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Canopy management strategies to control yield and grape composition of Montepulciano grapevines

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Abstract

Background and Aims: Higher temperature during the season is forcing growers in Central Italy to explore ways to reliably control vine yield and grape ripening, while maintaining grape composition. The most common approaches include altering winter pruning, shoot thinning (St), leaf removal and bunch thinning. These studies, however, rarely evaluated these practices in concert and over multiple seasons.
Methods and Results: From 2009 to 2013, five treatments were applied to *Vitis vinifera* L. cv. Montepulciano: winter pruning only (Wp, Control); Wp plus St; St plus pre-flowering defoliation (St + Dpa); St plus pre-veraison defoliation (St + Dpv); and St + Dpv plus bunch thinning (St + Dpv + Bt) applied prior to veraison. Effects on canopy architecture, yield, ripening and berry composition were measured. Compared to Wp, St, St + Dpv and St + Dpv + Bt treatments reduced leaf area and leaf layer number in the fruiting zone, while St + Dpv + Bt reduced yield. No treatment slowed ripening. The treatment St + Dpa reduced yield and the incidence of *Botrytis cinerea*, and improved fruit composition, but increased TSS in berries. All treatments were ceased after 2013 and the vines were pruned in winter only. The treatment St + Dpa imposed in 2013 had a strong carry-over effect on yield but not TSS in 2014.
Conclusions: Shoot thinning alone reduced canopy density but failed to reduce yield or improve fruit composition. Both the St + Dpv and St + Dpv + Ct treatments provided a more open fruit zone, had no effect on yield and increased TSS in fruit at harvest. Shoot thinning plus pre-flowering defoliation decreased yield and improved berry composition in a Mediterranean climate; however, given its observed carry-over effects on yield this approach should be applied only in alternate years, suggesting the need for further research exploring additional viticultural practices.
Significance of the Study: Despite some benefits of St, defoliation and bunch thinning on their own or even in concert, no combination tested was consistently effective for controlling vine yield and grape ripening, while maintaining grape composition.

Keywords: Botrytis cinerea, *bunch thinning, canopy density, climate change, crop control, leaf removal*

Introduction

An increase in mean temperature has been observed over the past three decades in several important viticultural areas of Central Italy (Di Lena et al. 2012). Seasonal trends have been characterised by milder winters and more frequent summer drought conditions, leading to advanced phenology and higher sugar accumulation (Palliotti et al. 2014, Frioni et al. 2016) and higher alcohol content in resultant wines which is problematic, as the preferences of wine consumers are shifting towards wines of moderate alcohol content (Seccia and Maggi 2011). In addition, a continuing upward trend in mean temperature will make it difficult for growers in these regions to adhere to yield and TSS regulations according to the origin designation (Denominazione di origine controllata, DOC). Consequently, managing vineyard yield and berry ripening are critical, and the impact of winter pruning and canopy management techniques to manage yield and ripening has been the focus of many recent studies (Poni et al. 2006, Intrieri et al. 2008, Bravetti et al. 2012, Lanari et al. 2013, Gatti et al. 2015, Silvestroni et al. 2016). Variation between sites and cultivars in these studies, however, has made it difficult to generate general recommendations. By testing multiple treatment combinations over consecutive seasons, the relative contribution of individual techniques as well as the potential for each treatment or treatment combination can be assessed. Winter pruning is the most widely used viticultural technique to regulate crop yield and to achieve targeted grape composition, not withstanding the fact that node number per vine is not an accurate predictor of yield at harvest (Bernizzoni et al. 2011, Geller and Kurtural 2013). Winter pruning is often followed by shoot thinning (St) and/or leaf removal to decrease foliage density and encourage light and air penetration into the canopy (Bravetti et al. 2012, Silvestroni et al. 2016). Shoot removal also facilitates more desirable shoot spacing along canes and cordons, and more even leaf area distribution in the canopy (Naor et al. 2002, Bravetti et al. 2012, Silvestroni et al. 2016), thus improving penetration into the fruiting zone. This can, in turn, improve bud fertility and fruitset and mitigate against yield losses due to St. Often St increases yield and decreases vegetative growth, leading to higher yield to leaf area ratios. Increase of yield capacity is

