Effect of post-veraison source limitation on the accumulation of sugar, anthocyanins and seed tannins in *Vitis vinifera* cv. Sangiovese berries

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Abstract

Background and Aims: Climate change can alter the synchronous accumulation of sugar and other main berry compounds during ripening. The aim of this study was to determine whether post-veraison trimming could delay sugar accumulation and influence the production of anthocyanins and seed tannins in Sangiovese grapes.
Methods and Results: Shoots were trimmed in 2009, 2010 and 2011 when the berry total soluble solids (TSS) reached 15–17°Brix, leaving eight nodes on each main shoot. The accumulation of TSS, anthocyanins and seed tannins was measured during ripening, and yield parameters were recorded at harvest. Grapes from trimmed vines contained a lower TSS in 2009 and 2010, but there was no impact on the concentration of anthocyanins and seed tannins. In 2011, leaf area limitation was insufficient to reduce TSS accumulation, because yield constraints were observed and the leaf area/yield ratio was within the optimal range.
Conclusions: The lower rate of TSS accumulation in berries had no impact on the concentration of anthocyanins and seed tannins, suggesting that this approach could produce grapes with a lower TSS at harvest or delay harvest. In low-vigour vines suffering from water deficit, the post-veraison trimming repeated over the years could reduce yield, which may be responsible for the lack of TSS reduction compared with that of control vines.
Significance of the Study: Our study provides insight into the relationship between TSS accumulation and the production of anthocyanins and seed tannins in the berry in response to post-veraison leaf area reduction.

Keywords: anthocyanin, seed tannin, sugar accumulation, trimming, Vitis vinifera

Introduction

Grapevine (*Vitis vinifera* L.) production is highly sensitive to climate change, which has already caused phenological variations in some viticultural areas that may worsen in the future (Schultz 2000, Duchene and Schneider 2005, Jones et al. 2005). Improved vineyard management techniques have enhanced grape composition, but a warming climate may induce excessive concentration of soluble solids, low total acidity and atypical aroma. Furthermore, the colour intensity of red berries is often lower in warm regions, associated with increasing astringency linked to the unbalanced accumulation of phenolic substances during ripening.

The accumulation of sugars is probably more responsive to variation in temperature than anthocyanin synthesis, because the optimal temperature range for sugar synthesis and transport is 8–33°C, whereas anthocyanin synthesis is favoured at 17–26°C (Coombe 1987). High temperature (above 30–35°C) inhibits anthocyanin accumulation (Kliewer 1970, 1977, Mori et al. 2007a,b, Movahed et al. 2011). A recent report has suggested that elevated temperature may decouple the accumulation of anthocyanins and sugars in berries, thus affecting the colour–alcohol balance of wines (Sadras and Moran 2012).

The relationship between temperature and tannins synthesis is not clear, but seed tannin concentration, which influences wine astringency and sensory properties (Gawel 1998, Vidal et al. 2003, Fernández et al. 2007), is less susceptible to temperature change than that of anthocyanins (Cortell et al. 2007, Ristic et al. 2007). Although soluble solids and anthocyanins begin to accumulate at veraison, tannins are produced several weeks before veraison and their concentration declines thereafter, followed by a plateau during the later stages of berry ripening (Kennedy et al. 2000b, 2002) in which the extractability of grape tannin into wine may be modified due to tannin composition (Hanlin et al. 2010).

Climate change may therefore affect these berry traits in different ways, especially when heat waves occur after veraison, during the later steps of ripening. We therefore developed a hypothesis in which the reduced rate of sugar accumulation may delay technological ripening, allowing the achievement of an adequate evolution of phenolic substances, mainly seed tannins, which may be responsible of excessive wine astringency (Kennedy et al. 2000b).

Berry sugar accumulation can be reduced by increasing the bud number per vine left after winter pruning, as previously reported by several authors after mechanical pruning (Morris and Cawthon 1981, Anderson et al. 1996), but the resulting increase in yield may exceed appellation thresholds. An alternative approach is source limitation, because the rate at which soluble solids accumulate is closely related to the leaf area/yield ratio (Kliewer and Dokoozlian 2005). Several studies have

shown that the removal of the younger and more efficient part of the canopy around veraison may help to reduce sugar accumulation (Intrieri et al. 1983, Reynolds and Wardle 1989, Intrieri 1994, Palliotti et al. 2013).

Although the relationship between the leaf area/yield ratio and sugar accumulation is well documented (Kliewer and Dokoozlian 2005), it is not clear how the leaf area and berry sugar concentration affect the accumulation of phenolic substances. The build-up of anthocyanins in berries is stimulated by sugars (Pirie and Mullins 1976, Larronde et al. 1998, Hiratsuka et al. 2001, Gollop et al. 2002) mainly in the initial phase in which their accumulation is strongly correlated with the increase of soluble solids. Furthermore, it is reported that during the last stage of ripening accumulation of anthocyanins and sugar can be uncoupled (Guidoni et al. 2008). The increase of leaf area to yield ratio, when values do not exceed the threshold of 0.8–1 m^2/kg, has resulted in a higher concentration of soluble solids, anthocyanins and total phenols and increased colour intensity (Reynolds et al. 1994, Guidoni et al. 2002, Filippetti et al. 2007, Pastore et al. 2011). Despite these results, it is not clear how phenolic substances may be influenced by sugar accumulation rate and by the leaf area to yield ratio.

In view of this uncertainty, we set out to determine whether post-veraison trimming can reduce the rate of sugar accumulation and to measure its impact on the accumulation of anthocyanins and seed tannins in Sangiovese berries.

Materials and methods

Plant material and experimental design
The trial was undertaken in 2009, 2010 and 2011, on a Sangiovese vineyard in the experimental field of the University of Bologna, in Ozzano Emilia, Bologna, Italy (latitude 44°25′N; longitude 11°28′W). Sangiovese vines (12T clone), planted in 1994, were grafted onto the 3309 C rootstock. Vines were spaced 1.5 m within the row and 3 m between rows. They were spur-pruned (16 nodes per vine) and trained to the Free Cordon system. Shoots were trimmed after flowering removing just the apical part of the shoots (two to three nodes) as usually applied in the Free Cordon system in order to maintain upward growth. Pest management was carried out according to Emilia-Romagna region standard practices. The soil was silt–clay with a low content of nitrogen and organic matter and the vineyard had no irrigation system.

The experimental design included 24 vines in four blocks (three vines per block and per treatment) randomly assigned, within each block, to the following treatments: (i) untrimmed control vines (C); and (ii) shoot trimming, 1 week after full veraison, which left eight nodes and related laterals [trimmed vines (TRIMM)]. Vines were shoot trimmed manually when the berry total soluble solids (TSS) reached 15–17°Brix: day of the year (DOY) 218 in 2009 (17 days after veraison); DOY 222 in 2010 (17 days after veraison) and DOY 216 in 2011 (6 days after veraison).

