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ORIGINAL RESEARCH ARTICLE

Beneficial effects of bunch-zone late defoliations and shoot positioning on berry composition and colour components of wines undergoing aging in an organically-managed and rainfed Sangiovese vineyard

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ABSTRACT

In the context of climate change, where high temperatures are frequent in the first phases of ripening, protecting grapevine bunches from solar radiation is essential for preserving berry composition and wine colour. The effects of bunch-zone late defoliations (DEFs) and "semi-ballerina" shoot positioning (SB) on vine physiology and grape and wine quality of organic cv. Sangiovese wines during storage were assessed in two contrasting seasons (2013 and 2014). The treatments altered neither vine physiology (leaf photosynthetic activity and stomatal conductance, stem water potential) nor vine phenology, yield, budburst and fruitfulness. Defoliations imposed at post-veraison (DEF I) and pre-harvest (DEF II), but not shoot positioning imposed at post-veraison, enhanced the concentration of berry skin flavonols at harvest, compared to an untreated control. Late defoliations and SB did not change berry weight, anthocyanins, soluble solids, pH or titratable acidity at harvest. The severity of *Botrytis* bunch rot was assessed in both seasons. In 2013, it was negligible regardless of the treatment. In 2014 (characterised by higher rainfall and lower average temperatures than in 2013), late defoliations (DEF I and DEF II), especially DEF I, and SB to a minor extent, limited the severity of *Botrytis* bunch rot. The oenological benefits of late defoliations and shoot positioning were observed during wine storage. These canopy management practices positively influenced wine components (polymeric pigments; namely short polymeric pigments) that might have a marked effect on the final colour intensity, without altering the basic chemical characteristics of the wine. When choosing the timing for carrying out defoliation in order to improve grape quality and bunch rot containment, the meteorological conditions should be properly considered. Our results may contribute to providing further recommendations for canopy management for grape growers who produce organic Sangiovese wines that undergo aging.

KEYWORDS: canopy management, organic viticulture and wine, anthocyanins, flavonols, quercetin, *Vitis vinifera*, wine storage

INTRODUCTION

Canopy management plays a key role in the modulation of berry composition, as it contributes to controlling the decoupling of sugar and anthocyanins accumulation, which is particularly evident in some red varieties, such as Sangiovese, Cabernet Sauvignon, Merlot, Nero di Troia and Uva Longanesi (Rombolà *et al.,* 2011; Palliotti *et al.,* 2013; Poni *et al.,* 2013; Tessarin *et al.*, 2014; Baiano *et al.*, 2015; Filippetti *et al.,* 2015; Bondada *et al.,* 2016; Tessarin *et al.*, 2016; Pastore *et al.*, 2017; Tessarin *et al.,* 2018), and its effects depends on the terroir and climate conditions.

In the context of climate change, grape growers, especially those who cannot practice irrigation, need to carry out summer pruning practices (e.g., shoot topping/trimming, leaf removal and shoot positioning) in order to be able to preserve grape quality until harvest and to deliver healthy grapes to the winery (Palliotti *et al.*, 2014; Poni *et al.*, 2018). Defoliation is a common intervention whose effects in vine physiology and berry composition largely depend on treatment timing and intensity (Palliotti *et al.*, 2014; Poni *et al.*, 2018). Defoliation in the fruit zone is one of the most important and commonly applied canopy management interventions in viticulture (Ivanišević *et al.*, 2020). This technique is performed on grapevines to improve light penetration and air circulation around the clusters (Ivanišević *et al.*, 2020). The right moment for defoliation depends on the region, variety and type of wine produced (Ivanišević *et al.*, 2020).

Performed on different genotypes and in different growing conditions, early defoliation usually involves the removal of about 6–7 main basal leaves before flowering, resulting in a significant decrease in fruit-set, which in turn increases the loosening of clusters and tolerance to rot (Poni *et al.*, 2018). Moreover, this technique, irrespective of genotype, markedly improves grape composition (total soluble solids, anthocyanins, phenols and aromatic compounds) and wine sensory properties (Poni *et al.*, 2018). However, defoliation just before anthesis should be carefully applied, because it can decrease must titratable acidity, increase must pH (Risco *et al.*, 2014) enhancing malic acid degradation (Gatti *et al.*, 2015), increase berry sunburn (Lopes *et al.*, 2019) with negative consequences in terms of wine quality. Risco *et al.* (2014) and Lopes *et al.* (2019) reported a cumulative negative effect of early defoliation on vine bud fertility.

Late defoliation (carried out at the onset of veraison or later) is a frequently adopted practice that curtails the development of fungal diseases. It allows healthy bunches to be maintained for longer on the vine, resulting in the attainment of adequate levels of phenolic and aromatic compounds (Kalua and Boss, 2009).

Controlling the phytosanitary status of bunches during ripening is crucial, particularly in organic and biodynamic viticultural systems (Botelho *et al.*, 2016); this is mainly due to there being less effective active ingredients for controlling bunch rot, which has led to research on agroecological strategies, such as using essential oil vapour (Burggraf and Rienth, 2020) and carrying out post-veraison trimming (Bondada *et al.,* 2016; Tessarin *et al.,* 2018). In addition, the EC Regulation (EC, 2012) for the production of organic wines provides a series of restrictions on determinate oenological practices (e.g., dealcoholisation) and limits the amount of sulphites that can be added to the must during winemaking (Parpinello *et al.*, 2015). Due to such restrictions, organic wine producers face difficulties in managing grapes affected by rot in the cellar.

Basal leaf removal at veraison can induce changes in berry composition, especially a decrease in total soluble solids (Pastore *et al.*, 2013; Pastore *et al*., 2017; Tessarin *et al.*, 2014). The main drawback of basal leaf removal at veraison is the possible decrease in berry anthocyanins (Pastore *et al.*, 2013; Tessarin *et al.*, 2014), which is also mirrored in wines. However, basal leaf removal at the end of veraison does not seem to result in any significant changes in berries (Tessarin *et al.*, 2014).

Late bunch-zone defoliation carried out at post-veraison on cv. Aglianico did not change berry soluble solids or the alcohol concentrations of young wines without decreasing berry anthocyanin levels and the wine colour (Caccavello *et al.*, 2017). The post-veraison defoliation of leaves above the bunch zone of cv. Sangiovese potted vines (Poni *et al.*, 2013) and of those grown in field conditions (Palliotti *et al.*, 2013) without exposing the bunches to direct solar radiation caused a decline in photosynthetic capacity of the vines, resulting in a decrease in berry soluble solids and a reduction in wine alcohol levels without any changes in anthocyanins and polyphenols levels in the berries and wines (Palliotti *et al.*, 2013; Poni *et al.*, 2013). Physiologically, leaf removal is based on the fact that, around veraison, the leaves above the bunch on the apical two-thirds of the canopy are the most photosynthetically functional (Poni *et al.*, 1994).

In the context of climate change high temperatures are frequent in the first phases of ripening; therefore, protecting bunches from solar radiation is essential for preserving berry composition and wine colour (Tessarin *et al.*, 2014). Grapevines trained to vertical shoot positioning (VSP) systems were found to undergo more heat damage compared to those with sprawling, non-positioned canopies (Dry, 2011). Dry (2011) proposed creating a "semi-ballerina" effect on VSP canopies in warm to hot sunny climates; this consists in positioning long shoots downwards, thereby shading the bunches (Supplementary Figure 1) in the warmest hours of the day and reducing the risk of bunch damage. Specific research is required to determine the possible implications of this technique in terms of the effects on grapes and wine quality.

Sangiovese is one of the most widespread grape varieties in Italy, covering around 54000 ha, which constitutes 10.8 % of the national grape growing area (Focus OIV, 2017). Sangiovese has a relevant agronomic and economic role, since it is used for producing hundreds of different wines, such as Chianti Classico, Brunello di Montalcino, Romagna Sangiovese, which must undergo an aging period

of a minimum of 24 months before commercialisation (Rinaldi *et al.*, 2021). This cultivar displays a sensitive anthocyanin profile (Mattivi *et al.,* 2006; Castellarin *et al.,* 2012) due to its high percentage of unstable dihydroxy pigments, which is mirrored in wine quality parameters (Arapitsas *et al.*, 2012).

