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Defoliation of two-wire vertical trellis: effect on grape quality

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ABSTRACT
Basal leaves were removed from Cabernet Sauvignon vines trained to a two-wire vertical trellis at fruit set and at veraison. Leaf removal did not modify total soluble solids and titratable acidity at harvest. Defoliation at fruit set of lower cordon recovered the grape anthocyanin composition gap between upper and lower cordons and that produced a positive effect on anthocyanin synthesis. Hence, control of the upper cordon and defoliation of fruit set of the lower cordon treatments showed comparable values of anthocyanins. Defoliation at veraison did not produce any appreciable effect. This study shows that skin anthocyanin composition in a two-wire vertical trellis can be modified by leaf removal in the fruit zone of the lower cordon, reducing variability in the ripeness between the two cordons.

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Cabernet Sauvignon; anthocyanins; condensed tannins; canopy management; cordon position; leaf removal

Introduction
Trellis design is a major consideration in vineyard establishment and, once established, is difficult and costly to alter. The wide variety of training systems used for wine grapes around the world attests to a long history of trellis development driven by a range of factors from tradition to mechanised production. The versatility of the grape vine has contributed to adapt diverse canopies from single and double wire to the vertical trellis for facilitating mechanisation.

Vertical trellises can be broadly divided into two groups: non-divided and divided canopies. In each of these groups, we can find many different training systems. Vertical Shoot Positioned Trellis, Single Curtain and California Sprawl are the most widespread examples of a non-divided canopy. Divided canopy systems have many variations including vertically divided trellis systems, for example, Scott Henry, Smart-Dyson, TK2T (Te Kauwhata Two Tier) (Smart & Robinson 1991) and horizontally divided trellis system, like the Lyre system (Carbonneau 1984) and the Geneva Double Curtain (Shaulis et al. 1966). Generally, the driver for double cordon systems has been a better fruit composition and a greater yield (Reynolds et al. 1994; Reynolds & Vanden Heuvel 2009), mainly because of improved canopy volume and exposed leaf area (Gladstone & Dokoozlian 2003) and hence light interception, as well as the greater number of buds that are retained per unit row length (Reynolds & Vanden Heuvel 2009). Moreover, light interception in
these trellis systems, at the same canopy density, is mainly related to the height of the
cordons from the soil level (Hedberg & Raison 1982).

Although these divided canopy systems have become popular, vertical canopy division
has a few key drawbacks. The Scott Henry system frequently shows effects of dominance of
the upper canopy over the lower cordon canopy, such that shoots trained vertically
upward are normally much more vigorous than their downward-positioned counterparts (Reynolds &
Vanden Heuvel 2009).

With every training system, there is a change in the cluster zone of the micro-environ-
ment especially in terms of light (photosynthetically active radiation and Red/Far Red light
ratio) (Reynolds & Vanden Heuvel 2009), but wind speed, evaporation rate, temperature
and humidity can also be altered (Smart 1985). Canopy division reduces leaf area density
and improves sunlight exposure and penetration into the canopy interior by simply
increasing canopy volume or the amount of space available for foliage distribution (Glad-
stone & Dokoozlian 2003).

The relevance of vine photosynthesis within the context of training systems lies also in
the ability of the total leaf area to exploit all sources of photosynthetically active radiation
(PAR). The use of diffuse radiation and sunflecks by leaves in the interior of the canopy is
of paramount importance (Smart 1985; Poni et al. 1993). Thus, modifications in training
vines may not only increase the amount of leaf area exposed to high-intensity direct radi-
ation, but may increase the interception of diffuse radiation and improve the light micro-
climate of the remainder of the foliage (Reynolds & Vanden Heuvel 2009).

Canopy management practices, adjusted according to the chosen trellis system, that
create a canopy architecture where bunches receive sufficient diffuse light, but where
berries are protected from excessive direct sunlight exposure and berry heating, would
seem appropriate for the production of fruit with good ripeness and optimal levels of
anthocyanins for vines grown in hot climates (Haselgrove et al. 2000; Bergqvist et al.
2001). However, no unique effect of cluster sun exposure on sugar (total soluble solids,
TSS), anthocyanins, flavonols and phenolics, titratable acidity (TA), malate, juice pH
and berry weight compared to non-exposed fruits is documented (Kli ewer & Lider
1968; Smart et al. 1985; Crippen & Morrison 1986a, 1986b; Dokoozlian & K li ewer

Generally, defoliating consists in the elimination of basal leaves and lateral shoots in the
fruitt ing zone from fruit set to veraison, consequently reducing canopy density, improving
air movement, increasing the efficacy of fungicide treatments (Zoecklein et al. 1992) and
influencing in different manner the grape composition. Defoliation can be a useful tool to
achieve these goals especially in a dense canopy of the two-wire vertical trellis. The effects
of defoliation often depend on the ratio of leaves removed to those left and the phenolo-
gical stage at which defoliation is carried out (Kli ewer & Antcliff 1970). Defoliation can
also regulate the ratio between vegetative and productive activity, removing the old and
shaded leaves that consume the carbohydrates produced from the leaves exposed to the
sun (Intrieri et al. 2008). In the two-wire vertical trellis and in Cabernet Sauvignon
variety, an early canopy manipulation is necessary to maintain optimum light level
because the maximum foliage density is reached early in fruit development (Bowen
2009). However, in general, contradictory results regarding the influence of defoliation
on must quality have been obtained depending on vintage, cultivars and rootstocks,

Elimination of basal leaves and lateral shoots, beyond reducing canopy density, can affect TSS (Hunter et al. 1991; Haselgrove et al. 2000; Bergqvist et al. 2001; Spayd et al. 2002; Cortell & Kennedy 2006; Joscelyne et al. 2007; Ristic et al. 2007; Chorti et al. 2010; Pastore et al. 2017), potassium content in juice (Bledsoe et al. 1988; Jogaiah et al. 2013) and TA (Hunter et al. 1995; Reynolds et al. 1996; Main & Morris 2004; Barbagallo et al. 2007; Scafidi et al. 2010; Di Profio et al. 2011; Pastore et al. 2017).

