Influence of mechanical postveraison leaf removal apical to the cluster zone on delay of fruit ripening in Sangiovese (*Vitis vinifera* L.) grapevines

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Abstract

Background and Aims: Postveraison limitation of canopy photosynthesis delays grape berry ripening and reduces sugar accumulation, thus lowering the alcohol content of the subsequent wines. This study was designed to evaluate whether similar results could be obtained by defoliation apical to the bunch zone using a leaf-plucking machine when berry sugar content was approximately 16–17°Brix.

Methods and Results: In 2011 and 2012, defoliation treatments were applied postveraison to cv. Sangiovese vines (D) on either side of each row using a mechanical leaf remover, and these D vines were compared to a nondefoliated control (C). The machine removed 35% of the leaves on the vine and created a 50-cm vertical window without leaves above the bunch area, but retained a few leaves at the canopy apex (about 0.50 m^2/vine). In both years, leaf removal reduced the rate of berry sugar accumulation and led to a 1.2 lower harvest °Brix and consequently, a lower wine alcohol (−0.6%) content in D relative to that of C vines. In 2012, sugar content of D vines, monitored in a group of vines that was not harvested, had recovered to that of C vines 2 weeks after harvest. The concentration of total phenolic compounds in the grapes, the chemical and chromatic characteristics of the wines and the replenishment of soluble sugars, starch and total nitrogen in the canes and roots were similar in the D and C vines.

Conclusion: To achieve an effective delay in sugar accumulation in the berries, leaves should be removed at 16–17°Brix, and at least 30–35% of vine leaf area should be removed.

Significance of the Study: Mechanical removal of leaves postveraison above the bunch zone of Sangiovese can be an easy and economically viable technique for delaying sugar accumulation in the berries and for limiting the alcohol content of wines with no negative impact on desirable composition of either berries or wines.

Keywords: *berry composition, leaf area-to-fruit ratio, reserve storage, vine yield*

Introduction

The production of high-quality grapes is strictly related to technological (i.e. sugars and organic acids), phenolic and aromatic ripening, which is today subjected to new environmental challenges (Keller 2010). Over the last two decades, a trend towards overly fast grape ripening, often linked to advanced phenological stages including budburst, with excessive sugar accumulation in the fruit and high alcohol in the resulting wine has been the focus of research in several countries (Ganichot 2002, Duchêne and Schneider 2005, Godden and Gishen 2005, Petrie and Sadras 2008). In many cases, irrespective of grape cultivar, such features were also matched by unacceptably low acidity and high pH, and atypical flavours in the grapes. This pattern has been linked to several factors: (i) effect of global warming and a rise in canopy photosynthetic potential due to a steady increase of CO_2 concentration in the atmosphere (Schultz 2000, Bindi et al. 2001); (ii) improvements in vineyard management; (iii) law-enforced yield constraints in several appellation areas; (iv) increased planting of grapevine cultivars characterised by low cluster weight and/or grafted on low-vigour rootstocks; and (v) improved sanitary status of propagation material. Additionally, a global tendency towards 'light and responsible drinking' emphasises that consumers increasingly prefer wines with moderate alcohol content (Seccia and Maggi 2011), leading, in turn, to a modification of current models of production in viticulture. In the medium-to-long term, these factors will affect the geographical distribution of viticulture (Schultz 2000, Jones et al. 2005), whereas in the short term, new management techniques able to mitigate these negative impacts appear to be needed. Suitable vineyard strategies to slow down grape sugar accumulation are available to minimise costly, artificial winery interventions to reduce alcohol content in the wines, such as reverse osmosis, membrane techniques, supercritical fluid extraction and vacuum distillation, which

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have been recently made legal in all the countries of the European Union (European Commission 2009).

High-grape sugar concentration has significant impact on fermentation and subsequent wine composition, including changes in both sensory characteristics and in microbiological activity, linked mainly to growth inhibition or lysis of yeast cells, as well as sluggish and stuck fermentations. These latter phenomena are aggravated in hot years (Coulter et al. 2008), with a negative impact on wine composition. High sugar stress was found to up-regulate glycolytic and pentose phosphate pathway genes (Erasmus et al. 2003), leading to formation of undesirable by-products of fermentation, such as acetic acid and glycerol (Pigeau and Inglis 2005). Moreover, high alcohol content can negatively affect malolactic fermentation because *Oenococcus oeni* cells lose membrane stability, which leads to a delay in wine stabilisation and ageing and an increase in undesirable sensory modifications (Graca da Silveira et al. 2002).