Vegetative and yield data
In each year, before trimming and at harvest, leaf area was estimated after determining the linear relationship between main and lateral shoot length (cm) and the corresponding leaf area (cm^2) for 20 shoots, collected from TRIMM and C vines. The leaf area was measured with a LI-3000A leaf area meter (Li-Cor Biosciences, Lincoln, NE, USA). The eight regressions coordinates of the respective equations calculated in each year (data not reported) were then used to estimate leaf area of tagged vines by measuring the length of all main and lateral

shoots before trimming and at harvest. The main and lateral leaf area removed was measured just after trimming with a LI-3000A leaf area metre. The crop of each individual plant was harvested separately and the number of bunches and their mass were recorded. The number of berries was determined on three bunches per vine. Weather data (mean daily air temperature and rainfall) were recorded from April to September in the 3 years, at a meteorological station located close to the experimental site.

Estimation of gas exchange
The leaf assimilation rate (A) was recorded in 2010 and 2011 on one tagged shoot per vine. The readings were taken at the seventh well-exposed main leaf from the base of the shoot and at the fourth well-exposed lateral leaf with a CIRAS-1 portable gas-exchange system (PP Systems, Hertsfordshire, England). The readings were taken before trimming and after treatment at three dates during ripening.

Berry sampling
In each vintage, every 12 days from veraison to harvest, a sample of 20 berries per vine was collected by cutting through the pedicel with scissors. The collected berries were pooled per each block (60 berries) and then divided into three subsamples to determine the following parameters: berry mass and must composition [TSS, pH and titratable acidity (TA)], and the concentration of skin anthocyanins and of seed tannins. Therefore, 240 berries for treatment were collected at each sampling date.

Analysis of must biochemical parameters
Four samples of 20 berries per treatment were weighed and crushed in a manual press. The juice was analysed with a temperature-compensating CR50 refractometer (Maselli Misure Spa, Parma, Italy) to determine TSS. An aliquot of the same must (5 mL) was diluted with seven volumes of double-distilled water and titrated with a Crison Compact Tritator (Crison, Barcelona, Spain) with 1, 0.5 and 0.25 N NaOH (Sigma-Aldrich, St Louis, MO, USA) according to the stage of berry ripening to obtain pH and TA data (expressed as g/L of tartaric acid equivalents).

Analysis of skin anthocyanins
Skin anthocyanins were extracted and separated by HPLC as described by Mattivi et al. (2006) using a Waters 1525 instrument equipped with a diode array detector (DAD) (Waters Corporation, Milford, MA, USA) and a reversed-phase column RP18 250×4 mm (5-μm particle size) with a pre-column (Phenomenex, Castel Maggiore, BO, Italy). Anthocyanins were determined by measuring absorbance at 520 nm. A calibration curve was established with malvidin-3-glucoside standards (Sigma-Aldrich) and the anthocyanins were expressed as mg per berry and mg per kg of berries.

Analysis of seed tannins
Seeds were ground separately to a fine powder under liquid nitrogen, and total tannins were extracted and measured by HPLC as described by Downey et al. (2003) using a Waters 1525 instrument equipped with a DAD and a reversed-phase column RP18 250×4 mm (5 μm particle size) with a pre-column (Phenomenex, Castel Maggiore, BO, Italy). After free monomers were removed, the proanthocyanidin content was determined by acid-catalysed cleavage in the presence of excess phloroglucinol as described by Kennedy and Jones (2001). The abundance of free monomers and cleaved proanthocyanidins

was determined by individual reversed-phase HPLC separations and by measuring absorbance at 280 nm (Downey et al. 2003). The concentration of free monomers and hydrolysed terminal subunits was determined from standard curves prepared from commercial standards of (+)-catechin, (−)-epicatechin and (−)-epicatechin-3-O-gallate (Extrasynthese, Genay Cedex, France). The concentration of extension subunit-phloroglucinol adducts was calculated from published molar extinction coefficients (Kennedy and Jones 2001). The content of seed tannin fractions (monomers, extension subunits and terminal subunits) was expressed as mg per berry from veraison to harvest. Total tannins per berry and the proportion of flavan-3-ols were evaluated at harvest.

Statistical analysis

All data were processed by analysis of variance using the mixed procedure available in SAS v9.0 (SAS Institute, Inc., Cary, NC, USA). Treatment comparisons were analysed using the Tukey test with a cut-off at $P \leq 0.05$.

The results of trends of leaf assimilation, TSS, anthocyanins, berry and seed mass, free monomers, tannins and proportion of anthocyanins at harvest are shown as means ± standard error.

Results

Climatic data

The active heat summation calculated using base 10°C degree days from 1 April until 30 September (Figure 1) was highest in 2011 (2054) and lowest in 2010 (1718). Furthermore, the average daily temperature recorded from veraison to harvest in 2011 was mostly above 25°C (70% of the period). In 2011, the rainfall from April to September was only 136 mm, whereas in 2010 over the same period it was three times greater (Figure 1).

Vegetative and productive traits

All vines showed uniform leaf area development before trimming in all three vintages (data not shown). The TRIMM treatment reduced the leaf area by approximately 60% in 2009 and 2010 and by approximately 40% in 2011 compared with those of the control. The trimming predominantly affected the leaves on lateral shoots, especially in 2010 (Table 1). The leaf area in 2010 was higher than that in 2009, and there was no post-treatment lateral regrowth in any of the vintages.

Starting from a uniform bunch number per metre of cordon, the yield, bunch mass and berry mass were unaffected by trimming in 2009 (Table 1). In 2010 and 2011, the yield and bunch mass were significantly reduced in the TRIMM vines compared with those of the controls, whereas berry mass was unaffected (Table 1). We noted year-to-year variability in yield, and particularly in 2010, the yield was higher for both treatments (Table 1). The number of berries per bunch was also significantly lower in TRIMM vines compared with that of the controls in 2010 and 2011 (Table 1).

At harvest, the leaf area/yield ratio in TRIMM vines was substantially reduced to 0.38 and 0.39 m²/kg in 2009 and 2010, respectively, compared with 1.01 and 0.82 m²/kg in the C vines (Table 1). In 2011, the TRIMM leaf area/yield ratio 0.93 m²/kg was not significantly different from that of the C vines (Table 1).

