# Effect of water deficit and severe shoot trimming on the composition of *Vitis vinifera* L. Merlot grapes and wines

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## Abstract

**Background and Aims:** In recent years, increasing summer temperature, coupled with reduced and erratic rainfall during the growing season, has induced accelerated fruit ripening in several regions, resulting in an undesirable increase in wine alcohol concentration. This study was designed to evaluate the impact of canopy and water management on grape sugar and flavonoid accumulation, with the goal of reducing wine alcohol concentration while conserving or enhancing the concentration of phenolic substances.

**Methods and Results:** In 2011 and 2012, two irrigation treatments (I – irrigated and DI – deficit irrigated) and two canopy heights (HC – high canopy and SC – short canopy) were applied in a Merlot vineyard. No interactions between treatments were observed, and thus independent results were obtained; DI berries had significantly higher sugar concentration (+5%) than that of I in both years and higher wine alcohol concentration only in 2012. Short canopy berries had lower sugar concentration (−4%) and lower wine alcohol (−8%) (only in 2011) than that of HC. Anthocyanins and tannins in berry and wine were increased by water deficit and not affected by severe trimming.

**Conclusions:** Deficit irrigation did not reduce berry sugar concentration and wine alcohol concentration but did enhance desirable wine attributes. Berry sugar concentration and alcohol concentration in wine were reduced by SC in one of the two seasons. Water deficit and severe trimming showed independent effects on berry composition.

**Significance of the Study:** Severe canopy reduction at early stages of ripening can reduce sugars without affecting the accumulation of anthocyanins in Merlot. Conversely, DI applied before veraison, despite promoting anthocyanins accumulation, may also increase berry sugar concentration at harvest.

***Keywords:*** *deficit irrigation, hedging, leaf area/crop mass ratio, reserve storage, water stress*

## Introduction

In recent years, it has been observed that early and accelerated berry ripening is occurring in several viticultural regions of the world, a phenomenon linked to global warming (Petrie and Sadras 2008, Keller 2010). The rise in temperature and changes in seasonal precipitation are beginning to alter wine styles (Mira de Orduña 2010, Schultz and Stoll 2010). We are likely to see increased fruit sugar concentration at harvest resulting in wines of higher alcohol with lower acidity and reduced varietal aroma compounds (Keller 2010, Mira de Orduña 2010). Higher alcohol concentration appears to be particularly problematic given the trend of consumer preference for lower alcohol products, driven by increased awareness of alcohol-related health issues. All of these challenges concern viticulturists and winemakers, especially in historical wine regions where branded wine styles enjoy broad recognition in the international marketplace and contribute substantially to local, regional and state economies. To meet the new climatic conditions, the wine industry needs to adopt and integrate alternative viticultural and winemaking strategies (Clingeleffer 2010).

In response to this trend, modulation of sugar concentration in wine grapes through the reduction or delay of accumulation during ripening is a pivotal research objective in warm viticultural areas. The maintenance of both grape and wine quality, however, is related to preserving or increasing the concentration of phenolic substances associated with colour, flavour and health benefits, as well as the aromatic chemical make-up (Keller 2010). Desired levels of these characteristics are difficult to achieve at harvest as optimal phenolic concentration is often accompanied by high sugar concentration and low acidity. This issue has been explored by several researchers, focusing on the relationship between sugar and anthocyanins accumulation in red berries during ripening. Anthocyanins accumulation begins just after the onset of sugar accumulation and berry softening, approximately at 9 or 10°Brix, and a close relationship between sugar and anthocyanins accumulation has been hypothesised during ripening (Pirie and Mullins 1977). Sugars play a role as regulators in the synthesis of anthocyanins (Zheng et al. 2009, Dai et al. 2014), and they are also important substrates for anthocyanins formation (González-San José and Diez 1992). Environmental variables, however, influence their relationship. Water deficit increases the anthocyanins/sugar ratio (Keller et al. 2008, Sadras and Moran 2012), while elevated temperature decreases the same ratio (Sadras and Moran 2012).

Several viticultural practices have been proven to reduce berry sugar accumulation, and among these the removal of the

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medial and apical part of the canopy at veraison (Filippetti et al. 2011, Rombolà et al. 2011, Palliotti et al. 2013, Poni et al. 2013) is of great interest, particularly when the treatment can be mechanised. The lower photosynthetic performance of basal leaves when compared with that of medial and apical leaves during the ripening phase (Kriedemann et al. 1970, Poni et al. 1994) and the reduction of the leaf area/crop mass ratio, a key physiological parameter for determining sugar accumulation in the berry during the ripening process (Kliewer and Dokoozlian 2005), are the main factors for the lower sugar accumulation in the berry following medial-apical reduction of the canopy. Concomitantly with a reduction or delay of sugar accumulation, however, a decrease in colour accumulation in the berry was also observed in some cases (Peterson and Smart 1975, Reynolds and Wardle 1989, Martinez de Toda et al. 2013). Sugars are triggers of anthocyanins biosynthesis (Dai et al. 2014), and treatments that affect sugar concentration in the fruit before or at the onset of ripening may delay or limit the activation of anthocyanins biosynthesis at veraison, resulting in a lower concentration of anthocyanins at harvest.

Viticultural practices that promote anthocyanins concentration in grape berries have been extensively reviewed (Downey et al. 2006, He et al. 2010); irrigation management was reported to strongly affect the concentration of anthocyanins and other phenolic substances, as well as many aspects of grapevine physiology, such as grapevine growth and development and berry sugar concentration. Despite the fact that anthocyanins concentration is promoted by water deficit through the reduction of berry size (Matthews and Anderson 1989, Ojeda et al. 2002, Roby et al. 2004, Chaves et al. 2010), anthocyanins biosynthesis is also directly increased by water deficit (Ollé et al. 2011), as confirmed by the expression of key biosynthetic genes (Castellarin et al. 2007a,b, Deluc et al. 2009).

Our research investigated the effect of canopy reduction and irrigation strategies on the accumulation of berry sugar and phenolic substances and their impact on wine composition. We hypothesised that the combination of severe shoot trimming, applied after the inception of accumulation of anthocyanins, and deficit irrigation may induce a lower concentration of sugars and enhance the concentration of anthocyanins in the berries at harvest. This would result in the production of wine with lower alcohol and higher pigmentation, mitigating the effect of new climatic conditions with warmer and drier summers. With this objective, we tested our hypothesis in a 2-year experiment using a 2 × 2 factorial design with two levels of canopy height and two levels of water availability.

## Materials and methods

### *Vineyard site*

The experiment was conducted in 2011 and 2012 at the University of Udine experimental station A. Servadei, located in the Friuli region of north-eastern Italy (46°02′ N, 13°13′ E; 88 m asl). Merlot grapevines (clone R3) grafted on to SO4 rootstock (clone 31 OP) were planted in 1993 in soil (0% slope) with 12% gravel in the first 1 m of depth and a 2-mm-sieved fraction composed of 49.0% sand, 31.5% silt and 19.5% clay (Bucchetti et al. 2011). The field capacity was 29.3%, and the permanent wilting point was 19.3% (Bucchetti et al. 2011). Vines were planted with a spacing of 1.0 m within the row and 2.5 m between rows (a density of 4000 vines/ ha) with rows oriented north–south. Vines were spur-pruned during the winters of 2011 and 2012 to two nodes per spur and six spurs per vine, and trained to vertical shoot positioning with a single cordon at 0.8 m from the ground. Three sets of catch wires were used at 30-cm intervals from the cordon.