In partially defoliated vines, skin of berry receiving higher light levels showed an enhancement of anthocyanin concentrations (Kliewer 1970; Kliewer 1977; Main & Morris 2004; Matus et al. 2009). Instead, sunlight exclusion reduced anthocyanin synthesis (Jeong et al. 2004; Cortell & Kennedy 2006; Fujita et al. 2006; Barbagallo et al. 2007; Chorti et al. 2010; Guan et al. 2016) because of lower expression of structural and regulatory genes in berry skin (Jeong et al. 2004; Azuma et al. 2012; Guan et al. 2016). However, many studies, in contrast, showed that the anthocyanin accumulation in fully exposed berries may be similar to shaded berries (Dokoozlian & Kliewer 1996; Keller & Hrazdina 1998; Mabrouk & Sinoquet 1998; Downey et al. 2004; Jogaiah et al. 2013; Haselgrove et al. 2000) while some others reported that high light levels resulted in decreased anthocyanin levels (Bergqvist et al. 2001; Spayd et al. 2002; Chorti et al. 2010; Pastore et al. 2013). These results support the conclusions that the concentration of anthocyanins is not linearly related to the exposure of the grapes (Crippen & Morrison 1986a, 1986b), but that it is also dependent on temperature (Haselgrove et al. 2000; Spayd et al. 2002). Light and temperature are involved in gene expression of the anthocyanin biosynthetic pathway in grape skin. Nevertheless, when air temperature is higher than 30°C for some hours in a day and for a long period during ripening, skin anthocyanin accumulation can be decreased (Spayd et al. 2002, Yamane et al. 2006; Chorti et al. 2010). Berries subjected to high levels of temperature and sun exposure showed a diminution in skin colour (Mori et al. 2005, 2007; Chorti et al. 2010). As demonstrated by several authors (Mori et al. 2005, 2007; Yamane et al. 2006; Azuma et al. 2012; Movahed et al. 2016; Pastore et al. 2017), the reduction of anthocyanin accumulation in the skin berries depends on flavonoid synthesis inhibition and enhancement of degradation process. However, the modification of microclimatic conditions by leaf removal determines different intensity responses of the cultivars (Rustioni et al. 2014; Guan et al. 2016; Pastore et al. 2017) in relation to probably their various sensitivity to abiotic stresses (light and temperature) that could cause a different prevailing of anthocyanin biosynthesis on degradation and vice versa. Moreover, for Pastore et al. (2017), light has a higher effect than temperature on the synthesis of flavonoid compounds, while other authors (Spayd et al. 2002; Mori et al. 2005; Tarara et al. 2008; Chorti et al. 2010) suggested the contrary; hence, that temperature has more influence on anthocyanin accumulation than light.

Moreover, anthocyanin composition could be altered in response to light and temperature conditions. Results are concordant about the effect of light exclusion on higher proportions of esterified anthocyanins but with a different response of cultivars (Haselgrove et al. 2000; Downey et al. 2004; Ristic et al. 2007; Tarara et al. 2008; Rustioni et al. 2016; Guan et al. 2016). In fact, for Chorti et al. (2010), in Nebbiolo vines and in shaded berries,
the coumarate form was enhanced while for Ristic et al. 2007 in Shiraz vines the acetate form was produced in a higher quantity.

About variation of di-substituted and tri-substituted anthocyanin concentration in response to light exclusion, the results are not clear. In shaded bunches, some authors (Spayd et al. 2002; Downey et al. 2004; Ristic et al. 2007; Azuma et al. 2012; Guan et al. 2016) found an increase in the di-substituted, while others (Guidoni et al. 2008; Tarara et al. 2008; Chorti et al. 2010, Scafidi et al. 2011) showed an augmentation of tri-substituted anthocyanin. Furthermore, it seems that high temperatures decrease the di-substituted (Mori et al. 2007; Tarara et al. 2008; Cohen et al. 2012) and a greater bunch shading increase the coumaroyl or in general, acylated forms (Downey et al. 2004; Ristic et al. 2007; Tarara et al. 2008; Chorti et al. 2010; Scafidi et al. 2011).