Gas-exchange measurements

Leaf gas exchange was not affected by trimming in the main or lateral leaves in 2010 and 2011, remaining the same before
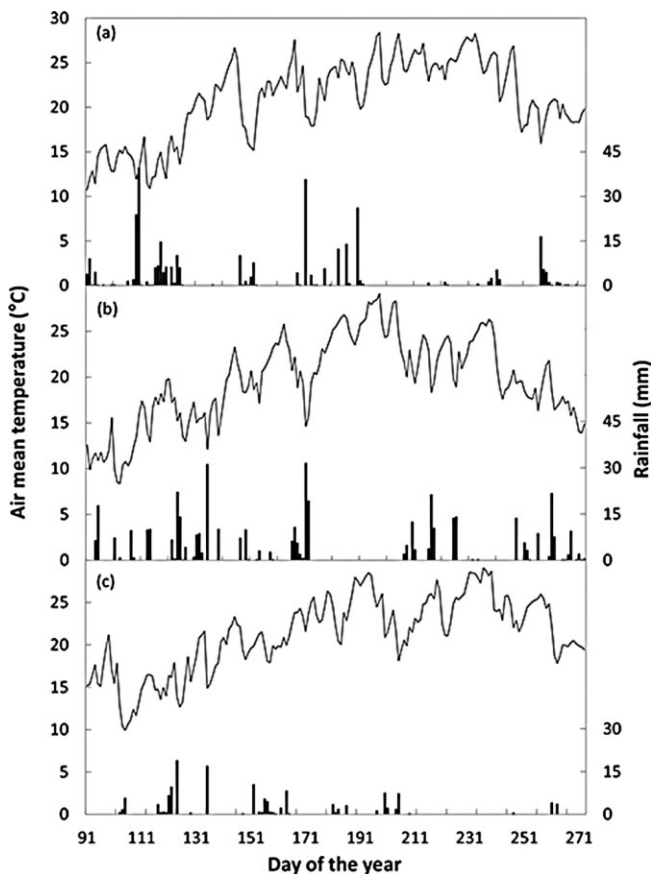
Figure 1. Seasonal trends (1 April–30 September) of diurnal air mean temperature recorded in (a) 2009, (b) 2010 and (c) 2011, close to the trial site. Vertical bars indicate daily rainfall. The degree days and the total rainfall from 1 April until 30 September were, respectively, 1963 and 317 mm in 2009, 1718 and 432 mm in 2010 and 2054 and 136 mm in 2011.

and after treatment (Figure 2). Mean assimilation (A) values remained stable during the season in 2010, but declined over the same period in 2011.

Total soluble solids, pH and total acidity

In 2009 and in 2010, TSS (°Brix) in berries from TRIMM vines at harvest were significantly lower than that of C berries, but no different to C berries in 2011 (Table 2, Figure 3). The TA and pH of the berries did not change following treatment in any of the vintages (Table 2). The daily TSS accumulation rate declined after trimming in 2009 by 8% and in 2010 by 16%, but there was no similar decline in 2011 (Figure 3).

The accumulation of TSS increased sharply under both treatments in 2011. The TSS of C berries reached 24°Brix 23 days after veraison in 2011, but 42 days after veraison in 2009 and 58 days after veraison in 2010 (Figure 3). Significant Y × T interaction and significant year effect were registered for TSS. The year effect was also statistically significant for TA and pH (Table 2).

Anthocyanins

The concentration of anthocyanins in the berries (mg/kg) did not vary among treatments at harvest in any of the vintages, but there were significant differences between vintages, with the highest concentration recorded in 2011 (Table 2). The evolution of anthocyanins during ripening, expressed on a per-berry basis,

Table 1. Vegetative growth parameters, yield components and leaf area/yield ratio recorded between 2009 and 2011 in control and post-veraison trimmed Sangiovese vines.

Parameters	2009		2010		2011		Year effect	Year × treatment interaction
	C	TRIMM	C	TRIMM	C	TRIMM		
Main LA after trimming (m²/m of cordon)	1.44 a	0.56 b	1.77 a	0.96 b	1.21 a	0.71 b	ns	ns
Lateral LA after trimming (m²/m of cordon)	0.79 a	0.25 b	1.12 a	0.18 b	0.59 a	0.30 b	ns	ns
Total LA after trimming (m²/m of cordon)	2.23 a	0.81 b	2.89 a	1.14 b	1.80 a	1.01 b	*	ns
Bunches (n/m of cordon)	10.5 a	10.6 a	11.2 a	10.2 a	10.1 a	10.2 a	ns	ns
Yield (kg/m of cordon)	2.2 a	2.1 a	3.5 a	2.9 b	1.4 a	1.1 b	**	ns
Bunch mass (g)	210.8 a	208.6 a	310.4 a	280.2 b	147.3 a	104.5 b	**	ns
Berry mass (g)	1.54 a	1.60 a	1.78 a	1.72 a	1.30 a	1.33 a	*	ns
Berries per bunch (n)	136 a	131 a	173 a	161 b	112 a	76 b	*	ns
Leaf area /yield† (m²/kg)	1.01 a	0.38 b	0.82 a	0.39 b	1.25 a	0.93 a	*	ns

*Significant at $P < 0.05$; ** significant at $P < 0.01$; ns, not significant. †This ratio was calculated from leaf area after treatment because no regrowth was observed at harvest on the C and TRIMM vines. Means within rows and years designated by different letters are significantly different according to the Tukey test. C, control vines; LA, leaf area; n, number; TRIMM, trimmed vines.
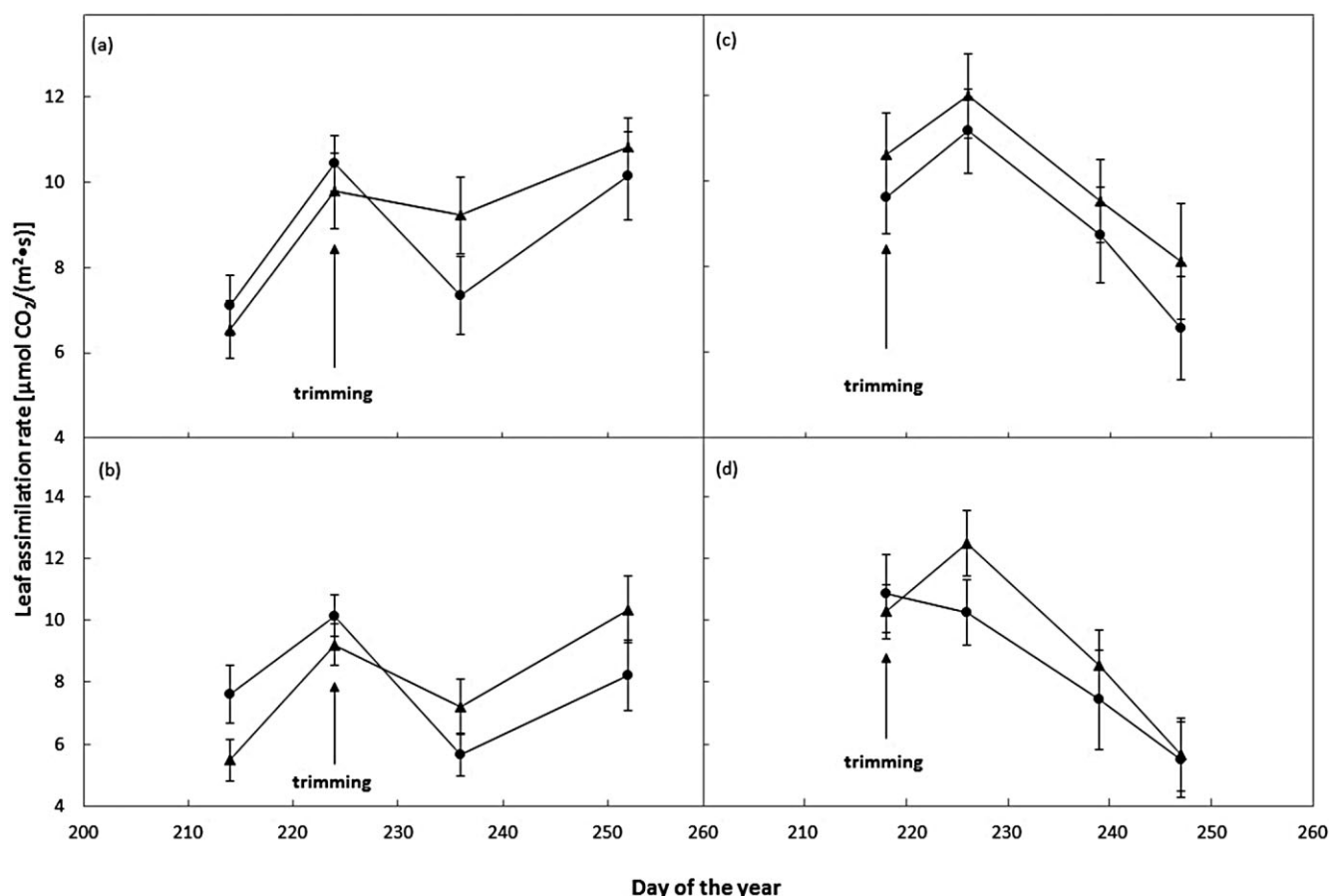
Figure 2. Leaf assimilation rate in (a,c) lateral leaves at fourth node and in (b,d) main leaves at seventh node in control (●) and post-veraison trimmed (▲) Sangiovese vines in (a, b) 2010 and (c, d) 2011. Veraison corresponds to day of the year (DOY) 205 in 2010 and DOY 210, in 2011. Arrowheads indicate time of trimming. Data are means (n = 12) ± standard error represented by bars.