Several management techniques have been tested to regulate sugar accumulation in the berries and/or to decelerate an overly quick and unbalanced ripening: (i) late winter pruning (Friend and Trought 2007); (ii) late antitranspirant sprays (Palliotti et al. 2012); (iii) application of shading nets on the canopy or portions thereof (Cartechini and Palliotti 1995, Downey et al. 2004); (iv) shoot trimming around veraison (Cartechini et al. 2000, Balda and Martinez de Toda 2011, Filippetti et al. 2011) or at fruitset (Stoll et al. 2009, Balda and Martinez de Toda 2011); (v) treatment with auxin, brassinazole, salicylic acid or cytokinin (Davies et al. 1997, Kraeva et al. 1998, Han and Lee 2004, Symons et al. 2006, Böttcher et al. 2010); and (vi) early harvest of part of the crop. The last technique produces a wine of low alcohol and pH with high acidity, which can then be blended with wines made from grapes harvested at optimum phenolic ripeness resulting in a wine with acceptable alcohol content and pH value (Kontoudakis et al. 2011).

Leaf removal is one of the most interesting canopy management techniques because of its simplicity and suitability to mechanisation. The assessment of its impact on ripening, however, is controversial, probably because of the variability in the timing and severity of its application, the cultivar response and interaction with crop load. Bubola et al. (2009) have reported a 1°Brix increase in soluble solids after the removal of basal leaves at veraison in Istrian Malvasia, but the same treatment did not significantly affect the soluble solids content and phenolics content in grapes of several other cultivars (Bledsoe et al. 1988, Hunter et al. 1995, Tardaguila et al. 2008). Conversely, severe leaf removal apical to the bunch zone prior to veraison in Riesling caused a delay of about 2 weeks in full grape ripening as compared with that of the control (Stoll et al. 2009). While much work has been done on the effect of basal leaf removal at different timings, to our knowledge, no data are available in the literature on the evaluation of the effect that late-season removal of leaves located above the bunch zone may have on the ripening pattern. Based on the relationship between leaf age and photosynthesis (Poni et al. 1994), leaves located in the apical third of the canopy are the most functional, having reached full expansion while still short of senescence.

Here we have examined the effect on grape and wine composition and replenishment of reserve in roots and canes in field-grown Sangiovese vines of a change in the postveraison leaf-to-fruit ratio induced by mechanical leaf removal applied to the apical two thirds of the canopy. A specific aim was to test whether, irrespective of the environmental conditions, an artificial reduction in total CO_2 assimilation capacity during the last stage of berry ripening would be able to delay grape ripening and sugar accumulation.

Materials and methods

Plant material and experimental layout

The trial was carried out over the 2011 and 2012 seasons in a nonirrigated commercial vineyard located in central Italy (Umbria region) near Magione (Perugia, 44°42' N, 12°57' E, elevation 272 m above sea level, sandy loam soil type). The vineyard is a 13-year-old planting of *Vitis vinifera* L. cv. Sangiovese, grafted onto 3309 C rootstock, planted at 2.5 m × 0.8 m inter-row and intrarow, and trained to a vertically shoot-positioned, spur-pruned cordon trellis with a bud-load of 9–10 nodes per metre of row length. Pest management practices were applied according to local standard practice, and shoots were mechanically trimmed when most started to grow above the top wire.

Six adjacent rows of 90 vines each were selected to form a completely randomised block design, with each row as a block. Within each row, half of the vines were randomly assigned to mechanical leaf removal (D), and the vines of the other half were assigned as a nondefoliated control (C). Therefore, each year, 12 experimental units were monitored. In 2011 and 2012, the defoliation treatment was applied on 23 August and 13 August, respectively, corresponding to phenological stage BBCH (Biologische, Bundesanstalt, Bundessortenamt und Chemische Industrie) 85 (Lorenz et al. 1995) and to an average grape soluble solids concentration of 16.7°Brix in 2011 and 17.2°Brix in 2012. Mechanical leaf removal was conducted with a tractor-mounted Binger EB 490 (Seilzug GmbH & Co., Bingen am Rhein, Germany) leaf remover, employing two runs per row, one on each side of the canopy (Figure 1). The machine travelled at approximately 2 km/h and removed leaves located apical to the cluster area, opening a window of about 50 cm height, while retaining leaves on top of the canopy (i.e. 10–20 cm below the top catch wire) (Figure 1). Weather conditions during the study were monitored by an automatic meteorological station located near the vineyard.

Leaf area development

In 2011 and 2012, just after leaf removal, 10 fruiting shoots per treatment were collected from ten buffer vines within the experimental blocks. Shoots were randomly chosen, and the total leaf area per shoot was measured using a surface area meter (AAM-7, Hayashi-Denko, Tokyo, Japan). The contribution of primary and lateral leaves was measured separately. The total leaf area per vine was calculated by multiplying the mean leaf area per shoot by the number of shoots per vine.