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related to a lower Ravaz index [RI (Ravaz 1911)], which can be measured as the ratio of yield to pruning mass (Naor et al. 2002, Myers et al. 2008). Ravaz index values ranging from 5 to 10 kg/kg indicate a good balance between yield and vine vigour (Kliewer and Dokoozlian 2005). Effects on fruit composition are variable, with some studies reporting an improvement in some parameters from a variety of sites with different cultivars (Keller et al. 2008, Sun et al. 2012, Susaj et al. 2013), while others show no change (Morris et al. 2004, Silvestroni et al. 2016). Leaf removal (defoliation) has been one of the most widely studied viticultural practices for reducing canopy density and yield, and the resultant outcomes appear to depend on application timing and severity. Removal of the first six basal leaves at the pre-anthesis stage has been shown to be an effective strategy for controlling yield capacity via source–sink relationships (Poni et al. 2006, Intrieri et al. 2008, Acimovic et al. 2016), which promotes looser bunch compactness, and subsequently lowers rot susceptibility while increasing grape sugar concentration (Sabbatini and Howell 2010, Bravetti et al. 2012, Silvestroni et al. 2016). Furthermore, Silvestroni et al. (2016) found that the source limitation induced by early defoliation impacts bunch size due to a minor shortening of the first rachis branch and overall bunch length. As reported previously by Poni et al. (2006) and Gatti et al. (2015), the berry skin and flesh mass are also influenced by this treatment, modifying final berry size. This can improve the concentration of phenolic substances, including anthocyanin (Poni et al. 2006, Pastore et al. 2013). Conversely, defoliation carried out pre-veraison removes leaves with lower photosynthetic activity, resulting in little effect on yield (Bravetti et al. 2012, Silvestroni et al. 2016). Vineyards are frequently bunch thinned to manage for premium quality wine, and it is an additional technique used in high-yielding cultivars to reduce yield within the limits imposed by the DOC regulations. Bunch removal typically increases the leaf area to yield ratio, improving grape composition by avoiding overcropping (Reynolds et al. 1996, Prajitna et al. 2007, Bravetti et al. 2012, Susaj et al. 2013). Yet, among these techniques, there are no clear recommendations for growers to reliably control yield and fruit composition. This investigation was conducted over 5 years (2009–2013) on Montepulciano vines to evaluate and compare the effect of the aforementioned canopy management techniques on canopy density, yield, ripening and grape composition. Our goal was to identify the most effective strategy for managing yield and grape composition. During the last season of the trial (2014), all vines were pruned in winter with no other treatments imposed in order to monitor any potential carry-over effects of previous treatments on yield capacity and grape composition at harvest.