Sangiovese is rich in skin flavonols, in particular quercetin. Quercetin increases with cluster exposure to light and thus after defoliation, especially early (pre-flowering) defoliation (Lanati *et al.*, 2021), which is performed to regulate yield and reduce cluster compactness and susceptibility to fungal pathogens (Poni *et al*., 2006). Quercetin is beneficial for wine quality (Boulton, 2001) and human health (Derosa *et al.*, 2021). However, its precipitates in wines are detrimental to wine quality; wines from Sangiovese are among those that are very sensitive to quercetin haze (Gambuti *et al*., 2020; Lanati *et al.*, 2021).

For the above-mentioned reasons, the achievement of multiple goals, especially the attainment of Sangiovese wines suitable for aging, constitutes a considerable challenge for organic grape growers.

Therefore, our research on cv. Sangiovese aimed to achieve the following main goals: 1) improve the grape sanitary status, 2) preserve the berry skin anthocyanins and technological parameters, 3) enhance the berry flavonols, 4) improve the quality of the wines undergoing aging, and 5) achieve the former goals without altering the yield, budburst and fruitfulness in the season being studied and the following ones.

We assessed the effects of post-veraison and pre-harvest defoliation and post-veraison shoot positioning on vine physiology and grape quality and sanitary status, as well as wine composition throughout storage, in an organically-managed and rainfed Sangiovese vineyard.

The experimental hypothesis was that post-veraison and pre-harvest defoliation and post-veraison shoot positioning are all able to trigger changes in berry composition, leading to the formation of compounds beneficial for the quality of wine undergoing aging without altering vine yield, budburst and fruitfulness.

MATERIALS AND METHODS

1. Plant material and experimental layout

The experiment was conducted in 2013 and 2014, in a mature vineyard planted in 2003 with *Vitis vinifera* L., cv. Sangiovese (clone FEDIT 30 ESAVE) grafted onto Kober 5BB and trained to a cordon de Royat training system with an 80 cm high cordon (vertical shoot positioning, VSP). The vineyard was located in Tebano (Faenza, RA), Italy (44°17′7″ N, 11°52′59″E, 117 m a.s.l), on a medium slope (< 8°), with southeast/northwest- and downhill-oriented rows. The climate of this area is subcontinental temperate, with hot, humid summers and cold winters. Rainfall averages 600 to 700 mm per year. Vines were spaced 1.0 m apart within the row and 2.8 m between rows, for 3571 vines/ha. From 2007 onwards, the vineyard was managed as organic in accordance with Reg. EC 834/2007 (EC, 2007), with neither irrigation nor fertilisation.

The loamy clayey and alkaline soils of the vineyard comprise 2.2 % organic matter, 1.5 ‰ nitrogen concentration, 14.7 % total carbonates, 6.7 % active lime, 10 µg/g assimilable phosphorus and 188 µg/g assimilable potassium. Spontaneous vegetation was present in the alternate planting rows. Annually, at the end of each growing season, herbaceous species were sown in alternate planting rows, such as fava bean (*Vicia faba*), barley (*Hordeum vulgare*) or subterranean clover (*Trifolium subterraneum*). The ground cover was managed by mowing the vegetation in late spring, which maintained the biomass on the soil surface.

The vineyard was treated against diseases and pests using products conforming to EC Regulations (EC, 2002). Copper (an average of 6 kg/ha/year) and sulphur (an average of 70 kg/ha/year) broad-spectrum fungicides were applied. Vines were spur-pruned to two count nodes, with 12-14 nodes per vine being retained at the end of February. The non-count shoots (shoots arising from the base buds of the spur) were removed at the beginning of the season, with 12 uniformly distributed shoots per meter of cordon being kept. The bunch number was adjusted to 16 per vine by bunch thinning at veraison. The experimental design included four treatments: an untreated control (CK); two different defoliation times: post-veraison defoliation (DEF I) and pre-harvest defoliation (DEF II) (Figure 1); and shoot positioning through the "semi-ballerina" effect (SB) (Supplementary Figure 1). The DEF I treatment was imposed at post-veraison, when the berries had reached an average of 15 °Brix (86 and 92 days after flowering, DAF, in 2013 and 2014 respectively), which was measured by refractometer (Digital Refractometer HI 96811, Hanna instruments, Milan, Italy). The DEF II treatment was imposed at pre-harvest (101 DAF, 19.8 °Brix in 2013 and 106 DAF, 17.4 °Brix in 2014; Supplementary Table 2). The SB treatment was performed at 86 and 92 DAF in 2013 and 2014 respectively. The control treatment (CK) comprised long canes possessing around 24 nodes trailing over both sides of the canopy. Defoliation treatments were performed by manually removing all main leaves and laterals up to the eighth node. The "semi-ballerina" treatment (Dry, 2011) consisted in positioning long canes downwards (around 24 nodes) on one side of the canopy exposed to the south-west. The treatments were applied to the same vines in each season. Each treatment was replicated three times in a randomised-block design. The treatments were applied on vines within three parallel rows that were divided into three consecutive blocks with 30 vines per repetition. For each repetition, the measurements were made on 10 vines positioned in the central row of the three parallel rows. All vines included in the experiment were subjected to the same soil management with alternate cover crops.

Photosynthetic Active Radiation (PAR) at bunch level was measured in the afternoon at post-veraison using a Skye Quantum Sensor (Llandrindod Wells, Powys, UK).

FIGURE 1. Vines subjected to late defoliations in the organically-managed and rainfed Sangiovese vineyard.

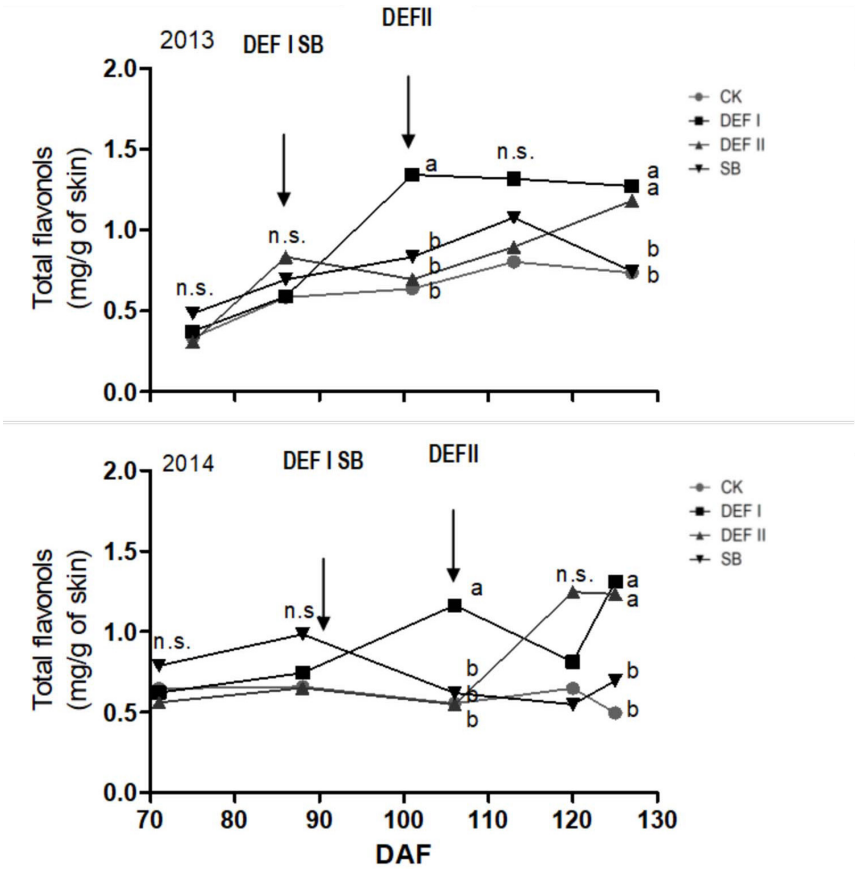

FIGURE 2. Seasonal trends of berry skin glucosylated flavonols (mg/g of skin), measured in 2013 and 2014 on cv. Sangiovese.