Recommended crop protection and mineral nutrition practices were followed, and the program was based on scouting, experience and weather conditions. Climatological data were recorded during the experiment by an automated weather station located 100 m from the experimental site. Average daily temperature was used to calculate growing degree days (GDD, base temperature 10°C), and vine phenological stages were recorded according to Coombe (1995).

An open-sided transparent cover (height = 4.7 m; film material for covering EVA, ethylene-vinyl-acetate) enclosed the experimental block encompassing four rows of vines that are 85 m in length. Differences in temperature and relative humidity with the outside were irrelevant; the EVA film was renewed each year of the experiment, and transparency for photosynthetically active radiation was 90%. The experiment was established only in the two central rows under the tunnel (172 vines), and the first and last three vines of the rows were not included in the trial to avoid the influence of precipitation at the tunnel perimeter. Thus, vines were maintained under a fully controlled water regime starting approximately 25 days after anthesis (DAA). Water was supplied by a subsurface drip irrigation system positioned 15 cm from the row with emitters set at a 2.5 L/(m$^2$ · h) application rate. The distance between emitters within the line was 0.6 m and 2.5 m between lines.

### *Experimental design*

Two water regimes were established at approximately 25 DAA, when the berries had reached the pea-size (E-L 31) phenological stage (Coombe 1995) and applied until harvest: (i) irrigated (I) treatment, in which vines were irrigated weekly 100% of evapotranspiration (ETc) to maintain midday stem water potential ($\Psi_{stem}$) between −0.4 and −0.6 MPa; and (ii) deficit irrigated (DI) treatment, in which irrigation was withheld until vines reached a $\Psi_{stem}$ value of −1.4 MPa. When $\Psi_{stem}$ was lower than −1.4 MPa, irrigation was managed to maintain it between −1.0 and −1.4 MPa. Water was given to DI vines three times in 2011 (17 mm total) and four times in 2012 (20 mm total). Vine water status was estimated weekly using midday measurements of $\Psi_{stem}$. On each side of the row (east and west part of the canopy) and for each experimental plot, two leaves from different vines were covered with aluminium foil-coated plastic bags for 1 h to allow stem and leaf water potential to equilibrate. Leaves were then removed, and $\Psi_{stem}$ was measured using a Scholander pressure chamber (Choné et al. 2001).

At fruitset, the vine canopy height was managed by manual trimming at approximately 120 cm above the cordon, corresponding to approximately the 12th node of the primary shoot. At the end of veraison (>80% of berries had changed colour), two canopy heights were set: (i) high canopy (HC), where vine canopy height was maintained approximately 120 cm above the cordon (standard trimming treatment); and (ii) short canopy (SC), where shoots were manually trimmed to maintain a canopy height of 65 cm above the cordon (severe shoot trimming treatment), corresponding on average to the sixth node of the primary shoot. No further trimming treatments were applied until harvest.

Treatments were applied in a 2 × 2 factorial design generating the following combinations: I-HC (irrigated-high canopy), DI-HC (deficit irrigated-high canopy), I-SC (irrigated-short canopy) and DI-SC (deficit irrigated-short canopy), each of which was replicated four times in randomly assigned plots of 10 vines each.

### *Leaf area measurement and yield related parameters*

The leaf area of primary and secondary shoots was measured at veraison (just before severe shoot trimming) and at harvest. At

each date, the leaves of primary and secondary shoots were independently harvested from one vine per plot. For SC vines, the leaves of the top area of the canopy (removed by the treatment) were kept separated from those of the bottom. Samples were brought to the laboratory in plastic bags. Leaf area measurements were carried out using a leaf area meter (LI-3100, LI-COR, Lincoln, NE, USA), and expressed as total leaf area, primary leaf area and lateral leaf area ($m^2$/vine). After the leaf area measurements, vines were not used for any additional sampling or measurements.

All vines (except those used for leaf area measurements) were harvested by hand the same day. Yield and number of bunches per vine were recorded during harvest. Mean bunch mass was calculated as yield/bunch number per vine.

### *Berry sampling and berry juice analysis*

Berry samples were collected every 12–14 days from approximately 30 DAA until harvest. At each sampling date, two sets of berry samples were collected from each plot. A set of 30 berries was harvested to measure juice total soluble solids (TSS), pH and titratable acidity (TA), and another set of 30 berries for analysis of anthocyanins and tannins. Samples were immediately stored in an insulated cooler and transported to the laboratory within 1 h of being harvested. Berries for juice measurement were weighed and manually pressed at room temperature, and the juice was used to determine TSS (°Brix) using a manual refractometer (ATC-1, Atago, Tokyo, Japan), the pH by a pH meter (HI2211, Hanna Instruments, Woonsocket, RI, USA), and the TA (expressed as g/L tartaric acid equivalents) by titration with NaOH 0.1N until pH 8.2 (Iland et al. 2004). The second set of 30 berries was weighed and immediately stored at −80°C for analysis of the concentration of anthocyanins and tannins.

### *Determination of anthocyanins and tannins*

Berries stored at −80°C were peeled with a scalpel and seeds separated from the flesh while keeping the berry tissues frozen. Skins and seeds were immediately dropped into liquid nitrogen, weighed and ground to a fine powder under liquid nitrogen with a grinder (A11B Ika, Königswinter, Germany). Aliquots of 0.18 g of berry skin powder were added to 1.8 mL of 1% HCl in methanol for extraction of the anthocyanins at room temperature in an ultrasonic cleaner bath for 1 h (Downey et al. 2007). Samples were then centrifuged at 21 130 *g* for 15 min, and the concentration of anthocyanins was measured with a spectrophotometer (Uvikon 922, Kontron Instruments, Bletchley, England) reading the absorbance at 520 nm. Anthocyanins were quantified against a calibration curve constructed with oenin (malvidin 3-glucoside) (Extrasynthese, Genay, France) dissolved in 1% HCl in methanol. Anthocyanins were expressed as mg/berry and mg/g of berry fresh mass (FM). All of the anthocyanins measurements were made in duplicate from each sample, and the two values obtained were averaged.

For the extraction of skin and seed tannins, 0.18 g of berry skins or seeds were added to 1.8 mL of acetone/water solution (70/30) and shaken gently for 24 h. The sample was centrifuged (15 min at 21 130 *g*), then 1 mL of supernatant was removed into a new 2 mL-micro tube, and the acetone was evaporated via 1 h of speed vacuum. The residual aqueous extract was adjusted to 1 mL with deionised water. After this, tannins were measured by the protein precipitation assay (Harbertson et al. 2003). Skin and seed tannins were expressed as mg/berry and mg/g of berry FM. Measurement of tannins was carried out in duplicate from each sample, and the two values obtained were averaged.

### *Non-structural carbohydrate analysis*

To assess the effect of canopy reduction and irrigation regimes on vine reserves, wood samples were collected during dormancy (18 January 2013) from the cane, trunk, permanent cordon and roots. Samples were taken from two vines per plot, and values averaged within the plot. Cane wood was collected from three independent canes per vine using pruning shears. Wood samples from the trunk and permanent cordon were collected using a 5-mm drill bit (Makita LCT303, Makita Corp., Aichi, Japan). Bark was removed before drilling; four holes were applied randomly, two in the trunk and two in the cordon, and sawdust collected was mixed. Root samples were collected near the base of the vine from roots of 4–10 mm in diameter.

Before analysis, wood samples dried in an oven (ED 23, Binder, Tuttlingen, Germany) were ground into particles by the M20 Universal Mill (Ika) and then finely ground into a powder using a Mixer Mill MM 400 (Retsch, Haan, Germany). An aliquot (75 mg) of fine powder per sample was used for carbohydrate analysis. Alcohol soluble sugars were extracted in 80% ethanol and measured with an anthrone-sulfuric acid-based methodology (Yemm and Willlis 1954). Starch was extracted from the sugar-free residue with hydrochloric acid and determined with the same anthrone-sulfuric acid procedure. For both analyses, D-(+)-glucose was used for the calibration curve.