Materials and methods

Plant material, experimental conditions and experimental design

The study was conducted over a 6-year period, 2009–2014, in a hillside vineyard (~5% slope) located near Ancona (Marche region, Central Italy; latitude 43°32′N, longitude 13°22′E; elevation 203 masl). The vineyard was planted in 2004 with certified virus-free Montepulciano grapevines grafted onto Kober 5BB rootstock. The vines were spaced 1.20 m within rows and 2.75 m between rows, oriented north-northeast to south-southwest resulting in a density of 3030 vines/ha. Vines were cordon-trained and hand-pruned in winter leaving seven spurs of two nodes per vine and trained to a vertically shoot-positioned trellis (VSP). The cordon was set at 0.85 m above-ground with two pairs of catch wires providing trellising extending 0.9 m above the cordons. During the study, shoots were mechanically trimmed when their growth exceeded the top wires, usually near the end of June. Recommended crop protection practices were carried out according to local practices determined by field scouting, experience and weather conditions. The study was conducted on 40 contiguous uniform vines chosen along one row and organised into four blocks with ten vines each. Each block was divided into five plots of two vines each, and the same treatment was assigned to the vines in each plot to have two replicates per treatment per block and, therefore, a total of eight replicates per treatment. From 2009 to 2013, all vines were pruned during winter. The five canopy management treatments applied were: annual winter pruning (Wp, Control treatment: no St or defoliation) was applied to all vines; St (leaving 14 shoots per vine) was carried out each year at the end of May and the beginning of June; pre-anthesis defoliation (St + Dpa) consisted of manual removal of leaves and laterals from the first six basal nodes of each shoot, and was carried out during the rapid shoot elongation phase (the first 10 days of June); pre-veraison defoliation (St + Dpv) was carried out identically to defoliation at pre-anthesis and was applied at the full canopy and bunch closure (stage *E*-L 32) phase (during the last 10 days of July); and bunch thinning applied on St + Dpv vines (St + Dpv + Bt) during the same period of the pre-veraison defoliation. Bunch thinning was carried out by manually removing bunches, wholly or partly, to obtain a yield per vine between 3.6 and 4.2 kg, consistent with the limits imposed by the DOC regulation at the study site. In the last year of the trial (2014), all vines were subjected to winter pruning only.

Vine growth, canopy measurements and weather conditions

Each year (2009–2013) the number of shoots before and after thinning was counted and recorded. Canopy development was monitored during the season via point quadrat analysis (Smart and Robinson 1991) with insertions to the full height of the canopy, at 10 cm intervals with a thin metal rod using a sampling grid (n = 100–120). The canopy density, expressed as leaf layer number (LLN), was estimated considering the effects of treatments on LLN in the fruiting zone (at 20 and 40 cm from the cordon). Primary and lateral leaves were separately removed for each treatment and their surface area measured with a leaf area meter (LI-3100, LI-COR Biosciences, Lincoln, NE, USA). Climate data were supplied from an automatic weather station located approximately 100 m from the experiment site, and at the same elevation. Daily maximum temperature (Tmax) and minimum temperature (Tmin) from 1 April to October were retrieved for each experimental year. Mean temperature (Tmean) and growing degree day (GDD, base 10°C) accumulation from 1 April to 31 October were calculated as described by Baskerville and Emin (1969).