These contradictory results in terms of flavonoid composition and concentration can be probably ascribed to different ways in which temperature and light were studied. The effects of temperature and light frequently coexist together and could not be separated because they were studied in different environmental conditions and sites as latitude, altitude, row orientation or slope (Bergqvist et al. 2001; Mateus et al. 2002; Spayd et al. 2002; Ortega-Regules et al. 2006; Giacosa et al. 2015) or/and in different years (Pastor del Rio & Kennedy 2006; Guidoni et al. 2008; Edo-Roca et al. 2014) or in different canopy management (Main & Morris 2004; Downey et al. 2006; Joscelyne et al. 2007; Matus et al. 2009; Jogaiah et al. 2013, Pastore et al. 2013, 2017). Instead, when different artificial treatments (box, net, phytotron, multi-incubator, forced-air delivery system) were used, it was possible to test separately temperature and light roles on flavonoid compound biosynthesis, degradation and biosynthetic pathway (Haselgrove et al. 2000; Downey et al. 2004; Jeong et al. 2004; Mori et al. 2005, 2007; Yamane et al. 2006; Tarara et al. 2008; Ristic et al. 2010; Chorti et al. 2010; Rustioni et al. 2016; Scafidi et al. 2011; Azuma et al. 2012; Cohen et al. 2012; Scafidi et al. 2013; Guan et al. 2016; Movahed et al. 2016).

The forecast of climate change scenario shows an increase of temperature and light and a decrease of rainfall (IPCC 2007; Jones 2012) especially in the southern hemisphere countries (Australia, South America, South Africa and New Zealand). Research (Webb 2006) suggests that change in grapevine varieties could be a way to overcome the future climate variation effects in Australia’s major wine regions; nevertheless, a recent study analysed the impacts of climate change on sensitivity of viticultural practices (as pest and disease control, tillage and harvest) and the capacity of wine growers to adapt to changing conditions (Holland & Smit 2010; Neethling et al. 2013). Changes of viticultural practices (pruning technique, leaf thinning, vine under-row management) are more related to local conditions. Moreover, climate variability between vintages is always taken into account in annual wine growers’ practices. In the near future, a seasonal climate variability is predicted to become greater (IPCC 2007); thus the wine growers will try to minimise annual variation in grape yield and quality, by adapting optimal annual and traditional practices. Within all viticultural practices, adaptation in leaf removal and pruning techniques were influenced less by climate change than rootstocks, cover crops and interrow management, and more by physical site characteristics and technological advancements (Neethling et al. 2013).

Although management practices generally aim to minimise the variability in the vineyard, the existence of berry variation is often ignored entirely (Hunter et al.
2010) and many grape growers and winemakers seem not to take these aspects seriously into consideration. The physical and chemical variation between berries in a vineyard (Barbagallo et al. 2011; Pisciotta et al. 2013) can even modify wine style (Melo et al. 2015).

The aim of the research was to study composition of grapes located in two cordons level (upper and lower) and to investigate how canopy management practices (as leaf removal) could eventually reduce grape composition variability and improve berry ripeness between the cordons in Cabernet Sauvignon vines trained to a two-wire vertical trellis and growing in a warm climate condition of northwest Victoria (Australia).

Materials and methods

Field sites and treatments

The study was carried out in an E–W-orientated Cabernet Sauvignon vineyard, located in Mildura, Australia (34°25′ 28.70″ S/ 142°17′ 02.02″ E), trained to a two-wire vertical trellis (Figure 1), with wires 1.1 m and 1.6 m above the ground. Vines were approximately 15 years old. The row spacing was 3 m and in-row vine spacing was 2.44 m. The pruning system was mechanical and the spurs were upwards oriented. Vines were drip irrigated for a total of 3500 m³/ha/year. Weather data were collected through an automated weather station of the Australian Government Bureau of Meterology (http://www.bom.gov.au) located near the vineyard.

Defoliation was manually applied removing all leaves up to the last cluster of all shoots located in the four arms (two upper and two lower cordons). Each trial was randomly replicated three times on three adjacent rows. Leaf removal was applied in all vines (80 vines/row) from the selected three rows per replicate (3) at two different developmental stages: fruit set (23 November) and veraison (14 January). Defoliated treatments were compared to control (or not defoliated vines) represented by 40 vines per replicate (3). Measurements in the control were done in the four arms (two upper and two lower cordons).

Figure 1. Two-wire vertical trellis, vines bilateral cordon trained.
Fruit was analysed from six treatments: (1) Control (C) or not defoliated vines – Upper Cordon; (2) Control (C) or not defoliated vines – Lower Cordon; (3) Defoliated at fruit set (DF) – Upper Cordon; (4) Defoliated at fruit set (DF) – Lower Cordon; (5) Defoliated at veraison (DV) – Upper Cordon; and (6) Defoliated at veraison (DV) – Lower Cordon.

**Estimation of leaf area and light measurement**

To evaluate shoot leaf area, an indirect method was used. A sample of 20 shoots per treatment and per replicate was randomly collected from one of the three selected rows just before and after leaf plucking. Vines, from which shoots were taken, were excluded from following sampling. All leaves of each shoot were removed, counted and weighed, then stacked vertically and a sample taken, using a disc of 2 cm diameter, which was also weighed. To estimate the total shoot leaf area before and after defoliation the following proportion was used: disc area:disc mass = leaf area:leaf mass.

The percentages of removed leaf area for the defoliated treatments and the number of leaves, removed by defoliation and remained on shoot, were reported in all treatments. Shoot numbers per cordon (upper and lower) were counted and leaf areas (m²) per cordon and treatments were calculated.

Light interception in clusters zone was measured as PAR by ceptometer (AccuPAR LP-80, Decagon Devices, Pullman, WA, USA) at 13, 25 and 52 days after fruit set in sunny conditions. The values of PAR (µmol/m²/s) were recorded at midday, keeping the ceptometer horizontally. Light intensity was expressed as percentage of the ambient light level.