### *Microvinification and wine analysis*

In 2011 and 2012, wines were made with a standard microvinification protocol developed by the viticulture and oenology research group at the University of Udine, Italy. At harvest, 18 kg of grapes from each experimental plot were harvested manually and transported to the experimental winery of the University of Udine. Sixteen fermentations were carried out. Each lot was mechanically de-stemmed and crushed (Delta, Toscana Enologica Mori, Florence, Italy), transferred to a 20-L glass fermentation container, 35 mg/kg of sulfur dioxide ($SO_2$) added and inoculated with 0.2 g/L of a commercial yeast strain (Lalvin EC-1118, Lallemand Inc., Montréal, QC, Canada). Grapes and juice were fermented at 18°C for 10 days on the skins and punched down twice daily. After alcoholic fermentation, the wines were pressed and 25 mg/L of $SO_2$ added. 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. Bottles were stored at 10°C for 4 months, at which time chemical and sensory analyses were undertaken. Alcohol content, TA, pH and dry extract were determined according to Iland et al. (2004). Wine colour intensity ($OD_{420nm} + OD_{520nm}$), colour hue ($OD_{420nm}/OD_{520nm}$) (Ribéreau-Gayon et al. 2004), and the concentration of anthocyanins, tannins and small and large polymeric pigments (SPP and LPP) (Harbertson et al. 2003) were determined by spectrophotometric determination (Uvikon 922, Kontron Instruments).

### *Sensory analysis*

The wines were subjected to a descriptive sensory test in duplicate, as described by Lawless and Heymann (2010). The panel was composed of 12 subjects, recruited from among viticulture and oenology experts, and took part in two sessions. The first was to define the scorecard. Subjects tasted a subset of the experimental wines, describing them by a series of attributes and scoring each attribute on a 1–10 scale, according to the intensity perceived. After an open discussion, to harmonise the significance and the intensity of each attribute, the following were used for structuring the scorecard: colour intensity and

hue (visual attributes), aroma intensity, red fruit, jam, herbaceous and spicy (aroma attributes), acidity, bitterness, astringency, mineral, body (taste attributes), retronasal intensity, persistence, fruity and herbaceous (retronasal attributes). This scorecard was used in the second (evaluation) session. Wine samples were labelled by three-digit numeric codes, and 50 mL were served to each member of the panel, according to a balanced randomised service order. Subjects were asked to taste the wines, scoring each attribute on a 1 (low) to 10 (high) intensity scale, with the exception of the attribute 'colour hue', for which 1 represented red-violet and 10 red-brown hue.

### *Statistical analysis*

Basic statistics, ANOVA, regression and correlation analyses were undertaken with R software (R Foundation for Statistical Computing, Vienna, Austria). Data were analysed separately by year using two-way ANOVA appropriate for a fully randomised design for determination of the effects of the two factors (irrigation and shoot trimming). Results were tested for normality and homogeneity of variance prior to being subjected to F-test ($P < 0.05$). When no interaction effect was found between the two factors, values are presented as means over the treatments. Seasonal variations of berry mass, TSS and anthocyanins are shown as means over the treatments ± standard error.

For sensory analysis, each attribute rated by the judges was subjected to ANOVA to ascertain the effect of treatments and interactions using a mixed-model ANOVA and treating the judges as a random factor (Næs and Langsrud 1998).

## Results

### *Vine water status, leaf area and productive parameters*

In both seasons, irrigation significantly affected the midday stem water potential ($\Psi_{stem}$) of vines (Table 1). In general, $\Psi_{stem}$ decreased progressively in DI vines after irrigation was withheld. In 2012, the severe deficit occurred earlier and lasted longer (Table 1). At pre-veraison stages (30–60 DAA), DI $\Psi_{stem}$ was on average at −0.40 and −0.76 MPa in 2011 and 2012, respectively. At veraison, 69 DAA in 2011 and 60 DAA in 2012, $\Psi_{stem}$ of DI vines was −0.86 MPa and −1.27 MPa, respectively. Afterward, $\Psi_{stem}$ decreased further during fruit ripening in both seasons. Shoot trimming treatments did not have a significant impact on $\Psi_{stem}$.

Water management and shoot trimming treatments significantly modified vine leaf area in both years (Table 2). The impact of treatments was consistent across seasons. Water deficit reduced total leaf area (2-year mean) by 35 and 43% at veraison and harvest, respectively. Severe shoot trimming reduced total leaf area (2-year mean) by 47 and 45% at veraison and harvest, respectively. Differences in canopy size between irrigation treatments were already established before veraison and were similar across the two seasons. Total leaf area, however, did not change from veraison to harvest except in DI-SC, which decreased significantly during fruit ripening due to the abscission of senescent basal leaves of primary shoots observed at harvest.

Budburst was observed on 10 April in both seasons, with anthesis (50% cap fall) occurring on 22 May 2011 and 3 June 2012, with the earlier anthesis in 2011 related to the warmer weather in April and May (Figure S1). Veraison (50% of red berries) was recorded on 30 July 2011 (69 DAA) and on 2 August 2012 (60 DAA). Grapes were harvested on 14 September 2011 (115 DAA) and on 18 September 2012 (107 DAA).

Water deficit reduced the yield per vine by 30% in both seasons as a result of the reduction in berry mass (Table 3). Leaf area/crop mass ratio was significantly reduced by DI in 2011, but not in 2012. Severe shoot trimming did not affect yield in either year, but it significantly reduced the leaf area/crop mass ratio from 1.49 to 1.04 $m^2$/kg in 2011 and from 2.94 to 1.67 $m^2$/kg in 2012. In general, the 2012 vintage led to a lower yield (30–40% less production) than that in 2011 for all treatments. Consequently, although leaf area was similar within treatments in both years, leaf area/crop mass ratio was higher in 2012 than in 2011 for all treatments (Table 3).

### *Berry composition*

Water deficit significantly increased TSS in both seasons at harvest (Figure 1, Table 4). Severe shoot trimming significantly reduced TSS at harvest only in 2011 when TSS was recorded as 22.8 and 21.9°Brix for HC and SC, respectively. In 2012, however, TSS was reduced by SC in pre-harvest stages at 81 and 95 DAA (Figure 1). In both years, neither TA nor pH was significantly influenced by treatments during the season (data not shown) or at harvest (Table 4).

The concentration of anthocyanins (mg/g of berry FM) increased faster in vines under water deficit in both years, and became significantly higher in DI than in I from 87 DAA and 67

**Table 1.** Effect of water management and shoot trimming on stem water potential of Merlot grapevines in 2011 and 2012.

| | $\Psi_{stem}$ (MPa)† | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 25–45 DAA | | 45–60 DAA | | 60–75 DAA | | 75–90 DAA | | 90–110 DAA | |
| | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 |
| **Water management (WM)** | ns | * | ** | *** | *** | *** | *** | *** | *** | *** |
| Irrigated (I) | −0.35 | −0.49 | −0.47 | −0.49 | −0.46 | −0.54 | −0.52 | −0.46 | −0.47 | −0.45 |
| Deficit (DI) | −0.31 | −0.58 | −0.71 | −1.11 | −0.98 | −1.31 | −1.14 | −1.32 | −1.33 | −1.21 |
| **Shoot trimming (ST)** | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| High canopy (HC) | −0.33 | −0.54 | −0.59 | −0.80 | −0.72 | −0.93 | −0.83 | −0.96 | −0.95 | −0.90 |
| Short canopy (SC) | −0.33 | −0.54 | −0.59 | −0.80 | −0.72 | −0.91 | −0.83 | −0.82 | −0.84 | −0.75 |
| **WM × ST** | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

Veraison occurred 69 and 60 DAA in 2011 and 2012, respectively. The difference between treatments and interaction between factors was assessed with a two-way ANOVA. The level of significance is reported within the columns: *, **, *** or ns, significant at $P < 0.05$, 0.01, 0.001 or not significant, respectively. †Values are presented as 15-day means of $\Psi_{stem}$ (MPa). DAA, days after anthesis.