2. Climatic conditions

During the 2013 and 2014 seasons, climatic data (mean, maximum and minimum daily air temperatures (T), relative humidity (RH) and total rainfall) were recorded by a nearby meteorological station (Data logger Mhaster and Pluviometer PG10, CAE, Bologna, Italy; Hydrometer T039 TIDROM, SIAP+MICROS, Treviso, Italy) located 800 m from the vineyard.

During the 2013 season, maximum temperatures were recorded at the beginning of August (around 40 °C). The total rainfall from budburst to harvest was 433 mm, mainly occurring in spring, at the end of August (55 mm) and during the second half of September (52 mm). The vegetative season (from budburst to leaf fall) of 2014 was marked by average temperatures well below seasonal norms, as the most frequent average temperatures recorded in the season. The highest maximum temperatures (32 °C) were recorded at the end of

spring (20 DAF and 21 DAF) and on 20th July (59 DAF). From the second half of April to harvest the total rainfall was abundant (489 mm) and quite frequent during both spring and summer. Climatic data are reported in Supplementary Figure 2.

3. Leaf macronutrients

At veraison (75 DAF, 2013; 71 DAF, 2014), 20 mature, exposed and completely expanded leaves located at the fourth node above the first bunch were collected from each experimental plot (60 leaves per treatment), in order to monitor the leaf nutritional status. Leaves were sampled from shoots originating from true buds and bearing at least one bunch. Leaf blades, deprived of petioles, were washed in a detergent solution (HCl 0.1 N + Tween 20 0.1 %) to remove any nutrients present on the leaf. They were then rinsed with distilled water, dried at 65 °C until a constant weight was reached, weighed and finally milled. The leaf blades were successively ground (sieve < 0.5 mm). Total nitrogen was determined by the Kjeldhal method and phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), and boron (B) were analysed as described in García-Escudero *et al*. (2013).

4. Leaf gas exchange

Leaf gas exchange was measured on a leaf at the 12th node of a fruiting shoot on two vines per experimental plot (six vines per treatment) using an infrared gas analyser (LI-COR 6400 IRGA equipped with an integrated 6400-40 leaf chamber fluorometer, Li-Cor, Inc., Lincoln, NE, USA) (Covarrubias *et al.*, 2014; Tessarin *et al.*, 2018). The measurements were performed on leaves positioned at the 12th node of the season's shoot in the morning between 09:00 and 10:30 and in the afternoon between 15:00 and 16:30 (solar time). The leaves were illuminated by the LI-COR 6400 LED light source providing a photosynthetic photon flux density of ~1200 μmol/(m^2·s). The level of CO_2 was fixed at 380 ppm within the leaf chamber. Net photosynthesis was recorded when foliar CO_2 uptake was steady. The measurements were taken on three dates during ripening: at 75, 113, and 127 DAF at 12.6, 22.1 and 23.3 °Brix respectively in 2013; and at 70, 109, and 118 DAF at 10.4, 17.4 and 19.2 °Brix respectively in 2014.

5. Stem water potential

The stem water potential (Mpa) was measured on two vines per experimental plot (six vines per treatment) at 12:00 (solar time) during berry ripening (75 DAF in 2013; 109 DAF and 118 DAF in 2014) using the Scholander pressure chamber method (Botelho *et al.*, 2016; Scholander *et al.*, 1965; Tessarin *et al.*, 2018). Two mature, completely expanded, exposed and healthy leaves located at the 12th node of different fruiting shoots were selected from each vine. About 60 min before measurement, the leaves to be used for determining Ψ stem were enclosed in plastic bags covered with aluminum foil.

6. Vegetative-productive parameters

In order to assess the effects of the treatments applied in the previous seasons, in 2014 and 2015 the percentage of bud burst of the count nodes (number of shoots from count nodes (SCN)/count nodes (CN) and the fruitfulness of count nodes (number of inflorescences (INF)/SCN) was determined from 30 vines per treatment when the inflorescences were clearly visible (BBCH 53).

The contributions to leaf area of primary and lateral leaves were measured separately, per position, using a LI-3000A leaf area meter (Li-Cor Biosciences, Lincoln, NE, USA) on nine representative fruiting shoots per treatment, at the end of the vegetative growth by sampling shoots from three additional vines located in the same vineyard. The total leaf area per vine (TLA), the total main shoots leaf area (SLA) and the total laterals leaf area (LLA) per plant were estimated by multiplying the average leaf area per shoot by the number of shoots per vine. The number of laterals per shoot, the length and the number of nodes and the average leaf area of each lateral were also determined. Grapes were harvested at optimum technological maturity (potential alcohol above 12.0 %, titratable acidity higher than 6.0 g/L, and pH from 3.30 to 3.50). At harvest (127 DAF, 2013; 125 DAF, 2014), yield components, such as number of clusters per plant, yield per vine, bunch weight (Digital Dynamometer, Wunder SA-Bi S.r.l, Milan, Italy) and leaf area (LA) to fruit ratio (LA/yield), were calculated on 10 vines per experimental plot (30 vines per treatment).

After leaf abscission, the pruning weight (kg) was determined and the Ravaz Index (yield/pruning weight ratio) was calculated on 10 vines per repetition (30 vines per treatment).

7. Berry growth and technological parameters

Berry weight (g/berry; technical balance, Gibertini Elettronica S.r.l., Milan, Italy); total soluble solids (TSS; °Brix; Electronic Refractometer Maselli Misure S.P.A., Parma, Italy); titratable acidity (TA; expressed as g/L of tartaric acid) and pH (Crison Compact Titrator, Crison Instrument SA, Barcelona, Spain) were determined on a must sample crushed from 50 healthy berries collected from 10 vines per replicate every two weeks, from veraison until harvest, to monitor the evolution of berry growth and technological parameters during ripening. The berries were collected randomly from the tips, wings and middle of the bunches from exposed and shaded sides of the canopy.

8. Berry skin anthocyanin and flavonol analysis

Additional berry samples (20 healthy berries per repetition randomly collected from the tip, wing, and middle of bunches from exposed and shaded sides of the canopy) were collected at harvest to quantify anthocyanins and flavonols. The skin extract from each sample was analysed according to the method described by Mattivi *et al.* (2006) using an HPLC apparatus (Jasco, Tokyo, Japan) equipped with a photo diode array (PDA) detector and a reversed-phase column RP18 250 × 4.6 mm (5-μm particle size) (Phenomenex, Castel Maggiore, BO, Italy). Anthocyanins were determined by measuring absorbance at 520 nm. A calibration curve was established with malvidin-3-glucoside standard (Lab Service

Analytica Srl, Anzola Emilia, BO, Italy) and the anthocyanins were expressed as mg/g of skin. Flavonols were determined by measuring absorbance at 360 nm; a calibration curve was established with quercetin-3-*O*-glucopyranoside standard (Lab Service Analytica Srl, Anzola Emilia, BO, Italy) and flavonols were expressed as mg/g of skin.

9. Cluster morphological traits and sanitary status

At harvest (127 DAF, 2013; 125 DAF, 2014) the qualitative parameters of a cluster were measured on 10 clusters per repetition. For each cluster, the index of bunch compactness (according to the 1983 OIV classification) was determined. The incidence (number of affected clusters per vine) and severity (number of affected berries per cluster) of bunch rot (*Botrytis cinerea*) were determined on 10 vines (one cluster per vine) per repetition (30 vines per treatment).

One bunch per vine (30 per treatments) was sampled to determine bunch weight, length and width, the number of berries per bunch, and the rachis weight, length and width.

10. Cluster temperature

An assay of cluster temperature values was performed by the infrared thermometer Raytek RayngerTM ST (Santa Cruz, CA, USA). The temperature of the basal cluster on the same shoot was detected on the sunlight-exposed surface of two clusters per repetition (six clusters per treatment). The cluster temperature measurements were repeated twice a day (at 14:00 and 15:00 solar time) and made on 5 (in 2013) and 4 dates (in 2014) during ripening.