**Berry sampling and analysis**

One typical cluster per vine and per replicate was selected from five vines according to similar exposure conditions (visually estimated) and representative of the treatment. The clusters were sampled at veraison (14/01/2008), +21 (04/02/2008) and +42 (25/02/2008) days after veraison. Defoliated treatment at veraison were sampled only two times (+21 and +42 days from veraison/defoliation) All berries were removed from each of the five clusters and two samples of 30 berries were randomly sub-sampled and weighed. Each 30-berry sample represented one field replicate respectively for whole berry weight and skin weight. Skins from each sub-sample were collected by expulsion of the seeds and flesh and then were weighed. The skins were carefully blotted dried by paper towel, weighed and snap frozen in liquid nitrogen before being ground to a fine powder (All Basic grinder, IKA Works, Petaling Jaya, Malaysia), then stored at −80°C until analysed for anthocyanins, flavonols and condensed tannins.

The flesh, when separated from the skins, was crushed, centrifuged and juice TSS (°Brix) and pH measured. Clear juice (5 mL) was diluted in distilled water (25 mL) and analysed for TA. TA was expressed as g/L of tartaric acid (Iland et al. 2004).

**Extraction and analysis of anthocyanins, flavonols and condensed tannins**

Total anthocyanin and flavonol concentration and anthocyanin and flavonol composition were determined by HPLC (Downey et al. 2007; Downey & Rochfort 2008). Anthocyanins and flavonols were extracted in triplicate from weighed aliquots of skin (approx. 0.5 g) in
5 mL of 50% aqueous methanol (v/v). Extracts were mixed by vortex mixer, sonicated for 20 minutes at room temperature, vortexed again and then centrifuged (16,110×g) for 10 minutes. Two hundred microlitres of supernatant were transferred to a HPLC autosampler vial. Anthocyanins were separated by HPLC according to Downey and Rochfort (2008). Anthocyanins were quantified against an external calibration curve of malvidin-3-O-glucoside and expressed as malvidin glucoside equivalents. Flavonols were quantified against an external standard of quercetin-3-O-glucoside and expressed as quercetin glucoside equivalents.

Total tannin concentration from triplicate aliquots of skin and whole berries was determined by protein precipitation following extraction in 70% aqueous acetone, resuspension and reaction with ferric chloride (Harbertson et al. 2002) and expressed at catechin equivalents.

Standards of malvidin-3-O-glucoside, quercetin-3-O-glucoside and catechin were obtained from Extrasynthase, France.

**Statistical analysis**

Means and standard errors were reported. One-way analysis of variance (one-way ANOVA) and Tukey’s HSD test were used at a 5% level of significance (α = 0.05). Tukey’s HSD test was performed within each data sampling and used to compare means of six treatments as described above ((1) Control (C) — Upper Cordon; (2) Control (C) — Lower Cordon; (3) Defoliated at fruit set (DF) — Upper Cordon; (4) Defoliated at fruit set (DF) — Lower Cordon; (5) Defoliated at veraison (DV) — Upper Cordon; (6) Defoliated at veraison (DV) — Lower Cordon).

Two-way ANOVA was used at a 5% level of significance (α = 0.05) for technological parameters but only significant effects were reported. All statistical analyses were performed using SYSTAT 10.

**Results**

**Climatic data**

Weather data are reported in Table 1. In 2008, total rainfall was 202 mm. Rainy months were November and December with 42.6 and 34.8 mm, respectively. The hottest months were from veraison to harvest. January, February and March showed 24, 16 and 19 days with temperature higher than 30°C and with 13, 7 and 15 days with temperature higher than 35°C, respectively.

**Table 1.** Long-term monthly climatic average for temperature (T), number of days with temperatures ≥40°C, ≥35°C and ≥30°C and rainfall in Mildura, Australia.

<table>
<thead>
<tr>
<th></th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
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<tbody>
<tr>
<td>Mean max T (°C)</td>
<td>33.7</td>
<td>30</td>
<td>31.7</td>
<td>23.3</td>
<td>19.8</td>
<td>17.4</td>
<td>15.1</td>
<td>15.6</td>
<td>22.1</td>
<td>26.6</td>
<td>26.8</td>
<td>28.7</td>
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<tr>
<td>Mean min T (°C)</td>
<td>18.3</td>
<td>15.1</td>
<td>14.8</td>
<td>8.8</td>
<td>6.5</td>
<td>6</td>
<td>4.4</td>
<td>4.4</td>
<td>7.1</td>
<td>10.4</td>
<td>13.1</td>
<td>14.4</td>
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<tr>
<td>Rainfall (mm)</td>
<td>19.4</td>
<td>0</td>
<td>11.4</td>
<td>4.8</td>
<td>15.8</td>
<td>14.4</td>
<td>19.6</td>
<td>34.2</td>
<td>2</td>
<td>3</td>
<td>42.6</td>
<td>34.8</td>
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<td>Number of days with temperatures</td>
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<td>0</td>
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<tr>
<td>≥35°C</td>
<td>13</td>
<td>7</td>
<td>15</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>7</td>
<td>6</td>
<td>10</td>
</tr>
</tbody>
</table>