Vine yield components and grape composition

In 2011, beginning from 1 week before leaf removal, the soluble solids content of a 180-berry random sample (six samples per treatment and measurement date) was periodically assessed with a temperature-compensating refractometer (RX-5000, Atago-Co Ltd, Tokyo, Japan). In 2012, the monitoring of soluble solids accumulation in the berries started about 2 weeks before the defoliation treatment and ended about 3 weeks after grape harvest. The two last samplings were made 12 and 21 days after grape harvest on 30 vines per treatment (five per experimental unit randomly chosen) that were not harvested. The rate of soluble solids accumulation, expressed as °Brix/day, was also calculated.

Harvest date was 21 September 2011 and 27 September 2012; the C vines were harvested at a mean soluble solids content of about 24°Brix. Grapes from 50 experimental vines per treatment were individually picked; precisely nine vines per experimental unit were randomly chosen. The number of

Figure 1. (a) Mechanical leaf remover operating in the Sangiovese vineyard and (b) the window opened in the canopy above the bunch zone.

bunches per vine and the crop mass were recorded while the average bunch mass was calculated. Four samples of 300 berries per treatment were randomly collected and used to measure the berry fresh mass, and in the juice, obtained by pressing, soluble solids content, titratable acidity and pH were measured. Titratable acidity was measured with a Titrex Universal Potentiometric Titrator (Steroglass S.r.l., Perugia, Italy), titrating with 0.1 N NaOH to an end point of pH 8.2, and results were expressed as g/L of tartaric acid equivalent. Must pH was measured using a PHM82 standard pH metre (Radiometer, Copenhagen, Denmark). The content of berry skin anthocyanins and total phenolics were determined according to Ough and Amerine (1988) and Slinkard and Singleton (1977), respectively. From each treatment, 20 10-mm diameter discs of the grape skin were cut from the sun-exposed part of the bunch and separated from the pulp. Each skin disc (0.785 cm^2) was macerated in 25 mL of methanol containing 0.1% HCl (v/v) at pH 1 and incubated at room temperature (about 25°C) for 24 h in the dark with occasional shaking. The anthocyanins content of the juice was determined by measuring the absorbance at 520 nm at pH 1 using an extinction coefficient (molar absorbance value) of 28 000 and molecular mass of 529 (typical of malvidin-3-glucoside). Total phenolics were measured as follows: to each 0.2-mL sample, 1.8 mL of distilled water (diluted to contain 0–250 mg/L gallic acid equivalent) was added, followed by 10 mL of 10% aqueous Folin-Ciocalteau reagent (Sigma-Aldrich, Milan, Italy) and 8 mL of 7.5% (w/v) aqueous Na_2CO_3. The mixture was held at 24°C, and after 2 h, the absorbance was read at 750 nm and compared with a gallic acid standard curve. Anthocyanins and total phenolics are expressed as mg/cm^2 berry skin.

Microvinification and wine analysis

In 2011 and 2012, wines were made using a microvinification technique. At harvest, grapes from 150 D and 150 C vines were harvested manually and transported to the experimental winery in 20-kg plastic boxes. For each treatment, the total harvested grape mass was divided into two lots, each weighing about 140–150 kg. Each lot was mechanically crushed, destemmed, transferred to 100-L stainless-steel fermentation containers, sulfited with 35 mg/L of SO_2 and inoculated with 35 mg/L of a commercial yeast strain (Lalvin EC-1118, Lallemand Inc., Montréal, QC, Canada). Wines were fermented for 16–18 days on the skin and punched down twice daily, with the fermentation temperature ranging from 20 to 27°C. After alcoholic fermentation, the wines were pressed at 0°Brix and inoculated with 30 mg/L of *O. oeni* (Lalvin Elios 1 MBR, Lallemand Inc.). After completion of malolactic fermentation, the samples were racked and transferred to 60-L steel containers, and 25 mg/L of SO_2 was added. Two months later, the wines were racked again, bottled into 750-mL bottles and closed with cork stoppers. After 8 months in 2011 and 4 months in 2012, the wines were analysed for alcohol, titratable acidity and pH (Iland et al. 1993). Wine colour intensity ($OD_{420nm}+OD_{520nm}$), colour hue (OD_{420nm}/OD_{520nm}) and the concentration of total phenolics and of anthocyanins were determined by spectrophotometer. Total phenolics were quantified according to Ribéreau-Gayon (1970) by measuring the absorbance at 280 nm of wine diluted 1:100 with distilled water. Anthocyanins were analysed as reported by Ribéreau-Gayon and Stonestreet (1965). All determinations were carried out in duplicate.

Carbohydrate storage in permanent vine organs

At the end of December 2011 and 2012, the concentration of alcohol-soluble sugars and starch in canes (node 3) and roots (fine brown with 1.5 ± 0.2 mm of diameter) was determined on six replicates according to a colorimetric method (Loewus 1952) using the anthrone reagent (Merck, Darmstadt, Germany). Absorbance was read at 620 nm with a Jasco V-630 spectrophotometer (Jasco International Co. Ltd, Tokyo, Japan). On the same material, total nitrogen content was also determined using the Kjeldahl method.