**Table 2.** Effect of the combination of water management and shoot trimming on the leaf area of Merlot grapevines at veraison and harvest in 2011 and 2012.

| Irrigation | Trimming | Total leaf area ($m^2$/vine) | | | | | | Primary leaf area ($m^2$/vine) | | | | | | Lateral leaf area ($m^2$/vine) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2011 | | | 2012 | | | 2011 | | | 2012 | | | 2011 | | | 2012 | | |
| | | V | H | *P*-value | V | H | *P*-value | V | H | *P*-value | V | H | *P*-value | V | H | *P*-value | V | H | *P*-value |
| I | HC | 8.07 a | 7.25 a | ns | 9.17 a | 7.89 a | ns | 5.24 a | 3.66 a | 0.04 | 5.87 a | 4.66 a | ns | 2.83 a | 3.59 a | ns | 3.30 a | 3.23 a | ns |
| DI | HC | 5.29 b | 4.15 b | ns | 5.13 b | 4.85 b | ns | 3.70 b | 2.26 b | <0.01 | 3.89 b | 3.47 ab | ns | 1.59 ab | 1.89 b | ns | 1.24 b | 1.39 b | ns |
| I | SC | 4.11 bc | 4.47 b | ns | 4.41 bc | 4.08 b | ns | 3.17 bc | 2.77 ab | ns | 3.23 bc | 2.63 ab | ns | 0.94 b | 1.71 bc | 0.03 | 1.18 b | 1.45 b | ns |
| DI | SC | 2.85 c | 2.26 c | 0.01 | 3.42 c | 2.28 b | 0.02 | 2.33 c | 1.62 b | <0.01 | 2.59 c | 1.58 b | 0.02 | 0.52 b | 0.63 c | ns | 0.83 b | 0.70 b | ns |

The difference between treatments was assessed with a one-way ANOVA; means within a column followed by a different letter differ significantly at $P < 0.05$ by Tukey Honest Significant Difference (HSD) test. Differences in the leaf area between phenological stages were tested with a one-way ANOVA performed within each treatment, and when significant *P*-values are reported within rows; ns, not significant. DI, deficit irrigated; H, harvest; HC, high canopy; I, irrigated; SC, short canopy; V, veraison.

**Table 3.** Effect of water management and shoot trimming on yield and berry components of Merlot grapevines at harvest in 2011 and 2012.

| | Yield per vine (kg/vine) | | Leaf area/yield ($m^2$/kg) | | Berry mass (g) | | Relative skin mass (%)† | | Skin mass/berry (mg/berry) | | Relative seed mass‡ (%) | | Seed mass/berry (mg/berry) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 |
| **Water management (WM)** | ** | *** | * | ns | *** | *** | *** | *** | ns | ns | ** | ** | ns | ns |
| Irrigated (I) | 4.06 | 2.51 | 1.46 | 2.45 | 1.85 | 1.40 | 10.8 | 11.3 | 199.4 | 162.8 | 4.38 | 4.53 | 81.0 | 65.5 |
| Deficit (DI) | 2.92 | 1.74 | 1.07 | 2.16 | 1.41 | 1.08 | 12.8 | 15.1 | 181.8 | 169.0 | 5.16 | 6.11 | 73.2 | 68.5 |
| **Shoot trimming (ST)** | ns | ns | * | * | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| High canopy (HC) | 3.76 | 2.30 | 1.49 | 2.94 | 1.65 | 1.27 | 12.1 | 13.2 | 196.9 | 164.1 | 4.89 | 5.17 | 80.1 | 64.5 |
| Short canopy (SC) | 3.22 | 1.95 | 1.04 | 1.67 | 1.61 | 1.22 | 11.5 | 13.2 | 184.3 | 167.7 | 4.65 | 5.47 | 74.2 | 69.5 |
| **WM × ST** | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

The difference between treatments and interaction between factors was assessed with a two-way ANOVA. The level of significance is reported within the columns: *, **, *** or ns, significant at $P < 0.05$, 0.01, 0.001 or not significant, respectively. †100 × (skin fresh mass/total fresh berry mass); ‡100 × (seed fresh mass/total fresh berry mass).

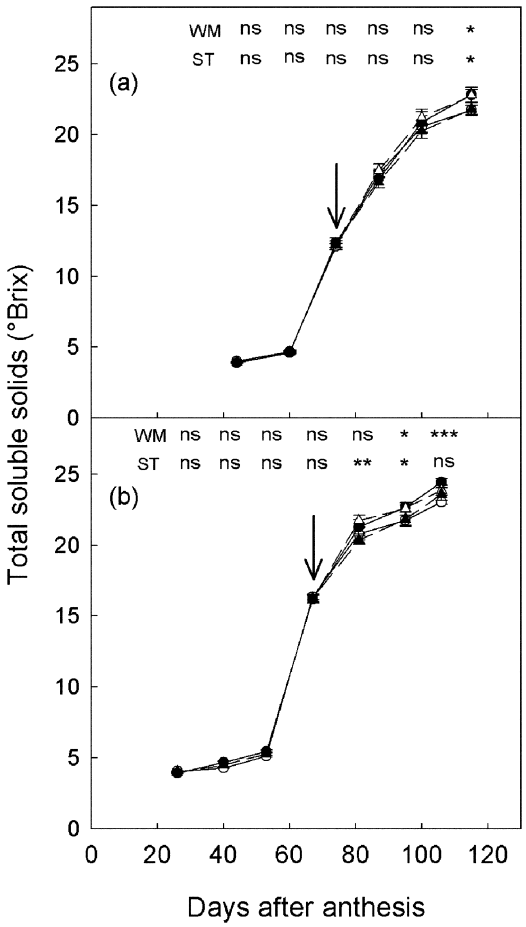


**Figure 1.** Seasonal trends of total soluble solids (°Brix) recorded in (a) 2011 and (b) 2012 for Merlot vines subjected to water management (WM) and shoot trimming (ST) treatments (mean ± SE, n = 8): I, irrigated (○); DI, deficit irrigated (●); HC, high canopy (△); and SC, short canopy (▲). The trimming date (end of veraison, 80% berries turned red) is indicated (↓). A two-way ANOVA was performed for assessing differences between factors at each date of sampling. *, **, *** or ns: significant at $P < 0.05$, 0.01, 0.001 or not significant, respectively.

DAA to harvest in 2011 and 2012, respectively (Figure 2a,b). A similar effect of DI was observed on the anthocyanins content per berry (data not shown). In contrast to water management, shoot trimming had no impact upon the accumulation of anthocyanins both during the season and at harvest in either year.

The concentration of skin tannins (mg/g of berry FM) was significantly higher in DI than in I grapes from 87 DAA and 40 DAA to harvest in 2011 and 2012, respectively (Figure 2c,d). Neither treatment, however, influenced skin tannins content per berry (mg/berry) during the season (data not shown) or at harvest (Table 5). As for anthocyanins, severe shoot trimming had no impact upon skin tannins concentration. Furthermore, the content of seed tannins (mg/berry) at harvest was not influenced by water management or shoot trimming treatments during either season (Table 5). Nonetheless, in 2012, the concentration of seed tannins (mg/g berry FM) was significantly affected by irrigation management from 53 DAA to harvest (Figure 2f) and was higher in DI than in I, while in 2011 a significant difference was detected only at 100 DAA (Figure 2e).