did not differ among treatments (Figure 4). The proportion of delphinidin-3-G, cyanidin 3-G, peonidin-3-G, petunidin 3-G and malvidin-3-G was typical for Sangiovese berries in both the TRIMM and C vines, with a high concentration of cyanidin-3-G and malvidin-3-G (Figure 5).

Berry and seed mass development
The change in berry mass from veraison to harvest did not differ significantly between treatments in any of the vintages. In 2009 and 2011, the gain in berry mass was slow compared with that in 2010. In 2009, C berries reached 0.14 g and TRIMM berries

Table 2. Berry composition recorded at harvest between 2009 and 2011 in control and post-veraison trimmed Sangiovese vines.
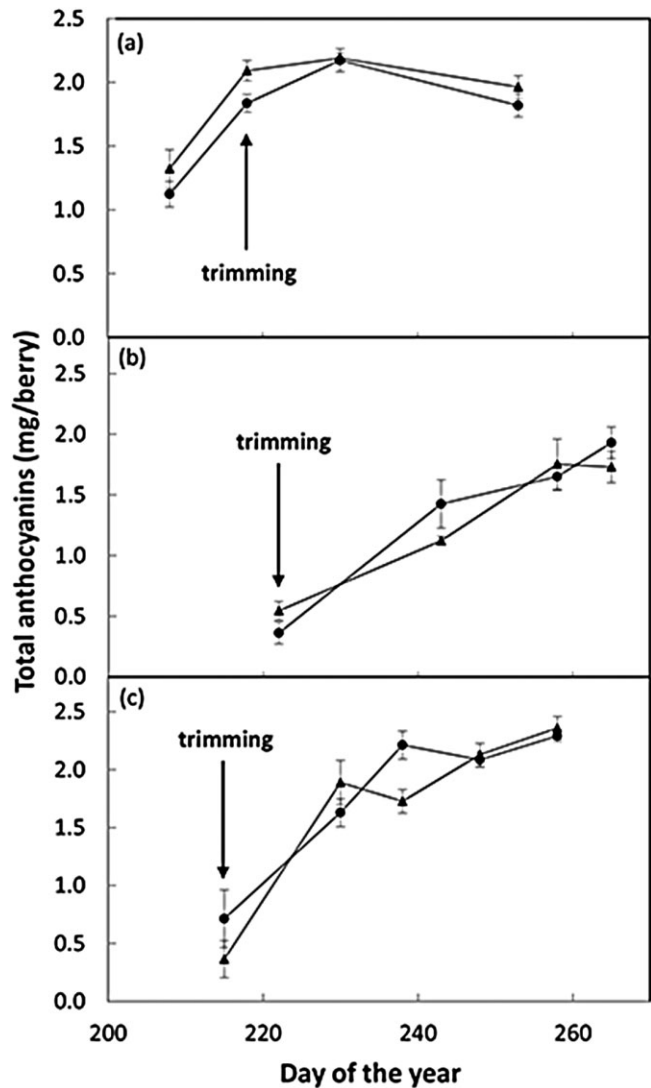
Parameters	2009		2010		2011		Year effect	Year × treatment interaction
	C	TRIMM	C	TRIMM	C	TRIMM		
Total soluble solids (°Brix)	25.2 a	24.6 b	23.7 a	23 b	26.4 a	27.2 a	*	*
Total acidity (g/L)	7.38 a	7.23 a	7.79 a	7.96 a	8.44 a	8.91 a	*	ns
pH	3.53 a	3.47 a	3.39 a	3.36 a	3.39 a	3.36 a	*	ns
Anthocyanins (mg/kg berries)	1194.1 a	1257.1 a	1117.7 a	1120.0 a	1708.0 a	1810.0 a	*	ns

*Significant at $P < 0.05$; ns, not significant. Means within rows and years designated by different letters are significantly different according to the Tukey test. C, control vines; TRIMM, trimmed vines.
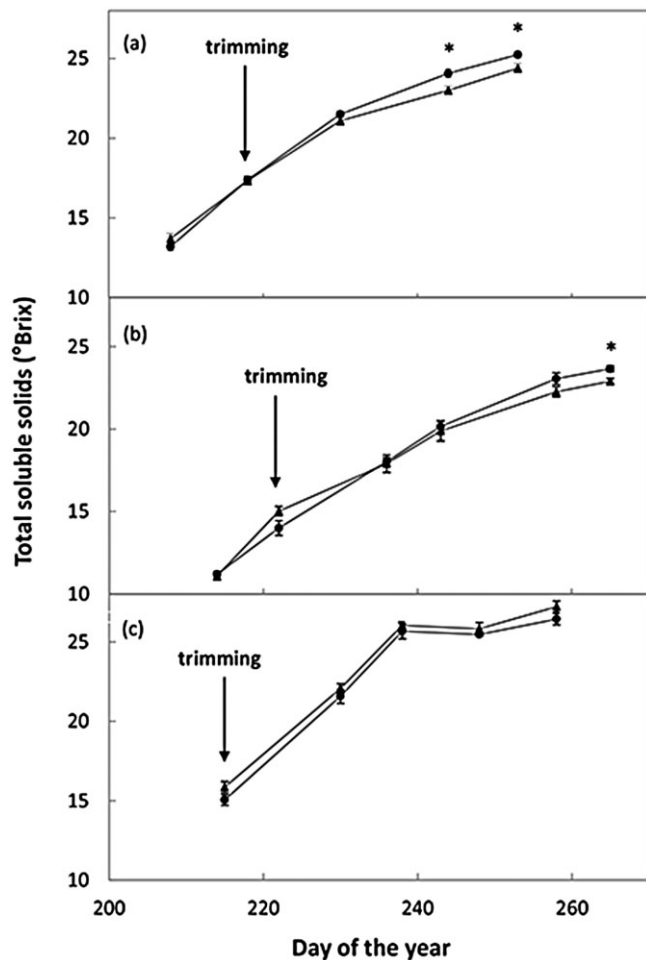

Figure 3. Total soluble solids accumulation in berries of control (●) and post-veraison trimmed (▲) Sangiovese vines in (a) 2009, (b) 2010 and (c) 2011. Veraison corresponds to day of the year (DOY) 201 in 2009, DOY 205 in 2010 and DOY 210 in 2011. Arrowheads indicate time of trimming. Data are means (n = 4) ± standard error represented by bars. Asterisks indicate significant differences between the treatments within data according to the Tukey test $P \leq 0.05$.