Statistical analysis

Two-way analysis of variance was used to examine defoliation treatment and year effects on vegetative parameters, yield components, and grape and wine composition using the SigmaStat 3.5 software package (Systat Software, Inc., San Jose, CA, USA). Mean separation was performed by Student–Newman–Keuls test. Unless a significant year × defoliation treatment interaction occurred, values are presented as means over the years and the treatments. Results of the seasonal evolution of sugar content, anthocyanins, total phenolics and the ratio of anthocyanins/sugar content are shown as means ± standard error.

Results

Environmental conditions

Heat accumulation expressed as growing degree days (GDD, calculated with base temperature of 10°C) from 1 April to 30 September was similar in 2011 and 2012, with 2234 and 2265

GDD, respectively. Total rainfall over the same period was slightly lower in 2011 (158 vs 168 mm in 2012) with no rain in August (Figure 2), whereas in 2012, rainfall was low with only 9.6, 4.0 and 15.2 mm in June, July and August, respectively. Despite a similar GDD summation, the summer of 2012 was marked by high daily maximum air temperature, on some days reaching 36°C in June, 40°C in July and 41°C in August (Figure 2). Despite such trends and the absence of irrigation, no visual symptoms of water stress or significant leaf yellowing were observed throughout the trial seasons.

In 2012, the early season from the end of April to the first half of May was relatively hot (temperature higher than 32°C) (Figure 2). Moreover, rainfall from October 2011 to March 2012 was only 122 mm and likely insufficient for the full restoration of the water reserve in the soil.

Defoliation treatment effects on leaf area and yield component

Mechanical leaf removal applied in 2011 and 2012 above the cluster zone set the final leaf area per vine at 2.8 m^2, while fractional reduction in leaf area, as compared with C vines, was around 35% (Table 1). In both years, no new leaves developed after leaf removal either from primary or lateral shoots.

Late mechanical defoliation had no effect on vine yield, average bunch number or berry mass regardless of year (Table 1). Final must soluble solids content in D vines was significantly reduced (1.2°Brix), whereas neither must titratable acidity and pH nor skin anthocyanins and total phenolics content showed any difference (Table 1). At harvest, the leaf-to-fruit ratio was lowered in D vines by about 36% (−0.64 m^2/kg) (Table 1).

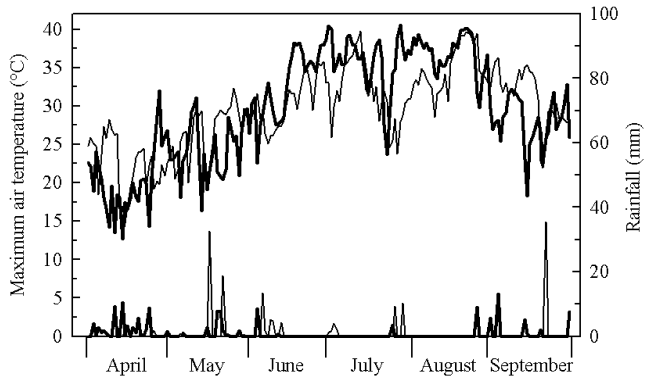

Figure 2. Seasonal trend of maximum air temperature and daily rainfall in (—) 2011 and (▬) 2012 seasons.

Year effects on leaf area and yield component

The environmental conditions recorded in 2012 led to a significant reduction in canopy total leaf area and was 18% lower than in 2011 (Table 1). Also, the growth of lateral shoots was negatively affected with a reduction of about 0.4 m^2 lateral leaf area per vine (−29%).

In 2012, yield per vine was significantly reduced as compared with that for 2011 (−42%) because of much lower bunch mass (−41% corresponding to −127 g per bunch) (Table 1), which, in turn, was driven by a marked reduction in berry mass (−1 g per berry). Conversely, estimated berry number per bunch was not modified.

Titratable acidity, total phenolics and pH value were similar in both years, whereas soluble solids and anthocyanins were significantly higher in 2012 compared with that in 2011 (Table 1). At harvest 2012, the leaf-to-fruit ratio was increased by about 40% (+0.49 m^2/kg) in comparison with that of 2011 (Table 1).