### *Wine composition and sensory attributes*

Water management significantly influenced most of the parameters analysed in wines, with the exception of the concentration of tannins (Table 6). No difference in the wine alcohol concentration was observed in 2011, but DI significantly increased it in 2012. As observed in the berries at harvest, wines obtained from DI grapes resulted in a higher concentration of anthocyanins, and consequently higher colour intensity and lower hue. Large polymeric pigments and SPP were also higher in DI than in I wines. Water deficit decreased wine pH in both years, as well as TA in 2011. Dry extract was found to be higher in DI wines in both years.

Severe shoot trimming significantly reduced wine alcohol concentration in 2011 by −1% v/v, while no difference was found in 2012. In contrast, severe shoot trimming influenced other wine parameters only in 2012, when SPP and colour intensity were lower, and colour hue was higher in SC than that in HC wines.

Sensory analysis revealed that water management significantly affected several sensory attributes, while as for most parameters analysed in the study severe shoot trimming did not affect the final sensory characteristics of wines (Table S1). Water deficit wines showed aroma intensity and jam-like notes higher than those resulting from I treatments in both vintages. Red fruit aroma and retronasal persistence were also recorded as higher in DI wines but in the 2012 vintage only; no effect of irrigation management was observed for most of the taste attributes. Interactions were observed between water management and severe shoot trimming in wine body, mouthfeel, colour intensity and hue. Wine body and colour intensity were consistently higher in deficit irrigated wines despite the level of canopy height, while colour hue was lower (Table S1).

### *Carbohydrate storage in permanent organs*

The effect of water management and severe shoot trimming on the concentration of non-structural carbohydrates (alcohol-soluble sugars and starch) in roots, trunk and canes of the vines was measured only after the second vintage (January 2013) (Table 7). Deficit irrigation reduced the starch concentration [g/kg dry mass (DM)] in the trunk, and a significant interaction was found between irrigation and severe shoot trimming treatments. There was no effect of water management on the starch accumulation in roots and canes. Deficit irrigation also generated an increase in soluble sugars (g/kg DM) in canes.

Severe shoot trimming reduced starch concentration (g/kg DM) in canes (−6%), trunk (−10%) and roots (−15%). As reported above, however, an interaction between water management and severe shoot trimming treatments was observed for the starch concentration in the trunk. As with irrigation, severe shoot trimming treatments generated differences in soluble sugars (g/kg DM) only in the canes where SC vines presented a higher value than that of their HC counterparts.

## Discussion

The aim of the late and severe shoot trimming treatment was de facto to reduce sugar accumulation without affecting bunch microclimate, and hence anthocyanins concentration. Combining this effect with water deficit, we expected to enhance the accumulation of anthocyanins and hence to produce wines with lower alcohol and higher pigmentation. The comparison of the wines obtained from vines subjected to the above treatments indicated that our objective was attained only in 2011, when severe shoot trimming decreased the alcohol concentration by 1% (v/v), and water deficit treatment increased anthocyanins concentration and other colour-related features (LPP, SPP, colour intensity) without affecting the alcohol concentration. Although in 2012 the same effect on anthocyanins content and colour features of wine was observed as in 2011, the wine alcohol concentration was not affected by SC and increased by

**Table 4.** Effect of water management and shoot trimming on the composition of Merlot grapes at harvest in 2011 and 2012.

| | TSS (°Brix) | | TSS per berry (g/berry) | | TA (g/L) | | pH | |
|---|---|---|---|---|---|---|---|---|
| | **2011** | **2012** | **2011** | **2012** | **2011** | **2012** | **2011** | **2012** |
| **Water management (WM)** | * | *** | ** | ** | ns | ns | ns | ns |
| Irrigated (I) | 21.8 | 23.1 | 0.40 | 0.32 | 6.18 | 5.81 | 3.50 | 3.51 |
| Deficit (DI) | 22.9 | 24.5 | 0.33 | 0.27 | 5.98 | 5.78 | 3.44 | 3.59 |
| **Shoot trimming (ST)** | * | ns | * | ns | ns | ns | ns | ns |
| High canopy (HC) | 22.8 | 23.9 | 0.38 | 0.30 | 5.94 | 5.96 | 3.49 | 3.58 |
| Short canopy (SC) | 21.9 | 23.7 | 0.34 | 0.29 | 6.21 | 5.63 | 3.45 | 3.52 |
| **WM × ST** | ns | ns | ns | ns | ns | ns | ns | ns |

The difference between treatments and interaction between factors was assessed with a two-way ANOVA. The level of significance is reported within the columns: *, **, *** or ns, significant at $P < 0.05$, 0.01, 0.001 or not significant, respectively. TA, titratable acidity; TSS, total soluble solids.

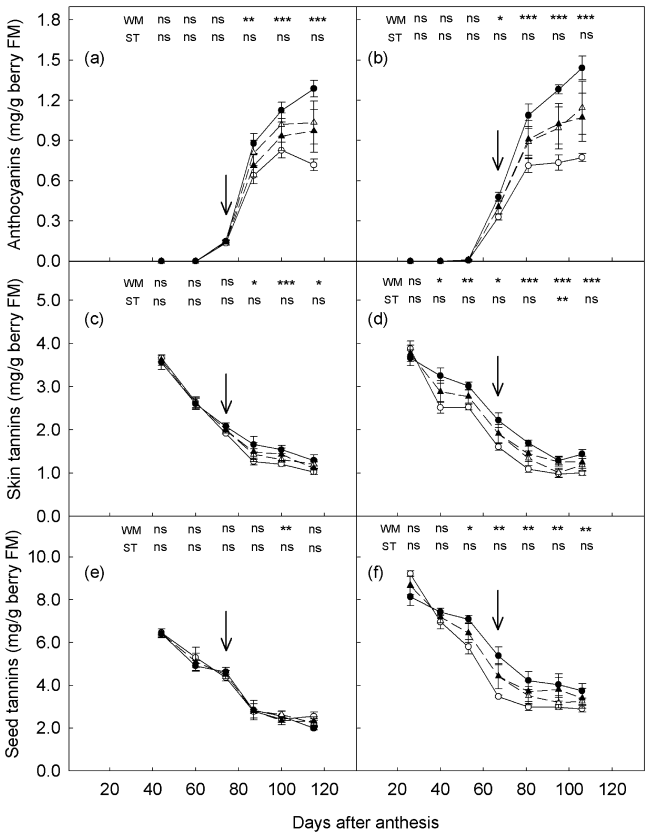


**Figure 2.** Seasonal trends of anthocyanins per berry (mg/berry) in (a) 2011 and (b) 2012, of skin tannins per berry fresh mass [mg/g berry fresh mass (FM)] in (c) 2011 and (d) 2012, and of seed tannins per berry fresh mass (mg/g berry FM) in (e) 2011 and (f) 2012 recorded for Merlot vines subjected to water management and shoot trimming treatments (mean ± SE, $n = 8$): I, irrigated (○); DI, deficit irrigated (●); HC, high canopy (△); and SC, short canopy (▲). The trimming date (end of veraison, 80% of berries turned red) is indicated (↓). A two-way ANOVA was performed for assessing the difference between factors at each date of sampling. *, **, *** or ns: significant at $P < 0.05$, 0.01, 0.001 or not significant, respectively.