11. Wine chemical analysis

In both vintages, grapes collected at optimum technological maturity (potential alcohol above 12.0 %, total acidity higher than 6.0 g/L and pH ranging from 3.30–3.50) were processed according to the organic winemaking protocol proposed by the Italian Association for Organic Farming (AIAB, Italy) in accordance with the requirements of Reg. CE N. 203/2012 and Reg CE n. 834/2007. Twelve vinifications (CK, DEF I, DEF II and SB repeated for three different vine blocks) of 20 kg of grapes harvested from all 10 vines per replicate were carried out. The 20 kg of grapes were chosen at random and the unhealthy clusters discarded. The winemaking process is described in Tessarin *et al.* (2018). Eighteen kilograms of grapes were destemmed and crushed for each vinification (2 replicates); afterwards, the skins and must were placed in stainless steel tanks, and sulfur dioxide (as potassium metabisulphite: 2 g/vinification, AEB, Brescia, Italy) and complex nutrients (6 g/vinification Nutristart, Laffort, Alessandria, Italy) were added. The contents of the tank were then inoculated with GMO-free yeasts (3.3 g/vinification Saccharomyces cerevisiae (F15, Laffort, Alessandria, Italy). During fermentation, sugar consumption was monitored by means of a Babo densimeter (Polsinelli, Frosinone, Italy). Moreover, the tank content was punched down manually on a daily basis to homogenise it and thus allow the skins to dissolve into the wine. At zero degrees Babo, raking was carried out using a piston press (2 bar, Vaslin, France). The duration of the fermentations ranged between 15 and 18 days. Twenty-nine to 31 days after the end of the alcoholic fermentation a final racking was carried out; then the wines were cold-stabilised, bottled and stored at 10 °C prior to chemical analyses.

The wines were analysed for alcohol strength (AS, %), dry matter (DM, g/L), pH (U), titratable acidity (TA, g/L), volatile acidity (VA, g/L), optical density (OD, AU) at 420, 520 and 620 nm, total colour intensity (CI, AU), hue (HUE, AU) and total polyphenols (TP, mg/L) at 280 nm according to European official methods (EC, 1990). Moreover, total (SO_2T, mg/L) and free (SO_2F, mg/L) sulphur dioxide (Ripper and Schmitt, 1896) and reducing substances (RS, g/L) (Lane and Eynon, 1923) were quantified.

Specific colour- and phenolics- related parameters, such as total anthocyanins (ANT, AU), total red colour (TC, AU), (COP, AU) (Boulton, 2001), large polymeric pigments (LPP, AU), small polymeric pigments (SPP, AU), tannins (TN, mg/L) and non-tannin total iron-reactive phenolics (IRP, mg/L) (Harbertson *et al.,* 2003), were measured by spectrophotometric assay (UV–Vis 1240 mini, Shimadzu, Milano, Italy). Large polymeric pigments (LPP, AU) and small polymeric pigments (SPP, AU) were measured at 520 nm, while tannins (TN, mg/L) and non-tannin total iron-reactive phenolics (IRP, mg/L) were measured at 510 nm (Harbertson *et al*., 2003). All analyses were carried out at the end of the alcoholic fermentation. However, in order to monitor the change in wine composition over time, the analyses of colour and phenolic components were repeated 4 and 16 months after end of fermentation for the vinifications performed in 2014, and 4, 16 and 28 months after end of fermentation for those performed in 2013. Data are presented as mean values obtained from two replicated analyses of each vinification.

12. Statistical analysis

Analysis of variance and comparison of means of parametric data were performed using SAS 6.04 software (SAS Institute, Cary, NC, USA) and Student-Newman-Keuls test ($P = 0.05$). Non parametric data (bunch weight, compactness and discoloration, Ravaz Index, LA/yield ration) were subjected to Kruskall Wallis test, followed by Dunn's comparison test ($P = 0.05$).

Wines analysis of variance for mean separation and Tukey as post hoc test were carried out with XLSTAT version 2011.1.05 (ADDINSOFT, Anglesey, UK). All statistics were performed with significance at $P = 0.05$.

RESULTS

1. Leaf macronutrients

The treatments did not modify the leaf nutritional status (Supplementary Table 1).

2. Stem water potential

The lowest values of stem water potential were detected in the first season due to the climatic conditions (low rainfall

and high temperatures), as no irrigation was applied. The treatments did not modify stem water potential (Mpa), which was recorded at 75 Days After Flowering (DAF) in 2013 (CK: -1.38; DEF I: -1.33; DEF II: -1.48; SB: -1.30); at 109 DAF (CK: -0.78; DEF I: -0.71; DEF II: -0.82; SB: -0.82) and at 118 DAF in 2014 (CK: -0.81; DEF I: -0.70; DEF II: -0.86; SB: -0.86) (Supplementary Table 3).

3. Leaf gas exchange

The treatments did not influence leaf photosynthetic activity during ripening (Supplementary Table 4). Furthermore, stomatal conductance was not affected by the treatments, the only exception being 118 DAF (15:00 - 16:30 solar time), when DEF II vines showed lower stomatal conductance (0.254 mol $H_2O/m^2\ s^1$) compared to defoliated (DEF) I (0.326 mol $H_2O/m^2\ s^1$) vines. However, similar values were registered when compared to the control (CK) (0.299 mol $H_2O/m^2\ s^1$) and SB (0.293 mol $H_2O/m^2\ s^1$) vines (Supplementary Table 4).

4. Vegetative and productive parameters

During both seasons, the defoliation treatments removed 30-35 % of total leaf area per vine (TLA). The results at harvest showed higher TLA and shoot leaf area (SLA) values in CK and "semi-ballerina" (SB) vines than DEF vines, whereas total lateral leaf area (LLA) was higher in 2013 only (Table 1). Moreover, during 2013, CK and SB vines showed a higher number of laterals per shoot, with higher length and lateral leaf area compared to defoliated vines (Table 1). In both seasons, the treatments did not modify plant productivity, pruning weight or the Ravaz Index (Table 2). The treatments also did not alter cluster weight; however, during the first season, DEF I vines had higher bunch weight values compared with DEF II and SB vines (Table 2). Leaf area (LA)/yield ratio was lower in the DEF II vines compared to CK and SB ones in both seasons, with season 2013 showing the highest values for this parameter (Table 2).

The treatments did not modify the percentage of bud burst (113 %, in 2014; 112 %, in 2015), expressed as shoots from count nodes/count nodes, and the fruitfulness of shoots from count nodes (1.55, in 2014; 1.47, in 2015).

5. Cluster morphological traits and sanitary status

Treatments influenced neither the number of berries per cluster (Table 2) nor the length and width of bunch and rachis (data not shown). Furthermore, in both seasons, the compactness of bunches was similar for all the treatments (Table 2). In 2013, the severity of bunch rot was negligible, regardless of the treatment. In 2014, the severity of bunch rot (57 % in control vines) was markedly reduced by DEF I (37 %) and to lesser extent by SB (39 %) and DEF II (42 %). The severity of bunch rot in DEF I vines was significantly different to SB and DEF II, and the values recorded for these two treatments were significantly lower than the control.

6. Berry growth and technological parameters

In 2013, all the treatments showed higher values for total soluble solids (TSS) and pH and lower values for titratable acidity at harvest than in 2014. Late defoliations and shoot positioning did not change the berry weight or the technological parameters during ripening (data not shown) and at harvest compared to the untreated control (Supplementary Table 5). At harvest, berry weight was around 2.5 g in 2013 and 2.7-2.9 g in 2014; TSS ranged from 23.6 to 23.9 °Brix in 2013, and from 18.8 to 19.6 °Brix in 2014; pH was in the range of 3.23-3.26 in 2013, and 3.14-3.20 in 2014; titratable acidity concentration ranged from 6.8 to 7.2 g/L of tartaric acid in 2013, and from 8.4 g/L to 8.6 g/L of tartaric acid in 2014 (Supplementary Table 5).