Dynamics of soluble solids and phenolics compounds

In both seasons, the mechanical defoliation postveraison did not modify berry fresh mass as compared with that of the C vines; the reduction in soluble solids content found in D vines appears to be linked to reduced canopy photosynthetic capacity and/or sugar translocation from leaves to bunches. From leaf removal until harvest, the rate of soluble solids accumulation, measured as °Brix in the berries, was lowered from 0.23/day in the C vines to 0.19/day in D vines in 2011, and from 0.16/day in the C vines to 0.13/day in D vines in 2012 (Figure 3). In 2011, at harvest, a

Table 1. Leaf area (total and lateral fraction), yield components, grape composition and leaf-to-fruit ratio recorded at harvest in Sangiovese vines subjected to mechanised leaf removal applied postveraison (D) and in control vines (C). Data averaged over treatments and years in the absence of significant interactions.

Parameter				Year		
	C	D	Significance†	2011	2012	Significance†
Total leaf area/vine (m^2)	4.28[a]	2.80[b]	**	3.88[a]	3.20[b]	*
Lateral leaf area/vine (m^2)	1.60[a]	0.72[b]	**	1.36[a]	0.96[b]	*
Bunches/vine	10.0	10.3	ns	10.6	9.8	ns
Yield/vine (kg)	2.51	2.63	ns	3.26[a]	1.88[b]	**
Bunch mass (g)	250.0	243.0	ns	310.0[a]	183.0[b]	**
Berry mass (g)	2.05	2.03	ns	2.54[a]	1.54[b]	**
Total soluble solids (°Brix)	23.9[a]	22.7[b]	*	22.9[b]	23.8[a]	*
Titratable acidity (g/L)	6.35	6.15	ns	6.23	6.40	ns
Must pH	3.26	3.31	ns	3.30	3.47	ns
Anthocyanins (mg/cm^2 skin)	0.419	0.411	ns	0.344[b]	0.486[a]	**
Total phenolics (mg/cm^2 skin)	0.59	0.57	ns	0.56	0.59	ns
Leaf-to-fruit ratio (m^2/kg)	1.77[a]	1.13[b]	*	1.21[b]	1.70[a]	*

*, **, ns indicate significance at $P \leq 0.05$ and 0.01 or not significant, respectively. †Means within rows designed by different superscript letters are significantly different by the Student–Newman–Keuls test.

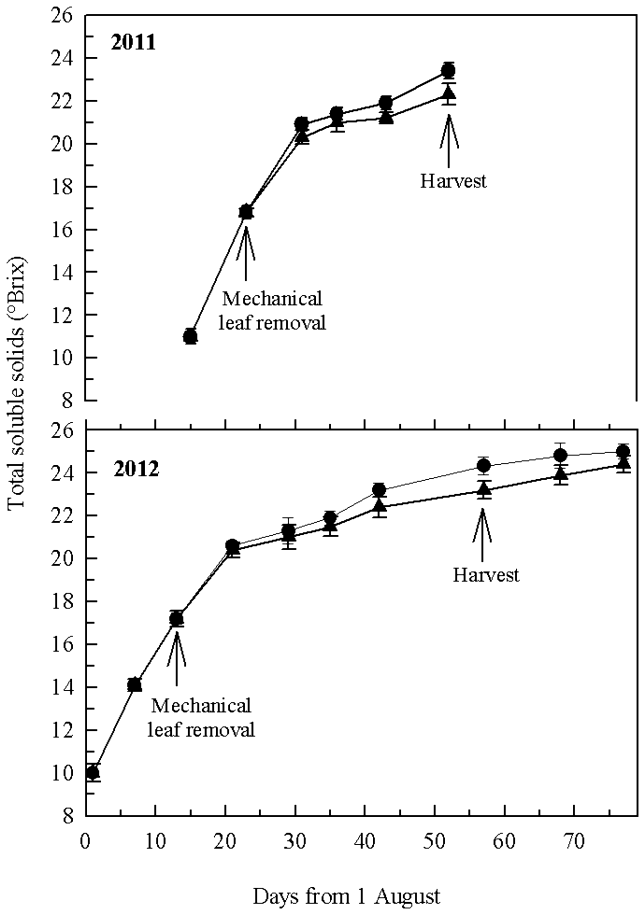

Figure 3. Seasonal trends of total soluble solids content recorded in 2011 and 2012 on Sangiovese vines subjected to (▲) mechanised leaf removal applied postveraison (D) or (●) with no leaf removal, control (C). Data are means ± standard error.