0.6% (v/v) under DI. Differences among treatments in the fruit at harvest were consistent with the ones reported for wine composition. The severe shoot trimming treatment was successful in reducing the sugar concentration in the berry at harvest only in 2011. In 2012, the severe shoot trimming had no effect on the final TSS content (Table 4), although it reduced sugar accumulation in the earlier stages of ripening (Figure 1b). The DI treatment confirmed the previously reported induction of anthocyanins concentration in the berry [among others, Castellarin et al. (2007a,b), Bucchetti et al. (2011) and Ollé et al. (2011)] but also increased berry TSS at harvest in both seasons. The observed TSS increase translated into higher alcohol concentration for DI than that in I wines in 2012, while it did not affect alcohol concentration in 2011.

The inconsistencies observed between seasons in the effect of the severe shoot trimming treatment on the sugar concentration of the berry at harvest indicate that factors other than just the canopy size affected the sugar accumulation process in the berry. Previous studies have shown that accumulation of sugars in the berry of hedged or selectively defoliated vines was related to the level of leaf area retained (Kliewer 1970, Kliewer and Antcliff 1970, Peterson and Smart 1975, Mansfield and Howell 1981, Reynolds and Wardle 1989). When experiments were repeated for consecutive seasons, however, inconsistencies on the effect of treatments were observed among seasons (Peterson and Smart 1975, Reynolds and Wardle 1989). In our study, differences in the leaf area/crop mass ratio observed between seasons within the same treatment may partially explain this inconsistent result. The leaf area/crop mass ratio generally increased from 2011 to 2012 (Table 3); this was observed and tested significantly (data not shown) across all the treatments. The increase was primarily due to the lower berry mass and vine yield observed in 2012 across treatments with respect to 2011, in parallel with no difference in the total leaf area. The potential higher photosynthetic capability per given amount of crop observed in 2012 was likely sufficient to deliver the same amount of sugars to the berries versus vines that were not severely trimmed. In previous studies, canopy manipulation treatments (e.g. pruning levels, defoliation and thinning) in vertical shoot positioned systems reduced berry TSS at harvest only when the leaf area/crop mass ratio was lower than 0.8–1.2 $m^2$/kg (Kliewer and Dokoozlian 2005, Palliotti et al. 2013, Poni et al. 2013). The severe trimming treatment in our study targeted this value in 2011 (1.0 $m^2$/kg) but not in 2012 (1.67 $m^2$/kg). Seasonal differences in berry mass and yield strongly altered the above ratio, and in turn the effect of the same canopy treatment on fruit TSS across seasons. Hence, in 2012, SC vines were not limited enough by the severe shoot trimming treatment to affect sugar concentration in the berry at harvest. A significant reduction of berry TSS, however, was observed in SC vines earlier during the season at 81 DAA and 95 DAA; at these stages, the reduction generated by the severe shoot trimming was similar to that observed in 2011 (−1°Brix). Therefore, the 2012 SC treatment determined a transient

**Table 5.** Effect of water management and shoot trimming on the concentration and content of anthocyanins and of skin and seed tannins of Merlot berries at harvest in 2011 and 2012.

| | Anthocyanins (mg/berry) | | Anthocyanins (mg/g FM) | | Skin tannins (mg/berry) | | Skin tannins (mg/g FM) | | Seed tannins (mg/berry) | | Seed tannins (mg/g FM) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 |
| **Water management (WM)** | *** | ** | *** | *** | ns | ns | * | *** | ns | ns | ns | ** |
| Irrigated (I) | 1.33 | 1.12 | 0.72 | 0.78 | 1.89 | 1.46 | 1.02 | 1.00 | 3.87 | 4.20 | 2.57 | 2.90 |
| Deficit (DI) | 1.83 | 1.63 | 1.29 | 1.44 | 1.84 | 1.64 | 1.29 | 1.44 | 4.07 | 4.25 | 2.24 | 3.74 |
| **Shoot trimming (ST)** | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| High canopy (HC) | 1.65 | 1.39 | 1.03 | 1.15 | 1.95 | 1.48 | 1.21 | 1.19 | 4.16 | 4.14 | 2.56 | 3.25 |
| Short canopy (SC) | 1.50 | 1.36 | 1.00 | 1.07 | 1.77 | 1.61 | 1.11 | 1.25 | 3.77 | 4.31 | 2.25 | 3.39 |
| **WM × ST** | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

The difference between treatments and interaction between factors was assessed with a two-way ANOVA. The level of significance is reported within the columns: *, **, *** or ns, significant at $P < 0.05$, 0.01, 0.001 or not significant, respectively. FM, fresh mass.



**Table 6.** Effect of water management and shoot trimming on Merlot wine composition in 2011 and 2012 vintages.

| | Alcohol % (v/v) | | pH | | TA (g/L) | | Dry extract (g/L) | | Anthocyanins (mg/L) | | Tannins (mg/L) | | LPP (AU) | | SPP (AU) | | Colour intensity† (AU) | | Hue‡ (AU) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 | 2011 | 2012 |
| **Water management (WM)** | ns | * | *** | * | * | ns | * | ** | ** | *** | ns | ns | *** | ** | *** | *** | *** | *** | *** | *** |
| Irrigated (I) | 12.3 | 13.1 | 3.53 | 3.57 | 5.82 | 6.86 | 27.4 | 32.7 | 127.0 | 117.7 | 264.2 | 304.3 | 0.61 | 1.03 | 0.79 | 1.08 | 0.57 | 0.67 | 0.79 | 1.00 |
| Deficit (DI) | 12.7 | 13.7 | 3.33 | 3.45 | 6.33 | 6.66 | 29.0 | 34.9 | 233.5 | 294.7 | 290.8 | 255.6 | 1.17 | 1.47 | 1.90 | 2.38 | 1.21 | 1.04 | 0.60 | 0.79 |
| **Shoot trimming (ST)** | ** | ns | * | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | * | ns | * | ns | * |
| High canopy (HC) | 13.0 | 13.5 | 3.47 | 3.52 | 6.00 | 6.75 | 28.4 | 34.3 | 203.2 | 209.3 | 296.5 | 261.9 | 0.90 | 1.23 | 1.38 | 1.91 | 0.91 | 0.94 | 0.70 | 0.85 |
| Short canopy (SC) | 12.0 | 13.2 | 3.39 | 3.39 | 6.15 | 6.77 | 28.0 | 33.2 | 149.7 | 183.6 | 252.2 | 313.7 | 0.88 | 1.23 | 1.30 | 1.31 | 0.86 | 0.68 | 0.68 | 0.97 |
| **WM x ×ST** | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |

Wines were analysed 4 months after alcoholic fermentation in both years. The difference between treatments and interaction between factors was assessed with a two-way ANOVA. The level of significance is reported within the columns: *, **, *** or ns, significant at $P < 0.05$, 0.01, 0.001 or not significant, respectively. † $OD_{420\ nm} + OD_{520\ nm}$; ‡$OD_{420\ nm}/OD_{520\ nm}$. LPP, large polymeric pigments; SPP, short polymeric pigments; TA, titratable acidity.