**Light microclimate**

At 13, 25 and 52 days after fruit set the upper cordon of control vines received more light than the lower cordon. Particularly, at 13 and 25 days after fruit set, the recorded values were around 27% and 13% respectively for the upper and the lower cordon while 52 days after, the values increase at 52% and 24% respectively in the upper and lower cordons (Figure 2). At 13 and 25 days after fruit set, in DF treatment, the leaf removal from the upper cordon further increased the light hitting the upper cordon fruit zone (more than 90% compared to the control). This light level was greater than the light hitting the lower cordon fruit zone, which was also increased (100% more than the control). After defoliation at fruit set and veraison of the lower cordon, similar light interception percentage was measured in both the lower cordon of defoliated vines and in the upper cordon of non-defoliated vines (Figure 2). Fifty-two days after fruit set, light interception in the control vines increases in both upper and lower cordons (52% and 24%, respectively) probably because of leaf fall (data not shown). Thereby differences between treatments decreased (Figure 2). At 52 days after fruit, lower cordon in DV treatment received 10% less of light interception than the upper cordon.

**Vegetative characteristics**

The study was conducted in two-wire vertical trellis subjected to mechanical pruning without any complementary manual intervention, hence the shoot number per cordon was high (115 and 61 in the upper and lower cordon). The lower canopies were inferior

![Figure 2. Midday PAR (µmol/m²/s⁻¹) in the cluster zone in the two cordons (upper and lower) of the three treatments (control – C, defoliated at fruit set – DF and veraison – DV) 13, 25 and 52 days after fruit set. Mean and standard error (± s.e.) (n = 3).](image-url)
to upper canopies with respect to shoot number and total leaf area per cordon. In particular, before defoliation, the lower cordon had less 37% of leaf area than upper cordon because of a higher shoot number. The number of leaves removed each time was around 1.5–2 per shoot (Table 2). The biggest relative leaf area reduction was at fruit set, due to the lesser number of leaves per shoot and smaller size of leaves above the clusters at that stage (higher ratio of removed/remaining leaves). For the same reason, the leaf area reduction of lower cordon was greater than the upper cordon. Particularly in C treatment at fruit set, the total leaf area was 858 cm² per shoot and 6.25 m² in the lower cordon, while 1032 cm² per shoot and 9.91 m² in the upper cordon. At veraison, the leaf area of control was 996 cm² per shoot and 9.91 m² in the lower cordon and 1045 cm² per shoot and 12.07 m² in the upper cordon (Table 2). After leaf removal, the lower cordon had always less leaf area than upper cordon (−37% and −52% respectively at fruit set and veraison).

**Berry characteristics**

Berry weight at harvest (42 days after veraison – 25 February) was higher in the upper cordon of non-defoliated vines than all other treatments. Defoliation at fruit set and veraison reduced the differences between the upper and lower cordon, through a weight reduction of berries coming from upper cordon. The skin weight of berries increased more during ripening in DF than in DV and C. Moreover, in defoliated treatments, from 21 to 42 days from veraison, the growth of skin was higher in berries coming from upper rather than those from lower cordons (Table 3).

All defoliated treatments did not affect TSS. The only significant differences were found at veraison, where the defoliated treatment at fruit set had the highest (lower cordon) and the smallest sugar values (highest cordon) (Table 4).

Juice pH was affected by cordon position as shown by two-way analysis results (Table 5). Lower cordon, especially at harvest, generally had higher pH than the upper cordon, whereas defoliation practices had no effect on pH or TA (Table 4).

The total anthocyanin content of berry skin showed no statistical differences between the two cordons in control treatment even if the value was higher in the upper than in the lower cordon. Defoliation at fruit set did not influence the anthocyanin content of the skins of clusters located in the upper cordon, but increased the anthocyanin content of grape skins from the lower cordon at harvest. In the upper cordon, defoliation at veraison showed a comparable anthocyanin accumulation in the skin as the control (Figure 3(a)).

At harvest, DF – Lower Cordon had also a positive effect on individual anthocyanins content of the skin (Figure 3(b–f)) showing the highest contents probably because of a prolonged period of synthesis. Instead, in the other treatments, the maximum values were reached earlier (Figure 3(b–d)). In fact, the content of all forms of anthocyanidins (delphinidin, cyanidin, petunidin, peonidin) except for malvidin decreased from 4 February to 25 February (harvest) in DV and in C – Lower Cordon (Figure 3(b–f)). In C – Upper Cordon, delphinidin, malvidin and peonidin remained stable while petunidin and cyanidin diminished from 21 days after veraison (4 February) to harvest (25 February), whereas DV – Upper Cordon decreased the content of all forms of anthocyanidins.

At harvest, DF – Lower Cordon showed significant different values of the total malvidin (Figure 3(d)) and trihydroxylated anthocyanins (Figure 4(a)) than other treatments in the
Table 2. Leaf area per shoot and per cordon before defoliation, number of leaves/shoot left after and removed by defoliation, shoot number and leaf area (m²) per cordon, leaf area reduction (%) at defoliation in the two cordons (upper and lower) of the three treatments (control – C, defoliated at fruit set – DF and veraison – DV on Cabernet Sauvignon).