TABLE 1. Leaf area of main shoots, laterals and total leaf area per plant, number of laterals per shoot, lateral length, number of nodes per lateral, lateral single leaf area at harvest, in controls, vines submitted to post-veraison or pre-harvest defoliations or post-veraison "semi-ballerina" effect through shoot positioning (cv. Sangiovese).

Year	Treatments	SLA (m^2)	LLA (m^2)	TLA (m^2)	Lateral/shoot (N°)	Lateral/Length (cm)	Lateral Nodes (N°)	Lateral leaf Area (cm^2)
2013 (127 DAF)	CK	4.41a	2.08a	6.49a	16.0a	4.81a	1.96	108.21a
	DEF I	3.01b	1.30b	4.31b	12.7b	3.50b	1.78	85.23b
	DEF II	3.03b	1.27b	4.30b	12.5b	3.64b	1.79	86.33b
	SB	4.44a	2.12a	6.56a	16.1a	4.80a	1.85	105.77a
	Significance	***	**	***	**	*	*n.s.*	*
2014 (125 DAF)	CK	4.25a	1.39	5.64a	13.7	3.53	1.60	86.73
	DEF I	2.97b	0.69	3.66b	11.6	2.30	1.30	58.83
	DEF II	3.07b	1.12	4.19b	12.0	3.60	1.69	78.88
	SB	4.23a	1.26	5.49a	13.6	2.93	1.47	69.50
	Significance	**	*n.s.*	*	*n.s.*	*n.s.*	*n.s.*	*n.s.*

*Significant at $P \leq 0.05$; ** significant at $P \leq 0.05$; *** significant at $P \leq 0.001$; n.s. = not significant ($P = 0.05$). Means followed by different letter in each row are significantly different according to the Student- Newman-Keuls test. CK, control vines; DEF I, vines defoliated in post-veraison; DEF II, vines defoliated in pre-harvest, SB, vines subjected to "semi-ballerina" effect through shoot positioning. SLA = main shoots leaf area; LLA = leaf area of laterals; TLA = total leaf area per plant (SLA + LLA). DAF = days after flowering.

TABLE 2. Bunch number per plant, plant productivity, bunch weight, number of berries per cluster, bunch compactness, pruning wood weight, Ravaz Index, LA/yield ratio in 2013 and 2014, in controls (CK), vines subjected to post-veraison (DEF I) or pre-harvest (DEF II) defoliations or post-veraison "semi-ballerina" (SB) effect through shoot positioning (cv. Sangiovese).

	2013					2014				
Parameters	CK	DEF I	DEF II	SB	*Significance*	CK	DEF I	DEF II	SB	*Significance*
Bunch (N°/plant)	13	13	13	13	*n.s.*	15	15	15	15	*n.s.*
Productivity (kg/plant)	4.4	4.4	4.0	3.8	n.s.	6.6	6.7	6.2	6.5	*n.s.*
Bunch weight (kg)	0.329ab	0.349a	0.312b	0.300b	*	0.434	0.445	0.426	0.420	*n.s.*
Berries (N°/bunch)	144	169	149	147	*	132	149	134	134	*n.s.*
Bunch compactness (OIV rating)	7.4	8.4	7.8	7.4	*	8.3	8.4	8.4	8.3	*n.s.*
Pruning wood weight (kg/plant)	0.67	0.69	0.63	0.65	*	0.62	0.69	0.60	0.59	*n.s.*
Ravaz index	6.7	6.6	6.9	6.1	*	11.1	10.2	10.6	11.1	*n.s.*
LA/yield	1.68a	1.05b	1.20b	1.75a	*	0.87a	0.55b	0.59b	0.88a	*n.s.*

*Significant at $P \leq 0.05$; ***significant at $P \leq 0.001$; n.s. = not significant ($P = 0.05$). Means followed by different letter in each row are significantly different according to the Student-Newman-Keuls test (bunch and berries number, plant productivity and pruning wood weight) and Kruskal-Wallis test, followed by Dunn's comparison test (bunch weight, compactness, Ravaz Index and LA/yield ratio).

TABLE 3. Concentration of total glucosylated anthocyanins; percentage of total glucosylated anthocyanins of delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside, di-substituted forms (3'4'-OH glucosylated anthocyanis), tri-substituted forms (3'4'5'-OH glucosylated anthocyanins); 3'4'-OH/3'4'5'-OH ratio; total glycosylated flavonols; percentage of total flavonols of myricetin-glucuronide and myricetin-glucoside; sum of quercetin-glucoside, quercetin-3-O-glucuronide and kaempferol–glucoside recorded at harvest in 2013 and 2014 in controls (CK), in vines subjected to post-veraison (DEF I) or pre-harvest (DEF II) defoliations or post-veraison "semi-ballerina" (SB) effect through shoot positioning (cv. Sangiovese).

	2013					2014				
Parameters	CK	DEF I	DEF II	SB	*Significance*	CK	DEF I	DEF II	SB	*Significance*
Total -G-anthocyanins (mg/g skin)	3.21	3.38	4.17	3.69	*n.s.*	2.18	2.34	2.29	2.37	*n.s.*
Delphinidin -3-G (%)	13.56	15.64	14.03	13.2	*n.s.*	11.37	13.38	11.02	12.38	*n.s.*
Cyanidin -3-G (%)	20.96b	29.22a	24.55ab	23.62ab	*	22.19b	33.48a	32.1a	23.21b	*
Petunidin -3-G (%)	14.30	14.43	14.16	13.86	*n.s.*	11.68ab	11.75ab	10.99b	12.67a	*
Peonidin -3-G (%)	13.70	13.08	14.36	15.39	*n.s.*	14.40	11.74	14.14	13.01	*n.s.*
Malvidin -3-G (%)	37.48a	27.62b	32.90ab	33.93ab	*	40.36a	29.65 b	31.75b	38.73a	*
3'4'-OH-anthocyanins (%)	34.66b	42.30a	38.91ab	39.01ab	*	36.59b	45.22a	46.24a	36.23b	*
3'4'5'-OH-anthocyanins (%)	65.34a	57.7b	61.1ab	60.99ab	*	63.41a	54.78b	53.76b	63.77a	*
3'4'5'-OH/ 3'4'5'-OH	0.53b	0.73a	0.64ab	0.64ab	*	0.58b	0.83a	0.86a	0.57b	*
Total – Flavonols (mg/g skin)	0.736b	1.273a	1.182a	0.745b	*	0.469b	1.311a	1.234a	0.696b	***
Myricetin – glucuronide (%)	1.30	1.33	1.57	1.89	*	2.29a	1.32ab	1.03b	2.23a	*
Myricetin – glucoside (%)	9.11	7.38	8.06	9.12	*	9.60a	5.61ab	4.58b	7.54ab	*
Sum of Quercetin glucoside and Quercetin glucoronide (%)	84.62	85.27	84.74	84.3	*	83.96b	85.27a	85.67a	85.03a	*
Kaempferolo glucoside (%)	4.97	6.02	5.63	4.69	*	4.15b	7 .80ab	8.72a	5.20ab	*

*Significant at $P \leq 0.05$; *** significant at $P \leq 0.001$; n.s. = not significant ($P = 0.05$). Means followed by different letter in each row are significantly different according to the Student- Newman-Keuls test (number of bunch and berries, and plant productivity) and the Kruskal-Wallis test, followed by Dunn's comparison test (bunch weight, compactness and discoloration).

TABLE 4. Chemical properties of wines produced from the control (CK), defoliated at post-veraison (DEF I), defoliated at pre-harvest (DEF II) and "semi-ballerina" shoot-positioned (SB) cv. Sangiovese vines.