**Table 7.** Effect of water management and shoot trimming on non-structural carbohydrate reserves in the different organs of Merlot grapevines in January 2013.

| | Sugar (g/kg DM) | | | Starch (g/kg DM) | | |
|---|---|---|---|---|---|---|
| | Cane | Trunk | Root | Cane | Trunk | Root |
| **Water management (WM)** | * | ns | ns | ns | *** | ns |
| Irrigated (I) | 67.4 | 59.5 | 35.0 | 225.2 | 189.1 | 178.5 |
| Deficit (DI) | 75.5 | 70.4 | 32.6 | 222.5 | 148.9 | 177.1 |
| **Shoot trimming (ST)** | * | ns | ns | ** | * | * |
| High canopy (HC) | 67.9 | 69.9 | 35.1 | 231.1 | 177.1 | 190.0 |
| Short canopy (SC) | 75.1 | 60.0 | 32.5 | 216.6 | 160.9 | 165.7 |
| **WM × ST** | ns | ns | ns | ns | * | ns |

The difference between treatments and interaction between factors was assessed with a two-way ANOVA. The level of significance is reported within the columns: *, **, *** or ns, significant at $P < 0.05$, 0.01, 0.001 or not significant, respectively. DM, dry mass.

reduction of sugar accumulation in the berry, but at a late stage of ripening the plant was able to reintegrate the sugar concentration in the fruit to the normal (HC) concentration. In Sangiovese, severe defoliation applied post-veraison determined a significant reduction of sugars at harvest; however, in the berries of the same treatment, unlike the control berries, sugar concentration kept increasing for 21 days after the harvest date (Palliotti et al. 2013). Kliewer and Antcliff (1970) and Smart (1985) similarly observed that treatments of leaf area reduction imposed approximately one month before veraison significantly reduced sugar accumulation in the berry during ripening, but the difference became smaller at harvest, and in the latter study not significant. The late recovery of sugars observed in this study was not related to a significant increase of leaf area due to the growth of lateral shoots (Table 2), but it may have been related to a higher supply of sugars to the berry at the expense of other sink organs, such as roots, trunk and canes (Table 7), as well as to a leaf photosynthetic compensation (Poni et al. 2013).

Climatic differences between seasons, in contrast, might have contributed to the different results observed in the effect of the severe shoot trimming treatment. In fact, despite the GDD curve being relatively similar between the two seasons, the year 2012 was characterised by a higher temperature in June, July and August (Figure S1). Such higher temperature probably contributed to accelerating the fruit developmental and ripening process during this year (Sadras and Petrie 2011). For example, in 2012, veraison was recorded 60 DAA, and berry sugar concentration reached on average across treatments 20°Brix at 87 DAA and 22°Brix at 95 DAA, while in 2011 20°Brix were reached only at 100 DAA. The more favourable conditions for sugar accumulation in 2012 could also have resulted in a higher sugar accumulation at late stages of ripening in SC treatment.

Despite the fact that in some cases reducing the leaf area/crop mass ratio by canopy reduction limited sugar accumulation in the berry (Kliewer and Bledsoe 1987, Kliewer and Dokoozlian 2005, Martinez de Toda et al. 2013), our study showed that manipulating the same ratio via water management may yield opposite results. In 2011, DI reduced this ratio from 1.46 to 1.07 $m^2$/kg, but TSS concentration in the berry actually increased (Figure 1a, Table 4). Similarly, SC reduced the same ratio from 1.49 to 1.04 $m^2$/kg, reducing TSS concentration. Water deficit, besides modifying the size of the canopy and hence leaf area/crop mass ratio, also affects several other physiological parameters that contribute to sugar concentration, such as leaf photosynthetic activity (Chaves et al. 2010), rate of import of sugar into the berry (Wang et al. 2003) and berry water budget (Keller et al. 2005).

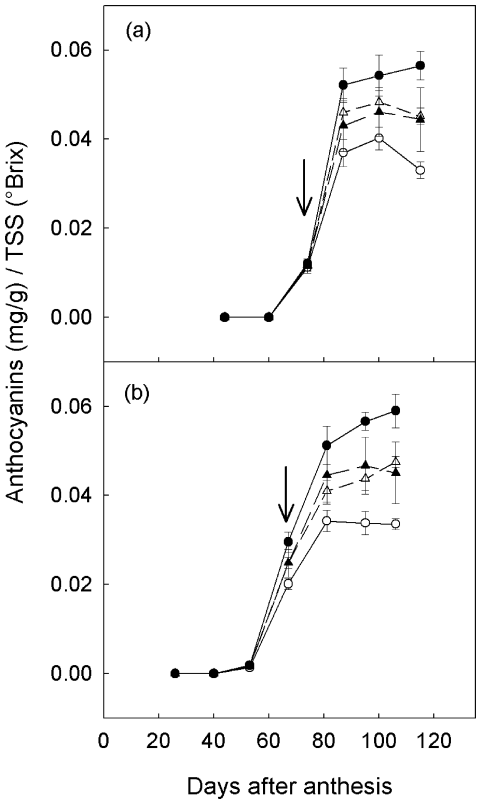


**Figure 3.** Seasonal trends of anthocyanins/sugar ratio expressed as anthocyanins (mg/g berry fresh mass)/total soluble solids (TSS) (°Brix) in (a) 2011 and (b) 2012 recorded for Merlot vines subjected to water management and shoot trimming treatments (mean ± SE, $n = 8$). I, irrigated (○); DI, deficit irrigated (●); HC, high canopy (△); and SC, short canopy (▲). The trimming date (end of veraison, 80% of berries turned red) is indicated (↓).

Furthermore, under our experimental conditions, the relationship between sugar and anthocyanins accumulation in the berry was not strict, and the two processes were differentially affected by the treatment tested. Water deficit decoupled sugar and anthocyanins accumulation, while the severe shoot trimming treatment did not. Higher anthocyanins/sugar ratios were achieved under water deficit conditions (Figure 3) as already reported in other red cultivars (Sadras et al. 2007, Sadras and Moran 2012); DI consistently enhanced anthocyanins accumulation during ripening in both years (Figure 2a,b, Table 6) through a stimulus of their biosynthesis as shown by Castellarin

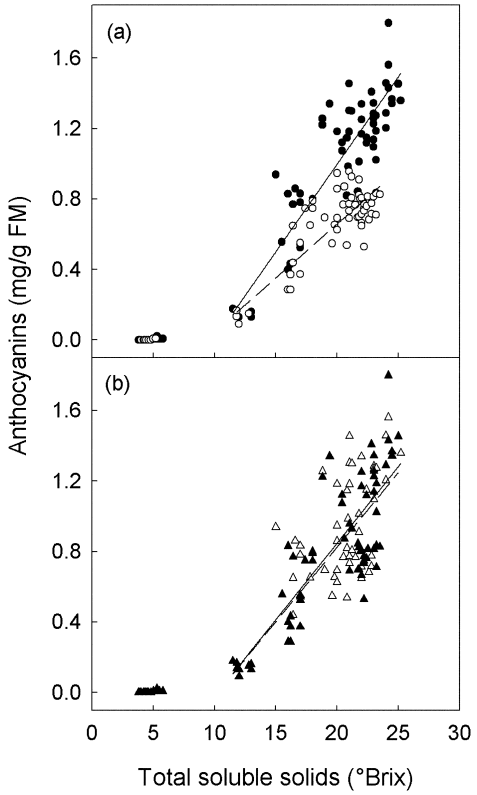


**Figure 4.** The relationship between anthocyanins [mg/g berry fresh mass (FM)] and total soluble solids (°Brix) during Merlot berry ripening as influenced by (a) water management I, irrigated (○) and DI, deficit irrigated (●), and (b) shoot trimming HC, high canopy (△) and SC, short canopy(▲), in experiments in 2011 and 2012.