<table>
<thead>
<tr>
<th>Phenological stage</th>
<th>Treatment</th>
<th>Cordon</th>
<th>Shoot</th>
<th>n</th>
<th>Shoot area before defoliation</th>
<th>Leaves/shoot left after defoliation</th>
<th>Leaves/shoot removed by defoliation</th>
<th>Leaf area reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit set</td>
<td>C</td>
<td>Upper</td>
<td>115</td>
<td></td>
<td>858 9.91</td>
<td>11.40</td>
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<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>61</td>
<td></td>
<td>1032 6.25</td>
<td>11.11</td>
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<td>0</td>
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<tr>
<td></td>
<td>DF</td>
<td>Upper</td>
<td>590</td>
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<td>Veraison</td>
<td>C</td>
<td>Upper</td>
<td>115</td>
<td></td>
<td>1045 12.07</td>
<td>12.33</td>
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<td>0</td>
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<tr>
<td></td>
<td></td>
<td>Lower</td>
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<td>996 6.07</td>
<td>11.83</td>
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<tr>
<td></td>
<td>DV</td>
<td>Upper</td>
<td>850</td>
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<td>9.78</td>
<td>10.9</td>
<td>1.43</td>
<td>18.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>775</td>
<td></td>
<td>4.73</td>
<td>9.9</td>
<td>1.93</td>
<td>22.18</td>
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</table>
lower and the upper cordon. Defoliation generally shifted the hydroxylation pattern of anthocyanins. There was a decrease in the ratio of tri/dyhydroxylated anthocyanins in the skins of berry from defoliated vines and in the lower cordon. Moreover, in fruits from the upper cordon and from all treatments, the ratio was lower than lower cordon (Figure 4(c)) because of the highest values of dyhydroxylated forms (Figure 4(b)), particularly at 21 days after veraison (4 February). At harvest (25 February) C – Lower Cordon showed significant differences than others treatments (Figure 4(c)).

Total non-acylated anthocyanin glucosides, acetylglucosides and coumarylglucosides showed the same trend reported for total anthocyanins. Early leaf removal in the lower cordon seems to increase the synthesis of non-acylated anthocyanin glucosides, acetylglucosides and coumarylglucosides at veraison (14 January). Non-acylated anthocyanin glucosides at harvest showed the highest content in DF – Lower Cordon, while acetylglucosides values were similar in C – Upper Cordon and DF – Lower Cordon. Coumarylglucosides values of DF – Lower Cordon were not statistically different from the C and DF – Upper Cordon (Figure 5). Furthermore, the percentages of acetylglucoside values (Table 6) were higher in C (both upper and lower) than defoliated treatments and generally decreased from lower to upper cordon from veraison up to 42 days. The percentages of coumarylglucosides anthocyanin remained always stable.

At harvest, there were no differences in tannin concentration between the skins of grapes located in DF – Lower Cordon, C and DF – Upper Cordon. Skin tannins of defoliation at fruit set from veraison to harvest declined less in the lower cordon with respect to the upper cordon. Hence, at harvest, the skin of clusters picked from the lower cordon of fruit set defoliated vines had the same tannin concentration as upper cordon of control vines. The same occurred for tannin concentration in whole berry (Figure 6).

Discussion

An aspect of primary importance in the double vertical cordon system is the shade that the upper cordon produces on the lower cordon also for a higher number of shoot per cordon. As was predictable, upper cordons received more light than lower ones. Defoliation of the lower cordon increased the amount of light exposure of clusters on that cordon to a similar level as clusters the upper cordon of undefoliated vines. At the same way, Wolf et al. (2003)
Table 4. Juice TSS (Brix), pH and TA (g/L) in the two cordons (upper and lower) of the three treatments (control — C, defoliated at fruit set — DF and veraison — DV) at veraison (0) (14 January), +21 days (4 February) and +42 days after (4 February) on Cabernet Sauvignon.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cordon</th>
<th>TSS</th>
<th>pH</th>
<th>TA</th>
<th>TSS</th>
<th>pH</th>
<th>TA</th>
<th>TSS</th>
<th>pH</th>
<th>TA</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>Brix</td>
<td>ab</td>
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<td>3.09</td>
<td>17.67</td>
<td>3.38</td>
<td>b</td>
<td>8.95</td>
<td>22.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>11.27</td>
<td>3.13</td>
<td>23.45</td>
<td>3.49</td>
<td>a</td>
<td>8.31</td>
<td>23.72</td>
<td>3.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Upper</td>
<td>10.97</td>
<td>3.07</td>
<td>23.45</td>
<td>3.43</td>
<td>ab</td>
<td>8.31</td>
<td>23.13</td>
<td>3.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>13.63</td>
<td>3.25</td>
<td>17.59</td>
<td>3.43</td>
<td>ab</td>
<td>8.70</td>
<td>22.77</td>
<td>3.65</td>
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<tr>
<td></td>
<td></td>
<td>Upper</td>
<td>—</td>
<td>18.67</td>
<td>3.43</td>
<td>ab</td>
<td>8.13</td>
<td>22.63</td>
<td>3.61</td>
<td>ab</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lower</td>
<td>—</td>
<td>18.60</td>
<td>3.50</td>
<td>a</td>
<td>7.77</td>
<td>22.47</td>
<td>3.72</td>
<td>a</td>
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<td></td>
<td></td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Note: Lowercase letters indicate significant differences at a 5% level of significance (Tukey’s HSD test); n.s. = not significant. Tukey’s HSD test was performed within each data. *Days after veraison.
showed that on Scott Henry trained vines, the lower canopies were generally inferior to upper canopies with respect to shoot number and total leaf area per shoot and per vine.