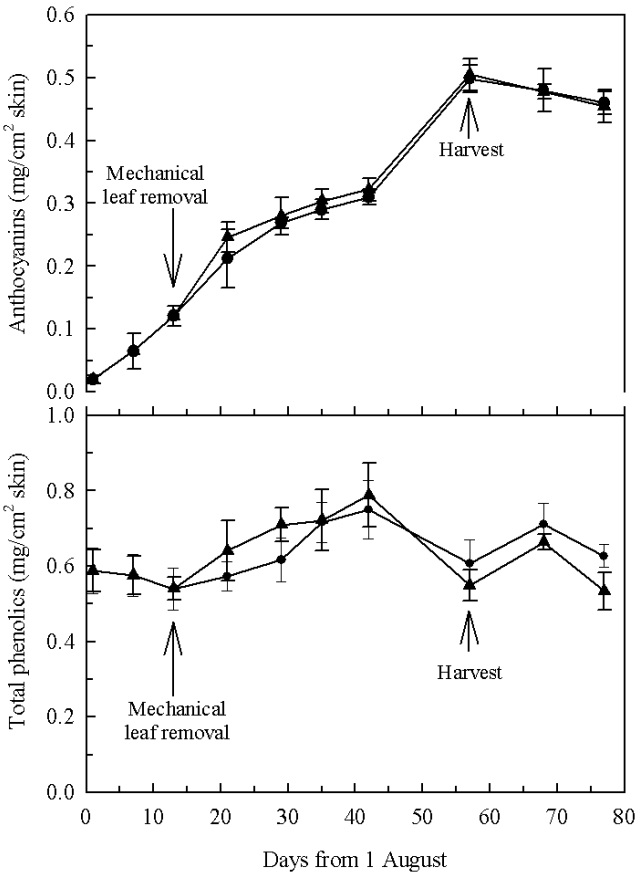

Figure 4. Seasonal trends of anthocyanins and total phenolics recorded in 2012 on Sangiovese vines subjected to (▲) mechanised leaf removal applied postveraison (D) or (●) with no leaf removal, control (C). Data are means ± standard error.

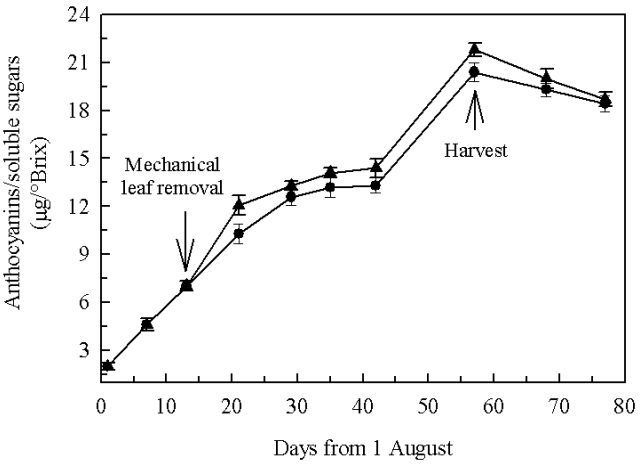

Figure 5. Seasonal trends of anthocyanins/soluble solids ratio recorded in 2012 on Sangiovese vines subjected to (▲) mechanised leaf removal applied postveraison (D) or (●) with no leaf removal, control (C).

reduction of 44 mg of soluble solids per berry was assessed in D vines compared with that of C vines, whereas in 2012, this limitation was 15 mg/berry. Regardless of season, sugar accumulation began to slow down about 1 month after leaf removal (Figure 3). Moreover, the containment of the sugar accumulation in the grapes following postveraison mechanical defoliation occurred in 30 days in 2011 and in 44 days in 2012.

In 2012, the evolution of berry composition after harvest showed that, unlike C vines, the soluble solids accumulation in the grapes of D vines kept increasing (Figure 3), without any concurrent change in anthocyanins and total phenolics content (Figure 4), suggesting that harvest delay can be offset for about 2 weeks. In C vines, the slight increase in the soluble solids content after harvest (an average of 0.034°Brix/day against 0.065°Brix/day assessed in C vines) suggests that a low export of carbohydrates from leaves into the berries occurred throughout this time.

The accumulation of anthocyanins and total phenolics in berry skins a for 2012 showed a similar trend with no change because of defoliation (Figure 4), whereas the anthocyanins/°Brix ratio showed a consistent increase in D vines until harvest and a tendency to decline to near that of the C vines' 2 weeks later (Figure 5).

Defoliation and year effects on wine characteristics and replenishment of reserves storage

The alcohol content of the wines produced from the D vines was reduced by 0.6% v/v. as compared with that of wines produced by the C vines, whereas no significant change was found in total acidity, pH, total dry extract, total phenolics concentration and chromatic characteristics (Table 2). Wine composition in 2011 and 2012 was similar (Table 2).

Sampled after the leaf fall in 2011 and 2012, the concentration of alcohol-soluble sugars and starch, as well as total

Table 2. Wine composition recorded over the 2011 and 2012 vintages in Sangiovese vines subjected to mechanised leaf removal applied postveraison (D) and in control vines (C). Data averaged over treatments and years in the absence of significant interactions. Wines were analysed 8 and 4 months after alcoholic fermentation in 2011 and 2012, respectively.