Figure 4. Content of anthocyanins in berries of control (●) and post-veraison trimmed (▲) Sangiovese vines in (a) 2009, (b) 2010 and (c) 2011. Veraison corresponds to day of the year (DOY) 201 in 2009, DOY 205 in 2010 and DOY 210 in 2011. Arrowheads indicate time of trimming. Data are means (n = 4) ± standard error represented by bars.

reached 0.22 g after 42 days, achieving final average mass of 1.54 and 1.60 g, respectively. In 2011, berries required 44 days to gain a similar amount of mass, achieving final average mass of 1.30 g (C) and 1.33 g (TRIMM). In contrast, the 2010 berries followed the typical trend for the third stage of ripening, with C berries increasing in mass from 1.35 to 1.78 g and from 1.27 to 1.72 g in the TRIMM berries (Figure 6).

The fresh seed mass did not differ significantly between treatments, although TRIMM seeds weighed less than C seeds (Figure 6). In 2009 and 2011, no gain in fresh seed mass was observed during ripening, and the values for both treatments at harvest were 0.04 g in 2009 and 0.035 g in 2011. A small
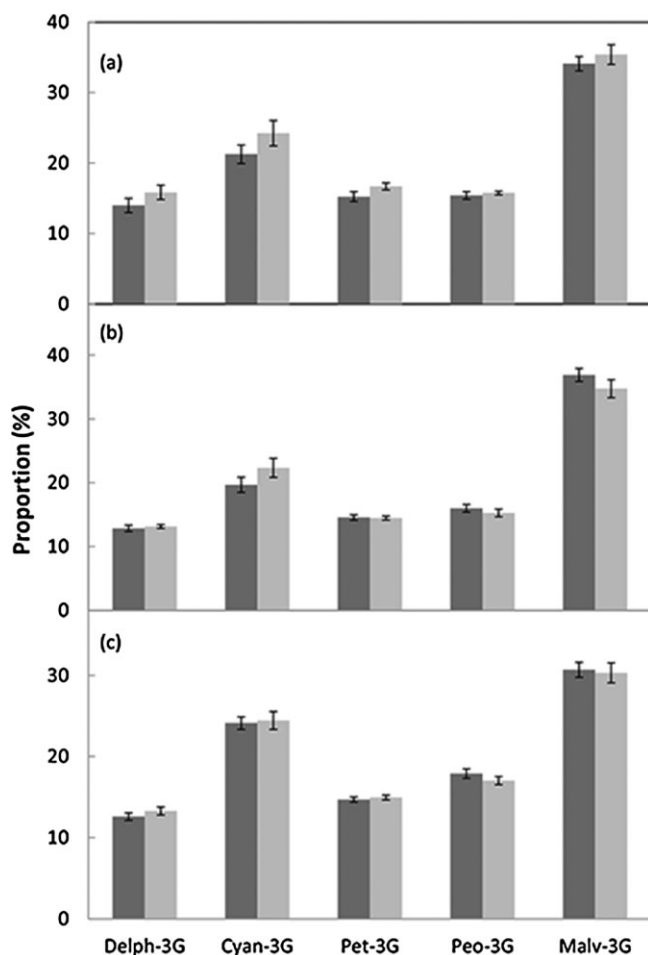
Figure 5. Proportion of individual anthocyanins at harvest in berries of control (■) and post-veraison trimmed (■) Sangiovese vines in (a) 2009, (b) 2010 and (c) 2011. Delphinidin-3-glucoside (delph-3G); cyanidin-3-glucoside (cyan-3G); petunidin-3-glucoside (pet-3G); peonidin-3-glucoside (peo-3G); malvidin-3-glucoside (malv-3G). Data are means (n = 4) ± standard error represented by bars.

Figure 6. Berry mass of control (●) and post-veraison trimmed (▲) and seed mass of control (○) and post-veraison trimmed (Δ) Sangiovese vines in (a) 2009, (b) 2010 and (c) 2011. Veraison corresponds to day of the year (DOY) 201 in 2009, DOY 205 in 2010 and DOY 210 in 2011. Arrowheads indicate time of trimming. Data area means (n = 4) ± standard error represented by bars.
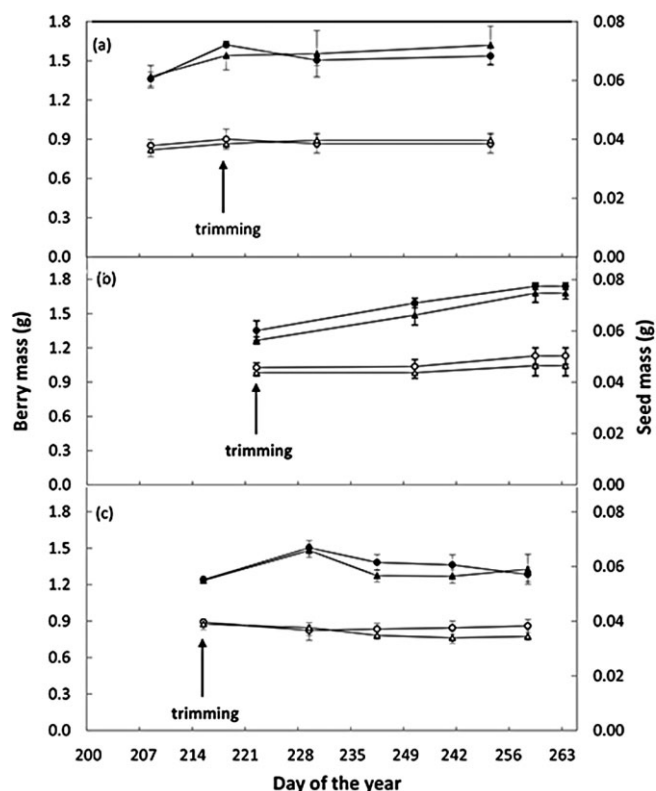
increase, however, in seed mass from veraison to harvest was observed in 2010 (Figure 6). The number of seeds per berry was unaffected by the treatments; the values for C berries were 1.92, 2.33 and 2.13 in the three consecutive vintages, whereas for the TRIMM berries, the equivalent values were 1.95, 2.13 and 2.08.