Defoliation, at fruit set as well as at veraison, produced, at the last stage of ripening, a decline in the berry mass of clusters located in the upper cordon. This was probably due to the excessive exposure of berries that increased fruit transpiration rates and subsequent berry dehydration (Bergqvist et al. 2001). Before ripening in the upper cordon, and in all sampling dates in the lower cordon fruit, the berry weight of defoliated plants (at fruit set and at veraison) was similar than undefoliated vines. Other authors have reported that defoliation reduced berry weight (Kliewer & Antcliff 1970), but the proportion of leaves removed in those trials was greater than in the current study. Changes in sugar

Table 5. Two-way analysis of variance: main effect (defoliation treatment and cordon position) on juice pH at 21 and 42 days from veraison (dav).

<table>
<thead>
<tr>
<th>Source</th>
<th>F-ratio</th>
<th>P</th>
<th>F-ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>1.589</td>
<td>0.248</td>
<td>1.180</td>
<td>0.341</td>
</tr>
<tr>
<td>Cordon position</td>
<td>11.644</td>
<td>0.006</td>
<td>8.268</td>
<td>0.014</td>
</tr>
<tr>
<td>Treatments × cordon</td>
<td>0.028</td>
<td>0.972</td>
<td>2.326</td>
<td>0.140</td>
</tr>
</tbody>
</table>

aDays after veraison.

Figure 3. (a) Total anthocyanins, (b) total delphinidin, (c) total petunidin, (d) total malvidin, (e) total cyanidin and (f) total peonidin in the two cordon (upper and lower) of the three treatments (control – C, defoliated at fruit set – DF and veraison – DV) at veraison (0) (14 January), +21 days (4 February) and +42 days after (25 February) on Cabernet Sauvignon. Mean and standard error (± s.e.) (expressed as mg/g of fresh skin) (n = 3). Lowercase letters indicate significant differences at a 5% level of significance (Tukey’s HSD test); n.s. = not significant. Tukey’s HSD test was performed within each data.
accumulation have also been previously reported (Bledsoe et al. 1988), with leaf area reduction advancing sugar accumulation. However, at harvest this did not result in an increase in TSS in the fruit.

Many previous studies have shown that shaded bunches have berries with less anthocyanins (Morrison & Noble 1990; Jeong et al. 2004; Cortell & Kennedy 2006; Fujita et al. 2006; Barbagallo et al. 2007; Chorti et al. 2010; Guan et al. 2016). Sun light exposition regulates anthocyanin biosynthesis in some varieties and light may be a limiting factor in the accumulation of anthocyanins during the early stages of ripening (Haselgrove et al. 2000).

Figure 4. (a) Total trihydroxilated, (b) total dihydroxilated and (c) trihydroxilated: dihydroxilated ratio in the two cordons (upper and lower) of the three treatments (control – C, defoliated at fruit set – DF and veraison – DV) at veraison (0) (14 January), +21 days (4 February) and +42 days after (25 February) on Cabernet Sauvignon. Mean and standard error (± s.e.), (n = 3) (expressed as mg/g of fresh skin). Lowercase letters indicate significant differences at a 5% level of significance (Tukey’s HSD test); n.s. = not significant. Tukey’s HSD test was performed within each data.

Figure 5. (a) Total non-acylated glucoside, (b) total acetylglicoside and (c) total coumaroylglicoside anthocyanins of the berry skins in the two cordons (upper and lower) of the three treatments (control – C, defoliated at fruit set – DF and veraison – DV) at veraison (0) (14 January), +21 days (4 February) and +42 days after (25 February) on Cabernet Sauvignon. Mean and standard error (± s.e.) (n = 3) (mg/g of fresh skin). Lowercase letters indicate significant differences at a 5% level of significance (Tukey’s HSD test); n.s. = not significant. Tukey’s HSD test was performed within each data.
In our trial, the lower cordons received about 50% of PAR of the upper cordon fruit zone. Defoliation at fruit set of the lower cordon reduced the gap in PAR with the upper cordon in undefoliated vines and that produced a positive effect in anthocyanin accumulation in fruit from the lower cordon, which was consistent with much of the previously published work.

Defoliation of the upper cordon at fruit set, and defoliation of upper and lower cordons at veraison, did not improve anthocyanin accumulation. Notably, in the upper cordon leaf