	2013					2014				
Parameters	CK	DEF I	DEF II	SB	*Significance*	CK	DEF I	DEF II	SB	*Significance*
ALC (%)	13.6	13.7	13.5	14.4	*n.s.*	9.9	10.3	10.5	9.6	*n.s.*
TA (g/L)	6.8	6.5	6.6	6.8	*n.s.*	6.1	6.2	6.1	6.7	*n.s.*
VA (g/L)	0.35	0.35	0.34	0.33	*n.s.*	0.27	0.23	0.25	0.27	*n.s.*
pH	3.45	3.47	3.49	3.47	*n.s.*	3.27	3.28	3.3	3.23	*n.s.*
DM (g/L)	25.0	25.3	24.9	27.6	*n.s.*	20.1	21.9	22.5	0.27	*n.s.*
RS (g/L)	1.3	1.4	1.3	1.4	*n.s.*	< 1	< 1	< 1	< 1	*n.s.*
SO2T (mg/L)	58	57	56	60	*n.s.*	37	31	30	40	*n.s.*
SO2F (mg/L)	25	25	25	26	*n.s.*	11	10	12	12	*n.s.*
CI (AU)	6.038	7.384	6.474	7.180	*n.s.*	1.791	2.103	2.427	2.010	*n.s*
HUE (AU)	0.647	0.648	0.666	0.630	*n.s*	0.848	0.888	0.763	0.868	*n.s.*
TP (mg/L)	1113	1206	1122	1280	*n.s*	617	665	739	677	*n.s.*

*Significant at $P \leq 0.05$; ***significant at $P \leq 0.001$; n.s. = not significant ($P = 0.05$). Means followed by different letter in each row are significantly different according to the ANOVA test followed by the post-hoc Tukey's test. Legend: ALC = alcohol strength; TA = Titratable acidity; VA = Volatile acidity; DM = Total dry matter; RS = Reducing substances; SO2T = Total sulphur dioxide; SO2F = Free sulphur dioxide; CI = Colour intensity; HUE = Colour hue; TP = Total polyphenols. Data are the mean value of three independent vinifications of 20 kg (replicates).

7. Berry skin anthocyanins and flavonols

The treatments did not modify total glucosylated anthocyanin concentration at harvest. A higher percentage of cyanidin-3-glucoside and forms of di-substituted anthocyanins, together with a higher 3'4'-OH/3'4'5'-OH ratio, was observed in both seasons in DEF I, as well as in the second season in DEF II berry skins compared with CK ones (Table 3). On the other hand, the percentage of malvidin-3-glucoside and forms of tri-substituted anthocyanins was lower in 2013 and 2014 in DEF I, as well as in the second season in DEF II berry skins compared to that of untreated vines (Table 3). Shoot positioning did not influence the percentage of berry skin glucosylated anthocyanins compared to CK in either season; however, in 2014 higher percentages of petunidin 3-glucoside were observed in SB berry skins compared to DEF II ones (Table 3). The treatments did not modify the total glucosylated anthocyanins concentration at harvest.

In both years, late defoliations increased the total concentration of berry skin flavonol glycosides at harvest, compared to CK and SB treatments (Table 3, Figure 2). In 2013, no differences in the percentage of glycosylated flavonols were observed among treatments, whereas in the second season lower percentages of myricetin-glucuronide and myricetin-glucoside and higher percentages of kaempferol-glucoside were observed in DEF II berry skins compared with CK; moreover, both DEF treatments and shoot positioning increased the percentage of the total of quercetin-glucoside and 3-*O*-glucuronide (Table 3). In both seasons, late defoliations increased the total concentration of berry skin flavonol glycosides at harvest, compared to CK and SB treatments (Table 3, Figure 1).

8. Cluster temperature

In both seasons, exposed clusters from vines subjected to late defoliations generally showed higher temperatures after treatment compared with those of the controls during the hottest hours of the day (Supplementary Table 6). The vines subjected to the "semi-ballerina" effect through shoot positioning showed similar cluster temperatures to CK vines, with the exception of some measurements performed at 85 and 101 DAF in 2013, when recorded values were higher and lower respectively than CK vines (Supplementary Table 6).

9. Wine chemical analyses

A one-way ANOVA performed on all treatments for wine chemical composition during storage did not show any significant differences between the parameters, regardless of the year of the vintage. However, the wines produced in 2014 were characterised by lower levels of all the chemical parameters than those produced in 2013 (Table 4), with range values below the regular/usual values: alcohol (range: 9.9-10.5 %), pH (3.23-3.30), dry matter (20.1-22.5), colour intensity (1.791-2.427 AU) and total polyphenols (617-739 mg/L); the hue, however, was high (0.763-0.888). In wines of 2013, the range was as follows: alcohol strength 13.5-14.4%, total polyphenols 1113-1208 mg/L, titratable acidity 6.5-6.8 g/L and pH 3.45-4.49.

The results of one-way ANOVA for phenolic and colour components of wines during storage are shown in Table 5. In 2013-wines, significant differences were found in polymeric pigments (PP) at 4 months of storage, recording the highest values for the DEF I treatment compared to CK. There was no significant difference after 16 and 28 months of storage, but it is worth noting that DEFI, DEFII and SB

TABLE 5. Phenolic and colour components of wines produced from the control (CK), defoliated at post-veraison (DEF I), defoliated at pre-harvest (DEF II) and "semi-ballerina" shoot-positioned (SB) cv. Sangiovese vines.

		2013					2014				
Parameters	Aged	CK	DEF I	DEF II	SB	*Significance*	CK	DEF I	DEF II	SB	*Significance*
TC (AU)	4	3.3	3.9	3.4	3.9	*n.s.*	0.8	0.9	1.2	1.0	*n.s.*
	16	2.3	2.9	2.8	3.0	*n.s.*	0.8	0.8	1.1	0.9	*n.s.*
	28	2.2	2.7	2.3	2.9	*n.s.*	-	-	-	-	*n.s.*
COP (AU)	4	0.7	0.7	0.9	0.8	*n.s.*	0.0	0.1	0.1	0.1	*n.s.*
	16	0.1	0.1	0.3	0.1	*n.s.*	0.0	0.0	0.1	0.1	*n.s.*
	28	0.1	-0.1	0.0	0.0	*n.s.*	-	-	-	-	*n.s.*
ANT (AU)	4	1.6	1.9	1.5	1.8	*n.s.*	0.5	0.5	0.8	0.6	*n.s.*
	16	1.0	1.3	0.9	1.3	*n.s.*	0.2	0.3	0.4	0.4	*n.s.*
	28	0.7	0.8	0.6	0.9	*n.s.*	-	-	-	-	*n.s.*
PP (AU)	4	1.0b	1.3a	1.0b	1.2ab	**	0.3	0.3	0.4	0.3	*n.s.*
	16	1.1	1.5	1.7	1.6	*n.s.*	0.5	0.5	0.6	0.5	*n.s.*
	28	1.4	2.0	1.7	2.0	*n.s.*	-	-	-	-	*n.s.*
LPP (AU)	4	0.5	0.7	0.5	0.6	*n.s.*	0.2	0.2	0.2	0.2	*n.s.*
	16	0.8	1.0	1.4	1.2	*n.s.*	0.3	0.3	0.4	0.3	*n.s.*
	28	2.7	3.9	3.4	3.8	*n.s.*	-	-	-	-	*n.s.*
SPP (AU)	4	0.5	0.6	0.5	0.6	*n.s.*	0.2	0.2	0.3	0.2	*n.s.*
	16	0.5b	0.7b	0.7ab	0.8a	*	0.2	0.3	0.3	0.3	*n.s.*
	28	0.7	0.7	0.7	0.9	*n.s.*	-	-	-	-	*n.s.*
TN (mg/L)	4	284.2	383.4	289.2	393.4	*n.s*	178.5	176.2	220.0	223.8	*n.s.*
	16	393.1	503.7	452.1	536.0	*n.s.*	164.6	155.8	186.7	187.7	*n.s.*
	28	267.6	492.2	294.9	433.8	*n.s.*	-	-	-	-	*n.s.*
IRP (mg/L)	4	1285.1	1487.5	1305.9	1511.6	*n.s.*	676.4	735.9	843.2	788.8	*n.s.*
	16	1307.8	1506.9	1322.2	1579.9	*n.s.*	642.3	686.2	768.0	729.1	
	28	1244.2	1357.5	1147.1	1484.8	*n.s.*	-	-	-	-	*n.s.*