Free monomer accumulation in seeds
The free monomers, catechin, epicatechin and epicatechin-gallate, were measured in acetone extracts that had not been subjected to acid-catalysis. In all three vintages, the free monomer content declined strongly after treatment (post-veraison) and continued to decline thereafter until harvest, with no significant difference between the treatments. The post-veraison losses of monomers in 2009, 2010 and 2011 were 0.45, 0.47 and 0.75 mg/berry, respectively. Despite the higher values observed on the first sampling date in 2011, the monomer content at harvest was similar in all vintages (Figure 7).

Seed tannin accumulation
Tannins were analysed following acid-catalysed hydrolysis in the presence of excess phloroglucinol to determine the quantity of extension and terminal subunits. There was no significant

difference in the accumulation of extension subunits between treatments, despite a marginal difference in the accumulation profile in the three vintages. In 2009 and 2011, the abundance of extension subunits declined slowly, whereas in 2010, there was a post-veraison decline followed by an increase at harvest, especially in the C group (Figure 8).

There was also no significant difference in the accumulation of terminal subunits between treatments. The abundance of terminal subunits declined in all three vintages, but whereas this trend was slow and steady in 2009 and 2011, there was a more dramatic post-veraison reduction in 2010 followed by a plateau that remained until harvest (Figure 9).

*Proportion of flavan-3-ols at harvest*
There was no difference between treatments in terms of the seed tannin content per berry and the proportion made up of flavan-3-ols (Table 3). In all three vintages, epicatechin was the most abundant tannin (representing more than 50% of the total) and catechin was the least abundant (less than 20%). The proportion of epicatechin-gallate ranged from 20 to 27%.

Discussion
The source limitation induced by post-veraison trimming in 2009, 2010 and 2011 proved to be effective in reducing the accumulation of TSS in 2009 and 2010, as expected following the late removal of approximately 50% of leaves of the shoots, mostly affecting the laterals. It is well known that the accumulation of sugar depends on the active leaf area available during
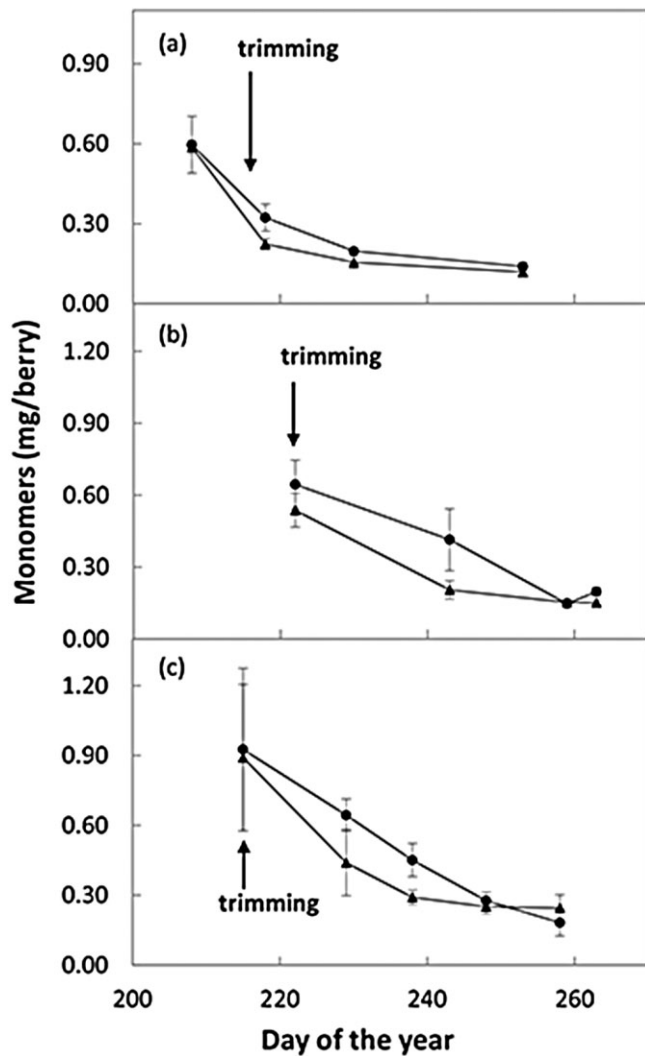
Figure 7. Free monomer content in seeds of control (●) and post-veraison trimmed (▲) Sangiovese vines in (a) 2009, (b) 2010 and (c) 2011. Veraison corresponds to day of the year (DOY) 201 in 2009, DOY 205 in 2010 and DOY 210 in 2011. Arrowheads indicate time of trimming. Data are means (n = 4) ± standard error represented by bars.
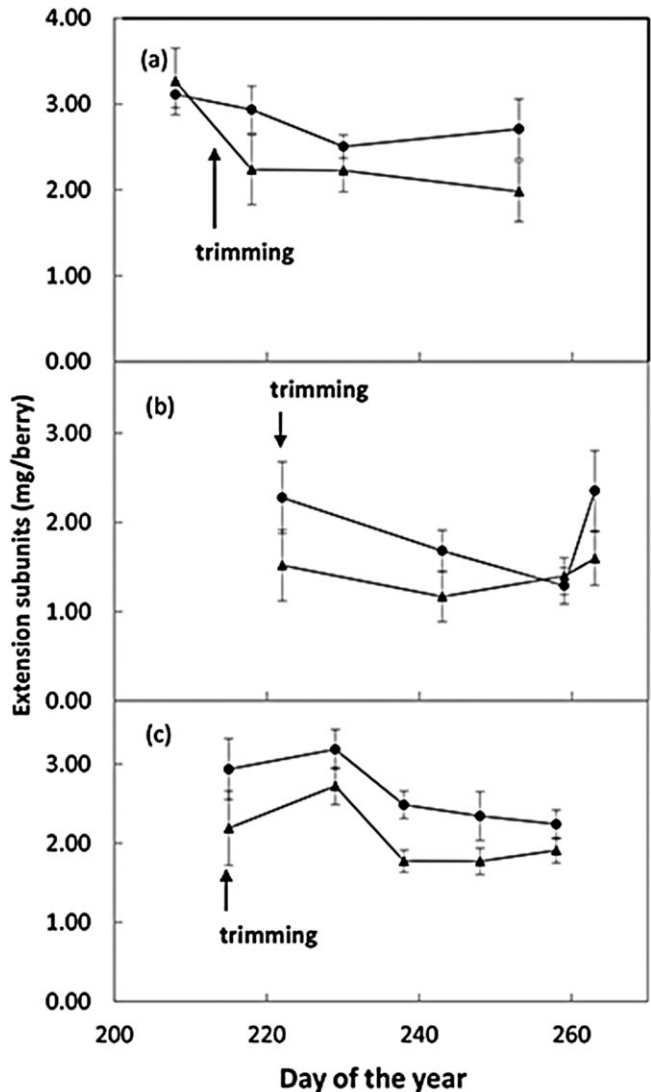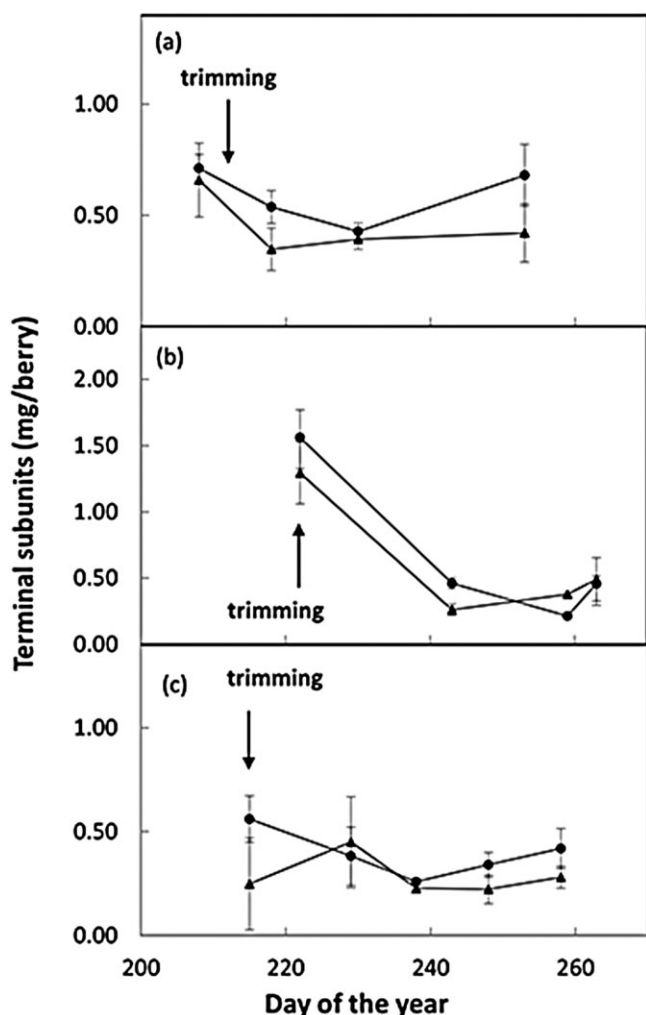
Figure 8. Content of extension subunits in seeds of control (●) and post-veraison trimmed (▲) Sangiovese vines in (a) 2009, (b) 2010 and (c) 2011. Veraison corresponds to day of the year (DOY) 201 in 2009, DOY 205 in 2010 and DOY 210 in 2011. Arrowheads indicate time of trimming. Data are means (n = 4) ± standard error represented by bars.