Table 6. The percentage of acetylglucoside and coumaroylglucoside anthocyanins in berry skins in the two cordons (upper and lower) of the three treatments (control – C, defoliated at fruit set – DF and veraison – DV) at veraison (0) (14 January), +21 days (4 February) and +42 days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cordon</th>
<th>Acetyl Glucoside</th>
<th>Coumaroyl Glucoside</th>
<th>Acetyl Glucoside</th>
<th>Coumaroyl Glucoside</th>
<th>Acetyl Glucoside</th>
<th>Coumaroyl Glucoside</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>+21 dav</td>
<td>+42 dav</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Upper</td>
<td>38.33</td>
<td>32.75</td>
<td>33.11</td>
<td>10.26</td>
<td>9.59</td>
<td>10.06</td>
</tr>
<tr>
<td></td>
<td>Lower</td>
<td>41.64</td>
<td>36.01</td>
<td>36.24</td>
<td>10.99</td>
<td>9.77</td>
<td>9.96</td>
</tr>
<tr>
<td>DF</td>
<td>Upper</td>
<td>36.32</td>
<td>30.96</td>
<td>30.33</td>
<td>9.88</td>
<td>10.15</td>
<td>10.33</td>
</tr>
<tr>
<td></td>
<td>Lower</td>
<td>39.05</td>
<td>34.32</td>
<td>32.89</td>
<td>10.64</td>
<td>9.92</td>
<td>9.22</td>
</tr>
<tr>
<td>DV</td>
<td>Upper</td>
<td>31.78</td>
<td>31.78</td>
<td>30.56</td>
<td>10.01</td>
<td>9.97</td>
<td>9.33</td>
</tr>
<tr>
<td></td>
<td>Lower</td>
<td>34.21</td>
<td>34.21</td>
<td>32.89</td>
<td>0.97</td>
<td>10.09</td>
<td></td>
</tr>
</tbody>
</table>

*Days after veraison.

Figure 6. Total tannins concentration in (a) berry (expressed as mg/g of fresh berry) and (b) skin (expressed as mg/g of fresh skin) in the two cordons (upper and lower) of the three treatments (control – C, defoliated at fruit set – DF and veraison – DV) at veraison (0) (14 January), +21 days (4 February) and +42 days after (25 February) on Cabernet Sauvignon. Mean and standard error (± s.e.) (n = 3). Lowercase letters indicate significant differences at a 5% level of significance (Tukey’s HSD test); n.s. = not significant. Tukey’s HSD test was performed within each data.
removal was detrimental to anthocyanin accumulation. It is considered likely that berries in that microclimatic situation received adequate light for anthocyanin production, but the higher temperature conditions were a limiting factor for anthocyanin accumulation, either through inhibition of synthesis and/or contribution to oxidative or photo-degradation (Haselgrove et al. 2000; Bergqvist et al. 2001; Mori et al. 2005; Yamane et al. 2006; Mori et al. 2007; Azuma et al. 2012; Movahed et al. 2016; Pastore et al. 2017).

Defoliation generally shifted the hydroxylation pattern of Cabernet Sauvignon grape skin anthocyanins.

The clusters located in the lower cordon received a positive effect from leaf removal at berry set. The better microclimatic condition in the bunch zone of DF – Lower Cordon did not support anthocyanin degradation probably due to the upper cordon shield.

The reduction of tri/dyhydroxylated ratio in the upper cordon was due to the greater increase of dihydroxylated forms. The light had stimulated the higher increase of dihydroxylated forms and, at harvest, tri-dihydroxylated ratio was similar in all treatments except C-Lower Cordon, the most shaded treatment. In shaded bunches, some authors (Spayd et al. 2002; Downey et al. 2004; Ristic et al. 2007; Azuma et al. 2012; Guan et al. 2016) found an increase in the di-substituted, while others (Guidoni et al. 2008; Tarara et al. 2008; Chorti et al. 2010; Scafidi et al. 2011) showed an augmentation of tri-substituted anthocyanins.

Furthermore, the most shaded treatments (C-Lower Cordon) exhibited always the highest percentage of acetylglucoside anthocyanins (Scafidi et al. 2011). Instead, the percentage of coumarate forms was more stable regardless of treatments, results in contrast with those obtained in several studies (Spayd et al. 2002; Downey et al. 2004; Ristic et al. 2007; Tarara et al. 2008; Chorti et al. 2010), probably because of different variety influence.

Defoliation at fruit set in the lower cordon increased the synthesis of tannin and reduced the degradation process. Several authors suggested this decline could be attributed to reduced extractability resulting from the conjugation of proanthocyanidins with other cellular components (Cheynier et al. 1997; Saint-Cricq de Gaulejac et al. 1997; Downey et al. 2003). Furthermore, Kennedy et al. (2000) asserted that oxidative cross-linking of polymers would decrease their extractability. However, at harvest, the skin of clusters picked from lower cordon of the fruit set defoliated vines had the same tannin content as fruit from the upper cordon of control vines.

The seed contribution to tannin content in the berry was not influenced by the different exposure conditions and so no differences were found in whole berry tannin concentrations. Also the generally higher seed tannin content may have masked any differences in skin tannin concentration if these were small. Overall, there was no impact of the defoliation treatments in tannin content consistent with previous reports (Downey et al. 2003).

**Conclusion**

The two-wire vertical trellis is a system that is characterised by a good yield and easy mechanisation of operations. However, a negative aspect of this trellis system, as we demonstrated in this study, is high anthocyanin composition variability between the upper and lower cordons. Flavonoid composition of lower cordon was modified by
defoliation at fruit set and clusters picked from this cordon had characteristics similar to those from upper cordon of non-defoliated vines. Positive effects on skin anthocyanin and tannin concentrations were reached by defoliation at fruit set of lower cordon.

The results reported here are from a single region, making broad generalisations unwise. It is uncertain if there would be regional differences in the observed impact of fruit set defoliation and that would need to be tested across different sites. It is also not known to what extent differences in grape composition would be sustained in wines without undertaking winemaking from a similar trial.

However, the general indication is that exclusively defoliation at fruit set of lower cordon could be a good management strategy to increase berry colour in the lower cordon and reduce variation in flavonoid composition between cordons in a warm climate two-wire vertical trellis production system.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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