Parameter				Year		
	C	D	Significance†	2011	2012	Significance†
Alcohol (% vol.)	14.0[a]	13.4[b]	*	13.8	14.2	ns
Total acidity (g/L)	6.16	6.39	ns	6.15	6.41	ns
pH	3.34	3.30	ns	3.22	3.37	ns
Total dry extract (g/L)	24.1	23.6	ns	23.9	24.0	ns
Anthocyanins (g/L)	0.27	0.26	ns	0.25	0.26	ns
Total phenolics (g/L)	1.60	1.57	ns	1.56	1.63	ns
Total tannins (g/L)	0.89	0.93	ns	0.83	0.94	ns
Colour intensity ($OD_{420nm} + OD_{520nm}$)	7.1	6.9	ns	6.5	7.6	ns
Colour hue (OD_{420nm}/OD_{520nm})	0.62	0.65	ns	0.62	0.65	ns

*, ns indicate significance at $P \leq 0.05$ or not significant, respectively. †Means within rows designed by different superscript letters are significantly different by the Student–Newman–Keuls test.

Table 3. Cane wood and root reserves recorded in Sangiovese vines subjected to mechanised leaf removal applied postveraison (D) and in control vines (C). Data averaged over treatments and years in the absence of significant interactions.

Parameter				Year		
	C	D	Significance	2011	2012	Significance
Cane wood						
Total nitrogen (mg/g DM)	545.0	595.0	ns	575.0	565.0	ns
Alcohol-soluble sugars (mg/g DM)	155.8	150.1	ns	157.1	148.9	ns
Starch (mg/g DM)	48.9	56.2	ns	40.3	64.8	ns
Root						
Total nitrogen (mg/g DM)	890.0	931.0	ns	978.0	885.0	ns
Alcohol-soluble sugars (mg/g DM)	82.0	91.6	ns	74.3	84.7	ns
Starch (mg/g DM)	109.9	98.8	ns	101.4	93.8	ns

DM, dry mass; ns, not significant.

nitrogen content stored in canes and fine roots, showed no differences between treatments (Table 3).

Discussion

Mechanical leaf removal applied late in the season, when canopy growth had ceased, opened a 50-cm high window above the cluster zone but did not reduce grape yield despite removing about one third of the total leaf area. The leaf removal treatment of D vines achieved a leaf-to-fruit ratio of 1.13 m^2/kg, a reduction of 36% compared with that of C vines. Although such a ratio is considered not limiting for vertical shoot positioned canopies (Kliewer and Dokoozlian 2005), the defoliation treatment still was effective at hindering net sugar accumulation in the berries. It is worth noting that the machine pulled out the most functional leaves, i.e. fully expanded median and apical leaves from either main and lateral shoots, located in the upper two thirds of the canopy and thus probably caused a significant limitation of canopy photosynthetic capacity. Defoliation did not affect the replenishment of carbohydrates in the roots and canes, and suggests that the recorded postharvest leaf area-to-fruit ratio is indeed not limiting as also previously suggested by Howell (2001) for cool climate grape production regions. Improvement in the light availability to the leaves located basal to the canopy window, in association with likely photosynthetic compensation in retained leaves (Poni and Giachino 2000), may have significantly contributed to the recovery of photosynthetic capacity of D canopies and, as a consequence, to their ability to achieve a soluble solids content similar to that of C vines upon a delayed harvest.

Defoliation treatments applied at veraison often lead to an increase in sugar accumulation because of a concomitant reduction in yield (Bubola et al. 2009), or the content of grape soluble solids and phenolics remain unaffected (Bledsoe et al. 1988, Hunter et al. 1995, Tardaguila et al. 2008, King et al. 2012). Our results are contrary to those findings in that postveraison defoliation above the fruit zone induced a delay in berry sugar accumulation (−1.2°Brix), equivalent to a harvest delay of 2 weeks, without any influence on the desired berry characteristics or wine composition. Ripening was delayed 14 and 20 days in cv. Riesling when the leaf-to-fruit ratio was artificially reduced by about 28 and 59%, respectively (passing from 1.95 of the control vines to 1.4 and 0.8 m^2/kg, respectively) (Stoll et al. 2009).

Therefore, this strategy of canopy management can be proposed as a practical tool to reduce the alcohol content of the