the period between veraison and harvest (Mansfield and Howell 1981, Candolfi Vasconcelos and Koblet 1990) and that the older and larger leaves in the bunch zone contribute less to post-veraison whole-vine photosynthesis than young leaves inserted at the top of the main shoots and on lateral shoots (Candolfi Vasconcelos and Koblet 1991, Poni et al. 1994). Candolfi Vasconcelos and Koblet (1990) showed that lateral leaves are the most important contributors to the accumulation of sugars in the fruit and starch in the wood.

The impact of the available leaf area on berry sugar accumulation may be conditioned by yield, and our data confirmed a strong correlation between the accumulation of TSS and the leaf area/yield ratio at harvest. In 2009 and 2010, the leaf area/yield ratio of vines trimmed after veraison fell below the level required to achieve adequate berry ripening (Table 1), ranging from 0.7 to 1.4 m²/kg (Smart and Robinson 1991, Jackson and Lombard 1993, Kliewer and Dokoozlian 2005). In 2011, the maintenance of normal sugar accumulation in treated vines may reflect the significant increase in the leaf area/yield ratio, which results from yield restriction. The analysis of yield

in 2011 showed that this effect might result almost entirely from bunch mass reduction and a lower number of berries per bunch, because berry mass was not affected by the treatment (Table 1). The yield of the TRIMM vines in 2010 was also reduced compared with that of the C vines, but this was not enough to counterbalance the leaf area reduction caused by post-veraison trimming. Previous studies have reported a yield reduction following the removal of all leaves (Howell 2001), but under other conditions, there was no response in terms of yield (Peterson and Smart 1975, Candolfi Vasconcelos and Koblet 1990, Palliotti et al. 2013) because defoliation may be partially compensated by an increase in the physiological efficiency of the remaining leaves or by the development of leaves on lateral shoots.

The vines in this study were not fertilised or irrigated and generally showed low vigour. No lateral shoot regrowth was observed after late leaf removal, and no compensatory increase in photosynthesis, based on single leaf gas-exchange readings, was recorded in either the leaves of the main shoots or lateral

Figure 9. Content of terminal subunits in seeds of control (●) and post-veraison trimmed (▲) Sangiovese vines in (a) 2009, (b) 2010 and (c) 2011. Veraison corresponds to day of the year (DOY) 201 in 2009, DOY 205 in 2010 and DOY 210 in 2011. Arrowheads indicate time of trimming. Data are means (n = 4) ± standard error represented by bars.

shoots in 2010 or 2011, as previously reported following earlier leaf removal (Candolfi Vasconcelos et al. 1994). The differential leaf assimilation rates in 2010 and 2011 probably reflected the climate, which was wetter and cooler in 2010. It should be noted that the dry and hot seasonal conditions in 2011 were associated with the lowest yield, bunch and berry mass registered in both treatments (Figure 1 and Table 1). In Semillon vines growing in hot arid climates, complete defoliation at harvest reduced the total amount of non-structural carbohydrates in the roots and also reduced fruit production in the subsequent two seasons (Holzapfel et al. 2006, Smith and Holzapfel 2009). It is likely that post-veraison leaf reduction may also have a similar effect, despite a recent report suggesting the similar replenishment of soluble sugars and starch in the canes and roots of control vines and those subject to post-veraison defoliation (Palliotti et al. 2013). Notably, the latter study involved the removal of a comparatively small amount of foliage (35%, compared with >50% in our experiments), and the canes and apical leaves were maintained after machine defoliation (Palliotti et al. 2013).

The potential depletion of carbohydrate reserves by trimming in 2009 and 2010 could be responsible for yield constraint,

reflecting the observed reduction of berry number per bunch (Table 1). The lower carbohydrate content in defoliated vines may have also reduced the number of flowers per inflorescence (Scholefield et al. 1977, Bennet et al. 2005) as well as the berry-set (May et al. 1969, Caspari and Lang 1996, Poni et al. 2006, Intrieri et al. 2008).

Vines in this study were not irrigated and were, therefore, dependent on rainwater, which was suboptimal for vegetative and productive growth between April and September (300 mm average, Figure 1). Water stress has a strong inhibitory effect on fruitset (Holt et al. 2010) and berry size (Roby et al. 2004, Girona et al. 2009). Under these conditions, it is likely that water deficit may have reduced the assimilation capacity of the remaining leaves (Figure 2) and may have thus exacerbated the negative impact on carbohydrate reserves and yield. The higher yield and leaf area recorded in 2010 for both treatments probably reflected the more adequate rainfall predominantly during the first phase of shoot and berry growth (Figure 1), which may have improved the final berry and bunch size. This is supported by the berry growth characteristics during the third stage of ripening, which followed the typical double sigmoidal profile in 2010 alone (Figure 6).