Legend: Aged = months from the end of fermentation; TC = total colour; COP = co-pigmentation; ANT = anthocyanins; TN = tannins; IRP = iron reactive phenolics. The letters represent the results of Tukey's comparison post hoc test: different letters in the columns indicate the means that are significantly different ($\alpha = 0.05$) among wines.

wines maintained higher values for these colour components over time. In the same season, the small polymeric pigments (SPP) fraction of 16 months stored wines were significantly higher for the SB treatment than the control (CK); this difference was no longer significant but still evident after 28 months of storage. As expected, large polymeric pigments (LPP) increased over time during storage in all the treatments, and values were higher but not significant in DEF I (3.9 AU) and SB (3.8 AU) compared to CK (2.7 AU). In 2014-wines, phenolic and colour components did not show any significant differences between treatments. In 2013, wines from the post-veraison defoliation (DEF I) and "semi-ballerina" shoot positioning (SB), were also found to have a slight increase in total colour (TC) and anthocyanins (ANT) compared to CK wine. The total monomeric anthocyanins decreased significantly only 28 months after end of fermentation for all treatments. However, the highest values were still observed in DEF I (0.8 AU) and SB (0.9 AU), compared to CK (0.7 AU). Co-pigmentation (COP) decreased significantly after 16 months of storage, regardless of type of management (CK: 0.7 to 0.1 AU; DEF I: 0.7 to 0.1 AU; DEF II: 0.9 to 0.3 AU, SB: 0.8 to 0.1 AU), then a total loss of co-pigmentation was monitored in all wines after 28 months of storage (Table 5). The concentration of tannins (TN) was higher in DEFI and SB at 4 months of storage and after 28 months these compounds slightly increased in DEFI, DEFII and SB, while a slight decrease was registered in CK. Likewise, the concentration of iron-reactive phenols (IRP) was higher in DEFI and SB at 4 months, compared to CK. After 28 months, IRP did not show any significant differences between treatments. In conclusion, the TC, COP, ANT, LPP, SPP of DEF I, DEF II and SB were higher than CK which lends support to the colour intensity data (Tables 4 and 5).

DISCUSSION

In previous studies, a reduction in total soluble solids (TSS) at harvest was obtained only when the leaf area (LA)/yield ratio was lower than 0.8–1.2 m^2/kg (Kliewer and Dokoozlian,

2005; Poni *et al.*, 2013). In our work, the decrease in total leaf area per vine (TLA) (-30-35%) that occurred in the defoliated (DEF I and DEF II) vines did not result in enhanced photosynthetic activity in the remaining leaves compared with the control (CK) vines. These different results may be explained by the limited influence of removing old leaves in our experiment (134 days in 2013 and 147 days in 2014 for DEF I leaves; 149 days in 2013 and 161 days in 2014 for DEF II leaves), which are characterised by low photosynthetic activity compared to that of intermediate and apical leaves (Kriedemann *et al.,* 1970).

Moreover, in our study, the plant nutritional status (Supplementary Table 1) and the physiological status were similar for all treatments, and the photosynthesis values were within the standards of Sangiovese vines (Penazzi *et al.,* 2011; Tessarin *et al*., 2018). This suggests that the reiteration of late defoliations or shoot positioning did not have any adverse effects on these parameters.

Our data suggest that in a season characterised by high rainfall during the ripening period it is important to improve the bunch microclimate by avoiding over-shading and by removing leaves and laterals from the bunch zone, preferentially at post-veraison. The possibility of reducing the attack of *Botrytis* on cv. Sangiovese bunches is of paramount importance for organic growers, lacking effective products against this fungus. Therefore, the implementation of these agricultural practices, while taking into account the meteorological conditions of each season, is of great importance in organic viticulture.

Berry weight and technological parameters (TSS, pH and titratable acidity (TA)) at harvest were not affected by the treatments; this result is consistent with other studies on late defoliation (Caccavello *et al.,* 2017; Pastore *et al.*, 2017). However, some studies have produced different results: post-veraison defoliation of leaves above the bunch zone in cv. Sangiovese potted vines (Poni *et al.*, 2013) or in field conditions (Palliotti *et al.*, 2013) have been found to induce a decrease in soluble solids of the berry (Palliotti *et al.*, 2013; Poni *et al.*, 2013) and a reduction in alcohol in the wines (Palliotti *et al.*, 2013), without changing anthocyanins and polyphenols in berries (Palliotti *et al.*, 2013; Poni *et al.*, 2013) or wines (Palliotti *et al.*, 2013). As discussed earlier, the removal of the basal leaves did not induce any compensation phenomena in the photosynthetic activity of the remaining younger leaves. Presumably, the sugars stored as plant reserves were translocated into the berries (Candolfi-Vasconcelos *et al.,* 1994; Rossouw *et al*., 2017). The exposure of bunches to direct solar radiation and the consequent higher berry temperature did not result into any reduction in TA (or pH increase) in the berries, possibly because the removal of the leaves resulted in a decrease in the organic acid-neutralising cations (particularly K) that were translocated via the phloem to the berries (Villette *et al.*, 2020).

Flavonols may play a crucial role as co-pigments in young red wine by stabilising the anthocyanins and creating a stable association to form polymeric pigments, whose importance for the colour of older red wines is well-known. When changes in the abundance of individual flavonols were observed (2014), a higher percentage of the sum of quercetin-glucoside and glucuronide was recorded in berry skins from the vines that underwent late defoliation and shoot positioning treatments; moreover, DEF II vines showed a higher percentage of kaempferol-glucoside and a lower percentage of myricetin-glucuronide and myricetin-glucoside compared with the CK vines (Table 3). In a study by Pastore *et al.* (2013), an increase in the concentration of cv. Sangiovese berry skin flavonols was also observed following the removal of leaves and laterals at pre-bloom and veraison, and similar to our study, higher quercetin and kaempferol percentages were detected compared to the control vines. This phenomenon occurs, because the synthesis of flavonols is promoted by the exposure of clusters to direct sunlight (Downey *et al*., 2004).

The concentration of flavonols in berries have been shown to strongly correlate with degree of fruit exposure (Price *et al.,* 1995; Haselgrove *et al.*, 2000) and bunch shading to significantly reduce flavonols levels (Downey *et al.,* 2004; Matus *et al.,* 2009). However, flavonol concentrations have also been reported to decrease following leaf removal treatments (Matus *et al.*, 2009; Baiano *et al.*, 2015); it is likely that the high temperatures within the berry skin after exposure to direct sunlight adversely affected flavonol accumulation, thus overriding the positive effects of the light.

The effects of late DEFs on total flavonol concentration at harvest may be partially due to the increased light condition in the bunch zone. In the measurements performed in the afternoon, the Photosynthetic Active Radiation (PAR) (Skye Quantum Sensor, Llandrindod Wells, Powys, UK) at bunch level varied in the range 30-250 μmol/(m^2·s) in vines subjected to the SB effect, 150-250 μmol/(m^2·s) in CK vines and 1200-2200 μmol/(m^2·s) in DEF vines. In these hottest hours of the day, the clusters of DEF vines showed higher temperatures than those of CK and SB vines (data not shown). However, previous research has shown that temperature may have little or no impact on berry flavonol biosynthesis (Price *et al.*, 1995; Haselgrove *et al.*, 2000, Spayd *et al.*, 2002; Mori *et al.,* 2005).