wine (–0.6% v/v), especially in viticultural areas where berry ripening is taking place during warm seasons that are quite often associated with hot periods, leading to accelerated ripening. Such seasons tend to promote above-average sugar content and pH, while retaining an unfinished or atypical phenolic and aromatic profile requiring grapes to be left to hang longer on the canopy. In contrast, for red grape cultivars, an early harvest cannot be proposed because of likely poor phenolic and aromatic ripeness, leading to wines with an excessive herbaceous and bitter taste due mainly to higher extractability of procyanidins from seeds (Kennedy et al. 2000). Importantly, the reduction of sugar accumulation in the berry achieved with postveraison defoliation left the accumulation of berry skin colour and phenolics unaffected. Recently, Kotseridis et al. (2012) have shown a different response of Merlot, Cabernet Sauvignon and Sangiovese to basal leaf removal postflowering applied at several levels of severity. Colour accumulation in Sangiovese, in particular, was lowest when full leaf removal was applied, while pigmentation improved when some leaf cover around the bunches was maintained. Overall, their experiment showed that colour accumulation was sensitive to local bunch microclimate and that cv. Sangiovese is especially prone to variation of berry pigmentation depending upon light and temperature regimes influenced by leaf removal. Our experimental approach did not alter the microclimate of the fruiting area as leaves were removed from only the canopy area located apical to the bunch zone, and this may at least partially explain why berry colour was similar in D and C berries. In contrast, the maximum coloration in the grape skin is reached when the leaf-to-fruit ratio is between 1.1 and 1.4 m^2/kg (Kliewer and Dokoozlian 2005).

In several white and red grape cultivars, Sadras and Petrie (2011) reported that the early ripeness associated with higher temperature is primarily driven by an early onset of veraison. Fortuitously, an earlier onset of veraison materialised in 2012 and was assessed (Figure 3). On 1 August 2012, and therefore before defoliation, 30% of berries showed pigmentation, whereas soluble solids was 10°Brix and the titratable acidity was 35.5 g/L. One week later, the fraction of coloured berries in D vines increased up to 80%, the soluble solids content reached 14.1°Brix and the titratable acidity was 20.3 g/L. The time elapsing between veraison and harvest, however, increased from 39 days in 2011 to 54 days in 2012, underlining a slowdown of berry ripening. Indeed, in 2012, a lower sugar accumulation capability occurred during the ripening stage, which, at harvest, reached 390 mg/berry against almost 600 mg/berry recorded in 2011. This behaviour may be linked to partial inhibition of the photosynthesis and translocation processes required for ripening, following air temperature and leaf-to-air vapour pressure differences often higher, respectively, than 35°C and 5 kPa (Palliotti et al. 2009), as frequently occurred in June, July and August 2012. In 2012, the seasonal course of environmental parameters was responsible for the strong limitation in the bunch and berry mass, whereas no significant symptoms of water stress or leaf yellowing were observed. Reduced total leaf area and yield recorded in 2012, however, might have mitigated the impact of soil water shortage and high leaf-to-air vapour pressure deficit. Moreover, the total rainfall in April and May 2012 was higher compared with that in 2011 (precisely 61 and 58 mm, respectively, against 9 and 51 mm of April and May 2011).

In Sangiovese vines, Pastore et al. (2011) have recently found that doubling the leaf-to-fruit ratio from 0.6 to 1.2 m^2/kg via bunch thinning at veraison will increase the sugar accumulation in the berries (+1.9°Brix). They conclude that the action is due to 68 highly modulated genes involved in the primary carbohydrate metabolic pathways, including sucrose and starch metabolism, glycolysis, the pentose phosphate pathway and the Krebs cycle. They further suggest a large-scale reprogramming of carbohydrate metabolism in response to bunch thinning and the resulting change in leaf-to-fruit ratio. Similarly, we speculate that leaf removal may down-regulate different genes involved in the synthesis and/or degradation and transport of starch and sugars in berries, such as sucrose synthase, sucrose-phosphate synthase, invertase, α-amylase, isoamylase and trehalose, because of a reduced source/sink ratio. Another hypothesis that can help us understand, at least in part, the lowering of the sugar accumulation in the berries after leaf removal is the reduction of abscisic acid (ABA) production, which is synthesised inside the chloroplasts and is a well-known promoter of ripening in grapes (Coombe and Hale 1973). The possible limitation of ABA influx in the grapes, as a result of the significant reduction in the leaf-to-fruit ratio, may deactivate the expression of sugar transport pathway genes, mainly invertases (Pan et al. 2005) and a monosaccharide transporter (Cakir et al. 2003).

Conclusions

The results of this study show that a mechanical leaf removal postveraison on cv. Sangiovese vines apical to the bunch zone is a practical strategy to delay sugar accumulation in the berry by about 2 weeks as compared with nondefoliated vines. The technique proved to be also effective in 2012, a season marked by dry, hot spring and summer seasons leading to an early limitation in leaf area development and berry growth. The technique proved itself as an effective, easy-to-do and economically viable method (it requires only 3–4 h/ha to be achieved mechanically) to hinder berry sugar accumulation and to obtain wines of lower alcohol content. Importantly, the technique did not affect the content of total phenolics in grapes and wines or the replenishment of reserves storage in canes and roots. To be effective at significantly delaying sugar accumulation in the berries, it is advised to remove leaves apical to the bunch zone at around 16–17°Brix and ensuring that at least 30–35% of the leaf area is removed.

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