There was no variation in the concentration of anthocyanins (mg/kg of berries) among the treatments at harvest and no relationship between the reduced accumulation of TSS in the 2009 and 2010 TRIMM vines compared with that of C vines, and the concentration of anthocyanins at harvest (Table 2). The analysis of anthocyanins (mg per berry) accumulation during the season confirmed the overall lack of differences among treatments (Figure 4).

One of the goals of this study was to determine whether reducing the rate of sugar accumulation by post-veraison trimming had an impact on anthocyanins synthesis. Although the treatment was effective in 2009 and 2010, there was no consistent relationship between sugar and anthocyanins accumulation across the two seasons. Only our 2009 results agree with the previous report demonstrating that anthocyanins accumulate in two phases with initial rapid accumulation strongly correlated with the abundance of sugars, followed by a second phase where anthocyanins and sugar accumulation can be uncoupled (Guidoni et al. 2008). In the second accumulation phase, in fact, the abundance of anthocyanins is more profoundly affected by the climate, particularly the temperature, rather than differences in the source/sink ratio (Winkler et al. 1962, Kliewer 1970, Buttrose et al. 1971, Kliewer and Torres 1972, Spayd et al. 2002, Jones et al. 2005, Mori et al. 2005, 2007a,b, Yamane et al. 2006). High diurnal temperature after veraison generally reduces the concentration of anthocyanins of berries (Yamane et al. 2006, Mori et al. 2007a) and can delay their accumulation: when this occurs, the decoupling of anthocyanins and sugar biosynthesis can take place and thus, in berries ripened at high temperature, a lower anthocyanin/sugar ratio is present and manipulation of the source/sink ratio had no clear impact.

Our trimming treatment, applied after veraison at the end of the initial phase of accumulation of anthocyanins, had no impact on the accumulation of anthocyanins. Moreover, when post-veraison source limitation was effective in reducing sugar accumulation, as in 2009 and 2010, the anthocyanin/sugar ratio tended to increase at harvest (as it is possible to infer from Table 2 data), but this observation needs to be analysed more thoroughly.

We recorded different trends in the accumulation of anthocyanins (Figure 4) and different final concentration (Table 2) among the vintages, which were not correlated with

Table 3. Total tannins and proportion of flavan-3-ols recorded in seeds at harvest between 2009 and 2011 in control and post-veraison trimmed Sangiovese vines.

Parameters	2009		2010		2011		Year effect	Year × treatment interaction
	C	TRIMM	C	TRIMM	C	TRIMM		
Total seed tannins (mg/berry)	3.53 a	2.52 a	3.01 a	2.24 a	2.96 a	2.25 a	ns	ns
Catechin (%)	16.8 a	15.7 a	15.9 a	14.0 b	17.1 a	17.8 a	ns	ns
Epicatechin (%)	58.5 a	58.8 a	59.5 a	65.6 a	55.9 a	55.3 a	ns	ns
Epicatechin-3-O-gallate (%)	24.7 a	25.5 a	24.6 a	20.4 a	27.0 a	26.9 a	ns	ns

ns, not significant. Means within rows and years designated by different letters are significantly different according to the Tukey test. C, control vines; TRIMM, trimmed vines.

the reported climactic conditions. The average diurnal temperature was highest in 2011 (although comparable to 2009), but both the vine yield and the berry mass were the lowest in 2011. These conditions may explain the high concentration of anthocyanins registered in 2011 compared with those of the previous two vintages (Table 2). The anthocyanin composition at harvest did not vary significantly between the treatments in any of the vintages (Figure 5). The biosynthesis of individual anthocyanins therefore does not appear to be influenced by the sugar concentration in the berries at harvest, as observed in 2009 and 2010. Under our conditions, trimming does not appear to alter the bunch microclimate enough to influence the anthocyanin profile, unlike that reported after bunch shading (Downey et al. 2004) or with temperature variation (Mori et al. 2005).

We also investigated the accumulation of seed tannins from veraison to harvest, and found that the abundance of these compounds was not significantly influenced by trimming. Previous studies have shown that tannins accumulate rapidly to a peak value followed by a gradual decline during seed drying and maturation (Ristic and Iland 2005). Our data correspond to the second period and confirm this trend in 2009 and 2011. Interestingly, the seed tannins continued to accumulate throughout the last phase in the 2010 vintage.

Our seed compositional analysis also showed that the abundance of flavan-3-ol monomers declined after veraison, reaching a similar value in each of the three vintages. The observed changes were consistent with previous studies with different cultivars, where flavan-3-ol monomers accumulated before veraison and declined thereafter (Kennedy et al. 2000a,b, Jordao et al. 2001, Downey et al. 2003). These previous studies showed a similar decline in the abundance of extension and terminal subunits (Downey et al. 2003); however, although we also observed a post-veraison decline in extension subunits despite some seasonal variability, the decline in terminal subunits was most prominent in the 2010 vintage. The overall reduction in seed proanthocyanidins we observed between veraison and harvest probably reflected the oxidation of seed tannins, as reported in other cultivars (Kennedy et al. 2000b, 2002).

Our treatment did not affect the proportion of individual flavan-3-ol units at harvest, particularly in the case of epicatechin-3-O-gallate. This is relevant because seed tannins have a greater impact than skin tannins on wine astringency, probably due to the higher degrees of galloylation in seeds (Ricardo da Silva et al. 1991, Sarni-Manchado et al. 1999, Bacon and Rhodes 2000). Because the astringency traits of red wines improve with grape ripening, it will be interesting to investigate in more detail any correlation between tannin accumulation during ripening, extractability in wine-like solutions and the sensory properties of wines, although some authors have suggested that other factors may be also important in determining the perception of tannin astringency, such as the berry polysaccharide content (Pastor del Rio and Kennedy 2006).

Conclusions

The source limitation induced by the post-veraison trimming of Sangiovese vines in 2009 and 2010 produced berries with a lower concentration of TSS at harvest, but with no difference in pH, TA or the concentration and composition of skin anthocyanins and seed tannins, compared with that of control berries.

Under the dry conditions that prevailed during the study, leaves of unirrigated vines did not exhibit a compensatory increase in photosynthesis in response to trimming. Neither was there any compensation in leaf area through the growth of lateral shoots. Consequently, the resulting yield penalty in the TRIMM vines compared with that of the C vines probably may have reflected a decline in carbohydrate reserves. In 2011, characterised by high temperature and dry conditions, the leaf area reduction due to trimming was associated with a yield reduction, so the leaf area/yield ratio remained unaffected and had no significant impact on the rate of sugar accumulation in berries.

Nevertheless, post-veraison trimming should be applied, according to climatic seasonal conditions and preferably not over consecutive seasons, to achieve lower sugar accumulation in bunches at harvest without affecting the concentration and composition of anthocyanins and seed tannins in the berry.

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