In this study, the higher exposure to light of the bunches in both of the defoliated (DEF I and II) vines did not affect total anthocyanin concentration at harvest (Table 3). It has been reported that depriving bunches from light may have no effect on colour or, conversely may influence anthocyanin composition and/or concentration, depending, for instance, on the variety (Downey *et al.,* 2004; Cortell and Kennedy, 2006) or the duration of the treatment (Li *et al.*, 2013). The effects of light on anthocyanin concentration are also closely dependent on the increase in berry temperature as a consequence of increased sunlight exposure, since high berry temperature can inhibit colour development (Bergqvist *et al.*, 2001; Mori *et al.,* 2005; Mori *et al.,* 2007). Temperature has more effect on anthocyanin accumulation than light (Spayd *et al*., 2002, Mori *et al*., 2005, Tarara *et al*., 2008). Although the DEF bunches experienced higher temperatures, total

glucosylated anthocyanin concentrations were similar for all treatments, and changes in anthocyanin composition were observed (Table 3). While the profile and structure of skin anthocyanins are known to influence intensity and stability of wine colour, there is little information regarding the influence of agronomic practices (e.g., irrigation, defoliation) on single anthocyanins (Tessarin *et al*., 2014; Theodorou *et al*., 2019). The data obtained in this experiment seem to suggest that bunch exposition to higher temperatures may change the proportion of total glucosylated anthocyanins (Table 3). Concerning the relative abundance of the single anthocyanin glucosylated forms, some changes were observed among the treatments; in particular, the 3′4′-OH/3′4′5′-OH anthocyanin ratio at harvest was always higher in DEF I berries, as well as in the second season in DEF II, compared to the CK and SB ones. This increase in the di-substituted to tri-substituted ratio was in accordance with the effect of veraison defoliation in the study by Pastore *et al.* (2017). This could be explained by a metabolic shift leading to higher accumulation of cyanidin and a lower accumulation of malvidin-3-glucoside in vines subjected to late leaf and lateral removal in the bunch zone (Table 3). Similarly, in cv. Sangiovese vines subjected to veraison-leaf removal in the bunch zone, Pastore *et al.* (2013) found a higher percentage of cyanidin-3-glucoside and a lower percentage of malvidin-3-glucoside compared to CK at harvest, despite there being no changes in the concentration of total anthocyanins. The modifications to anthocyanin percentage may be, in part, explained by the increased light interception by bunches in the canopies that underwent late defoliation treatments, with an increase in di-substituted anthocyanins forms, particularly cyanidin-3-glucoside, and a decrease in the tri-substituted ones (Table 3). Such changes exhibit similarities to changes in the proportion of flavonols (Table 3) and may be partly associated with the latter; indeed, the synthesis of anthocyanins from flavonols occurs through specific metabolic pathways (Castellarin *et al*., 2012). The di-substituted to tri-substituted anthocyanin ratio is a potential indicator of the mode of the actions triggered by late defoliation treatments; its changes indicate a possible effect of light on the metabolic pathways involved in the synthesis of anthocyanins. The changes induced by DEF I and DEF II on the anthocyanin profile likely contribute to explaining the observed changes in the wines (Table 5).

The additional shading produced by SB shoot positioning on one side of the canopy did not have any affect on anthocyanins and flavonols levels. This indicates that the berries were sufficiently protected by leaves.

To our knowledge this is the first investigation on the multiple effects of the described practices on berries (Table 3) and on wine throughout storage (Table 4, Table 5). Moreover, the implications of the "semi-ballerina effect" through shoot positioning on wine quality were assessed for the first time (Table 4, Table 5).

Late defoliations (post-veraison or pre-harvest) and the "semi-ballerina" effect through shoot positioning significantly influenced some of the colour components studied in the wines, while maintaining the basic properties of the wines (e.g., alcohol strength, titratable acidity, volatile acidity, dry matter and pH) in the 2013 and 2014 vintages (Table 3). In particular, the SB treatment had higher small polymeric pigments than CK in wines after 16 months of storage, and DEF I wines showed higher total polymeric pigments than CK in wines after 4 months of storage (Table 5). Likewise, Tessarin *et al.* (2018) found that late trimming in Sangiovese resulted in a higher concentration of small and large polymeric pigments in stored wines. Data suggest that bending downwards lignified canes at post-veraison (Supplementary Figure 1), which involved some fractures, mimicked the action of trimming performed in the same phenological stage. Although the statistical analysis did not show any significant differences among treatments for the other parameters under investigation (Table 5), the data indicate that DEF I and SB treatments resulted in a colour enhancement in the wines of the 2013 vintage, the season being characterised by higher temperatures during ripening. This fact also shows the importance of the timing of the treatment, since the treatments that showed the highest values were DEF I and SB, both applied at post-veraison. Colour components, tannins and iron-reactive phenols are of great importance for the evolution of aging wines. Our results are consistent with those obtained for cv. Nero di Troia in a study by Baiano *et al.* (2015); however, in this variety, the late defoliations influenced the chemical composition of the wines.

In the 2014 wines, evaluating the effects of the treatments was not easy due to low alcohol and polyphenol concentrations; nevertheless, overall, a similar trend was observed. Collectively, these components might have contributed to an effect on the final colour intensity of the wine with a sensory impact on the wine. The loss of anthocyanins during storage may be due to the degradation of the anthocyanins or their incorporation into oligomeric and polymeric pigments - most likely into pigmented tannin-anthocyanin polymers (LPP) via anthocyanin-acetaldehyde cross-linked oligomers and pyranoanthocyanins (SPP) (Harbertson *et al.*, 2003), as evident from the data on polymeric pigments. In fact, after one year of storage, the total polymeric pigments, fractioned as LPP and SPP, increased significantly (Table 5).

It is worth nothing in this study that the positive effects of the late defoliation treatments on the colour of the wine are not due to an enhanced concentration of berry skin anthocyanins (Table 5), but rather due to LPP and SPP pigments (Table 5). This increase may also be related to variations in the concentration of polyphenols in the seed due to late defoliation and shoot positioning treatments. Most studies have focused on early defoliation treatments, with results showing contrasting effects on seed polyphenols at harvest. Talaverano *et al.* (2016) reported a decrease in the concentration of seed polyphenols after the pre-flowering defoliation of Tempranillo vines. Kotseridis *et al.* (2012) recorded different results for the amount of seeds phenols in vines defoliated after flowering compared to the control vines, depending on the cultivar and the severity of the defoliation. The ability to drive the polymeric pigment

reaction forward depends on many factors, including the levels of anthocyanins, tannins and acetaldehyde, the pH, the levels of SO_2 and the temperature (Harbertson *et al.*, 2003). Our results suggest that late defoliation, particularly DEF I, and SB can substantially improve the quality of Sangiovese wines produced organically, increasing the parameters correlated with colour in a warm season as 2013.

CONCLUSIONS

The late defoliations (DEF I and DEF II) applied in the two consecutive seasons on the same cv. Sangiovese vines did not alter budburst, fruitfulness, leaf photosynthetic activity, vine water status, yield, berry technological parameters or berry skin anthocyanins. However, there was a marked increase in the concentrations of total flavonols in the two contrasting seasons as a result of enhanced light conditions in the bunch zone after the removal of leaves and laterals. Furthermore, late defoliations, especially DEF I, and "semi-ballerina" shoot positioning (SB) to a minor extent, limited the severity of *Botrytis* bunch rot. The results clearly indicate that the defoliations at post-veraison or pre-harvest have a beneficial effect on berry composition and grape sanitary status.

Oenological benefits induced by late leaf removal at post-veraison and pre-harvest and by the "semi-ballerina" effect through shoot positioning were observed in cv. Sangiovese wines throughout storage. In particular, these canopy management practices positively influenced wine components related to the final colour intensity without altering the basic chemical characteristics of wine.

The positive implications of late defoliations on wine colour are not related to enhanced concentrations in berry skin anthocyanins, but to increased flavonol levels and polymeric pigments. The increase in flavonols after the imposition of late defoliations indicates this practice can have positive implications for berry and wine composition, even when applied at pre-harvest.

"Semi-ballerina" shoot positioning and post-veraison defoliation improved the wine colour, particularly in the first season, which was characterised by higher temperatures and lower rainfall; this technique therefore shows high potential in the context of climate change. A more detailed metabolic analysis is required to better understand which compounds contributed to the observed effects, and thus be able to improve wine properties related to the aging of Sangiovese.

The choice of the proper timing of late defoliation (post-veraison vs. pre-harvest), should consider meteorological conditions in order to successfully both improve grape quality and contain and cluster rot. Technological advances mean that mechanical defoliation is also possible at pre-harvest, without damaging the bunches. Our results may further contribute to providing useful recommendations on canopy management for grape growers who produce organic Sangiovese wines subject to aging.

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