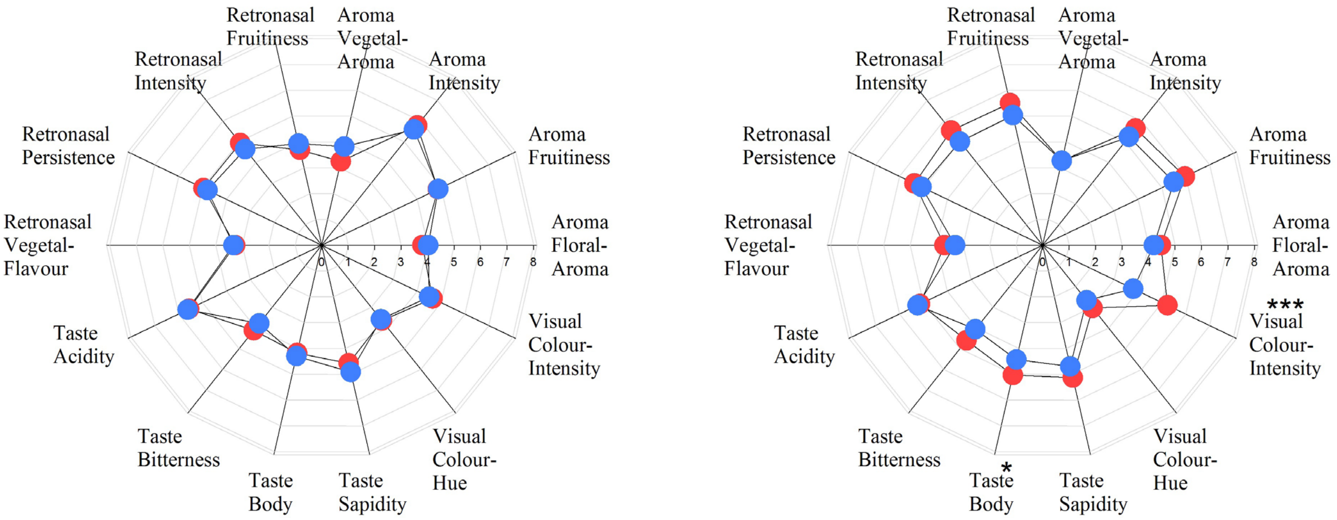


FIGURE 4. Descriptive sensory test results of Merlot and Tocai Friulano fully irrigated control (C) and deficit irrigated (D) wines in 2011 and 2012.

Data are represented in a radar chart. Blue dots represent the average observations among C wine while red dots the average observations among D wines. Asterisks placed near each attribute indicate significant differences (*, ** or ***: $P < 0.05$, $P < 0.01$ or $P < 0.001$) between treatments.

or to enzymatically hydrolyse the vast reservoir of glycosidically-bound monoterpenes by using ad-hoc strain yeast (van Wyk *et al*., 2019). However, up to now, the results that have been reported are debatable (reviewed in Hjelmeland and Ebeler, (2014); for example, the use of enzymatic or acid hydrolysis may lead to unwanted release of compounds (off-aromas), or to a reduction in the efficacy of glucosidase since it has been shown that their activity is limited by increasing ethanol and glucose concentration.

In Merlot wines, the differences in metabolite concentration had a big effect on the sensory wine attributes in 2011 and 2012 (Figure 4); for instance, D wines received higher scores for visual attributes, such as colour intensity (in agreement with the higher anthocyanins concentration measured in D grape berries and wines) and colour hue, namely a shift towards a purple-blue wine hue (in agreement with the increased tri-hydroxylated anthocyanins concentration observed in D grape berries and wines). In addition, Merlot D wines obtained higher scores for aroma attributes (such as intensity, fruitiness, and jam aroma) and retronasal attributes (such as intensity). Among the taste attributes, body was significantly higher in both seasons. Moreover, retronasal fruitiness and persistence were higher in D wines in 2012, and in terms of aroma, spiciness and sapidity were higher in 2011. This indicates that skin maceration favoured the transition of the attributes observed in the grape berries to the wines, as some glycosidically-bound VOCs were probably freed and released from the skins. In particular, it has been reported that the presence of C_{13}-norisoprenoids (compounds that we observed to be higher in D wines; Figure 3) leads to more fruity nuances and intense aroma in the wines (Escudero *et al*., 2007; San-Juan *et al*., 2011). Similar results for wine produced with deficit irrigated grapes have been reported in the literature (Koundouras *et al*., 2006; Casassa *et al*., 2015; Cáceres-Mella *et al*., 2018): the grapes and wines undergoing deficit irrigation showed a higher amount of anthocyanins with significant differences in sensory properties.

CONCLUSION

Taken together, our results indicate that fruit responds both physiologically and metabolically to the timing, duration and severity of water deficit during grape berry ripening (Chaves *et al*., 2010; Ripoll *et al*., 2014), as well as to the interaction of water deficit and other climatic conditions (e.g., light and temperature) (Herrera *et al*., 2017). We found that only an early and long-lasting moderate to severe water deficit positively impacted grape and wine composition for Merlot in 2011 and 2012 and for Tocai Friulano in 2012. Our results suggest that a controlled long-lasting deficit-irrigation can potentially positively impact the quality of red and white wines by increasing the accumulation of colour and aromatic compounds in the grapes, and hence in the wine, contributing to its much appreciated visual, olfactory and gustative properties. Numerous studies have been carried out on the effects of water deficit on grapes, but only a few have also focussed on the wines produced from them. While the present work has contributed to filling that gap, more research is necessary to fully understand the mechanisms of VOC extraction and release from its glycosidically-bound form during winemaking, and the end contribution of VOCs to wine flavour.

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