et al. (2007a,b) and a reduction of the berry mass as shown by Roby et al. (2004). The increase in sugar concentration observed at harvest in the same treatment was not proportional to the increase observed for anthocyanins. Moreover, the decoupling of the anthocyanins/sugar relationship by water deficit was consistent across seasons (Figure 4a). Conversely, the strong reduction of leaf area/crop mass ratio by severe shoot trimming did not affect the anthocyanins/sugar ratio (Figures 3,4b). Anthocyanins were not affected by the severe shoot trimming treatment (Figure 2a,b) in either of the seasons considered, and despite the significant reduction of TSS in the berry at harvest in 2011 and during fruit ripening in 2012, the anthocyanins/sugar ratio was never significantly affected by the same treatment. Previous studies reported that severe reduction of the leaf area determined the parallel decrease of the accumulation of sugar and anthocyanins in the berry (Peterson and Smart 1975, Reynolds and Wardle 1989). It appears that the timing on when the treatment is applied may be critical for this ratio. Reynolds and Wardle (1989) showed that severe defoliation imposed at the lag phase was significantly detrimental for the accumulation of sugar and anthocyanins, whereas the same treatments applied at veraison had a similar effect on sugar accumulation, while the reduction of anthocyanins was less severe. The timing of the severe shoot trimming application was designed at late veraison stages (more than 80% berries had turned red) in order to prevent the berries from experiencing the stress that this extreme treatment might generate before the beginning of colour accumulation. In grape cell and fruit cultures, sugars act as triggers for anthocyanins biosynthesis (Zheng et al. 2009, Dai et al. 2014). Source limitation for sugar accumulation in the berry before or at early stages of ripening may limit the hormonal stimulus of sugars on the anthocyanins biosynthesis. In a recent study, mechanical leaf removal on the apical part of the canopy applied at similar phenological stages on Sangiovese vines did not affect anthocyanins accumulation but significantly affected sugar accumulation in the berry and hence the anthocyanins/sugar ratio (Palliotti et al. 2013). Despite the lack in the effect on the anthocyanins/sugar ratio, our results confirm that severe reduction of leaf area applied after the beginning of ripening has no effect on the accumulation of anthocyanins in the berry. In Merlot, however, further research on this topic is deemed necessary to fully understand the complex physiological interaction between sugars and anthocyanins during fruit ripening upon leaf area reduction (i.e. testing different levels and timing of modification of the leaf area/crop mass ratio).

Finally, the absence of interactions between irrigation and severe shoot trimming treatments on berry composition was itself an interesting result. Vines under DI were already stressed when severe trimming was applied, and the severe reduction of the canopy did not cause any other effect than already reported in I-SC treatment combination. Indeed, even if both DI and SC lowered the photosynthate supply in the vines, their effect on sugar accumulation in the berry was independent; other research focused on the relationship between canopy and water management also found no interactions between treatments (Keller et al. 2008, Terry and Kurtural 2011, Williams 2012).

### *Wine composition and sensory attributes*

Water deficit affected several wine components, while severe shoot trimming had a limited effect on wine composition and sensory attributes in both vintages, consistent with results found in the berries (Table 6). Concomitant with the lower alcohol concentration obtained by trimming on 2011, the water deficit confirmed well-known positive effects on wine composition by enhancing wine aroma and increasing anthocyanins content, along with SPP and LPP, promoting higher colour intensity – a result confirmed with the sensory evaluation (Table S1) and in accordance with previous experience (Matthews et al. 1990, Salón et al. 2005, Ou et al. 2010). Wine hue was always lower in DI wines, meaning tonalities shifted more towards red-violet notes connected with a higher level of anthocyanins hydroxylation (Castellarin et al. 2007a,b).

Interestingly, in 2012, when SC yielded no effect upon berry composition, it did prompt a decrease in both wine SPP and colour intensity, as well as an increase in wine hue (Table 6), indicating a paler and red-brown colour for wines obtained from SC treatments. These results were confirmed by sensory analysis. This suggests that, despite the fact that anthocyanins concentration was not affected by severe shoot trimming, this treatment may have triggered compositional changes leading to differences in the anthocyanins profile in the berries (Guidoni et al. 2008) and/or to different co-pigmentation reactions between anthocyanins and other flavonoids in wines (Boulton 2001).

### *Grapevine carbohydrate reserves*

Carbohydrates of woody organs were analysed only during the second experimental year after irrigation and severe shoot trimming treatments had been applied for two consecutive seasons. Severe shoot trimming demonstrated a greater impact on carbohydrate storage in woody organs than water management. Where water deficit reduced the starch concentration only in the trunk (g/kg DM), severe shoot trimming significantly reduced the starch concentration in cane, trunk and root tissues (Table 7), although an interaction between irrigation and shoot trimming

was observed in the trunk specifically. When severe shoot trimming was applied in fully irrigated vines (I-SC), it strongly reduced starch concentration in the trunk from 204.2 in I-HC to 173.9 g/kg DM in I-SC. When the same treatment was applied to DI vines, there was no significant impact upon starch concentration with measurements of 149.9 and 147.9 g/kg DM in DI-HC and DI-SC, respectively. Such an interaction might be due to the severe limitation of DI on the carbon accumulation process; in DI, an already limited source was directing most of its sugars to the bunches while limiting the delivery to the trunk, and thus the severe shoot trimming treatment was not affecting the amount of reserves accumulated in this organ, whereas in irrigated vines severe trimming likely prevented the accumulation of a fraction of carbohydrates that could have been stored in the trunk.

Interestingly, both DI and SC vines displayed higher soluble sugar concentration in canes than that in I and HC vines. In grapevine, a large canopy reduction may stimulate longer photosynthetic activity (Howell 2001) and possibly a prolonged period of translocation activity than that in normal vines. Prolonged and higher leaf activity after harvest before leaf abscission may have been responsible for the increase of soluble sugars in DI and SC canes. The recovery, however, was insufficient for restoring long-term reserves in canes and lower organs.

## Conclusions

Water deficit increased berry sugar concentration in both years of the study and increased wine alcohol concentration only in 2012. Water deficit, however, positively affected the concentration of several wine phenolic substances and volatile aroma compounds that were detectable and desirable in the wine. Alternatively, severe shoot trimming reduced berry sugar concentration and alcohol in wine, but only in one of the two seasons, while the composition of the phenolic substances in berries and wines was not affected. This study indicates that, if applied at early stages of ripening, severe canopy reductions do not limit the accumulation of anthocyanins, and in general wine composition and sensory features.

The interaction between water deficit and shoot trimming treatments was not significant, and thus independent results were obtained. Water deficit significantly affected canopy, yield, fruit and wine components, while the reduction of the leaf area/crop mass ratio through severe shoot trimming had only a limited impact on several vine and fruit parameters analysed in this study. In particular, severe shoot trimming reduced leaf area similarly in the 2 years, but a reduction in TSS at harvest was reported only in 2011, although in 2012 sugar reduction was observed before harvest. Utilising shoot trimming techniques to modify the leaf area/crop mass ratio, and thus the source–sink balance, proved to be an effective tool in modulating sugar accumulation at harvest in Merlot berries only in the season when the leaf area/crop mass ratio was likely a limiting factor. Specific seasonal weather conditions may overcome the targeted ratio by compensating fruit size and/or yield, and consequently could require a different canopy leaf area to be removed accordingly. Moreover, the observed reduction of total non-structural carbohydrates in the woody organs of hedged vines suggests a possible long-term negative effect on vine vigour and productivity. Assessment of these two aspects, as well as the feasibility of mechanisation and costs associated with the treatment, is crucial to successfully transfer this technique to field application.

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## Supporting information

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**Figure S1.** Monthly mean air temperature and cumulative growing degree days (GDD) in 2011 and 2012 in the Merlot experimental vineyard. Mean air temperature in 2011 (○), 2012 (●) and 1990–2012 period mean (△). Cumulative GDD in 2011 (□), 2012 (■) and 1990–2012 period mean (◊).

**Table S1.** Effect of water management and shoot trimming on Merlot wine sensory attributes in 2011 and 2012 vintages. Wines were analysed 4 months after alcoholic fermentation in both years.