Vine yield, bunch morphology and grape composition

In all years, TSS was measured in grapes sampled weekly from mid-August (veraison) to harvest, and grapes were harvested when berry TSS plateaued above 20°Brix. Harvest date was 28 September 2009 and 26 September 2011, while the date was 15, 8 and 14 October in 2010, 2012 and 2013, respectively. To measure carry-over effects in 2014, grapes were harvested and analysed on 8 October. Yield per vine was measured at harvest and the total number of bunches was counted and weighed. Mean bunch mass was determined by dividing the yield per vine by the bunch number per vine. At each harvest, ten uniform bunches per vine selected on the basis of mean bunch mass were weighed separately and their size (rachis length and width) was measured, while bunch compactness and grape health status (rating of *Botrytis cinerea* and sunburn) were recorded. Bunch compactness was expressed as a ratio of bunch mass and bunch length (g/mm) and visually estimated with the Organisation Internationale de la Vigne et du Vin (OIV) code 204 (Organisation Internationale de la Vigne et du Vin 1983), which uses a numbered scale to rank 'berries in grouped formation with many visible pedicels' as 1 to 'misshaped berries' as 9. Commencing 5 days after the beginning of veraison in 2011, a drought year with above average temperature and little rain during most of the berry ripening phase, photoinhibition in basal leaves and millerandage was noted. Each year, samples of 100 berries per vine were randomly collected from each block and weighed to determine berry fresh mass. The berries were then crushed to obtain juice for measuring TSS (°Brix), pH and TA. A temperature-compensating Maselli LR-01 (Maselli Misure, Parma, Italy) digital refractometer was used to determine TSS. pH was measured with a Crison two-decimal pH meter (Crison Instruments, Barcelona, Spain) using a glass electrode, while TA was determined with a Crison Titrator (Crison Instruments) using 0.25 N NaOH to a pH 7.00 end point, expressed as g/L of tartaric acid equivalent. Tartaric acid concentration was determined using the 'colorimetric dosage method' via a reaction of tartaric acid with vanadium acid producing an orange colour measured with a spectrophotometer at 500 nm, while enzymatic kits (Enzyplus-Raisio, Raisio, Finland) were used to assess malic acid concentration. Each year, the concentration of anthocyanin and phenolic substances was determined according to Mattivi (2004) using the same berry sample analysed for must characteristics. These berries were further pressed to obtain a dried sample (skins and seeds only). Each dried sample was added to a buffer solution/extractive of hydrochloric acid, homogenised using an Ultra-Turrax T25 (Janke & Kunkel, IKA-Werke, Staufen, Germany) and subsequently centrifuged (model ALC 4218, Thermo Fisher Scientific, Milano, Italy) for 10 min at 3257 × *g*. The liquid phase was collected in dark glass bottles and used for anthocyanin determination; first diluted with ethanol hydrochloric acid and then the absorbance was registered with a spectrophotometer (UV-1601, Shimadzu Italia, Milan, Italy). Anthocyanin concentration was calculated as malvidin 3-glucoside chloride equivalents (mg/kg of grape). To determine phenolic substances, the extract was diluted with water. A 1 mL portion was transferred into a 20 mL calibrated flask, and 2 mL of methanol, 5 mL of water and 1 mL of Folin–Ciocalteu reagent were added. After 3 min, 4 mL of sodium carbonate (10%) was added and the solution was left to stand for 90 min. Absorbance was then registered at 700 nm using a 10 mm cuvette. Concentration was determined using a calibration curve and expressed as mg (+)–catechin/kg of grape. From 2009 to 2014, the concentration of yeast assimilable nitrogen (YAN), including ammonium and α-amino acid concentration, was assessed by formol titration to pH 8.10, according to the Ogorodnik and Merkureua procedure reported by Gump et al. (2002). In 2012 and 2013, data collection was expanded to include analysis of berry components (berry mass, skin mass, flesh mass, seeds mass, seed number per berry and skin-to-flesh ratio) to determine the influence of summer pruning treatments on their growth.

Gas exchange measurements

Leaf gas exchange in vine leaves subjected to the St and St + Dpa treatments was evaluated to identify any treatment-induced compensation effects. During the 5-year trial, leaf gas exchange measurements were taken during the period of full canopy development from June to September. In 2011, three measurements were made during the growing season (June, July and September) to characterise photosynthesis in vines subjected to the St and St + Dpa treatments. The 2012 season included measurements examining the evolution of photosynthesis at three times during the season (May, August and September) and were taken from old, mature and young leaves developed at the basal, medial and distal positions of the shoots, respectively. Measurements were conducted in the morning (from 0930 to 1130) on clear days, monthly until harvest, using a portable, open-system LCA3 infrared gas analyser (ADC BioScientific, Hoddesdon, England). The system had a broad leaf chamber with a 6.25 cm^2 window, and all readings were taken at ambient RH with the airflow adjusted to 350 mL/min. For each treatment, three fully expanded leaves at nodes six to ten from the base were sampled under saturating light [photosynthetically active radiation (PAR) > 1400 photons mmol/(m^2·s)].

Statistical analysis

Results were tested for homogeneity of variance and subjected to ANOVA using Statistica (version 4.3, StatSoft, Tulsa, OK, USA) and Sigma Plot (version 10, SPSS, Chicago, IL, USA). Treatments in each trial year (2009–2013) were compared using Duncan's multiple range test at the $P \le 0.05$ and 0.01 levels. Values are shown as the average of 5 years (2009–2013) and the significance of the treatment was reported. Year was considered as a random variable and effects of year × treatments were tested using the pooled error mean square as an error term (Gomez and Gomez 1984). Year × treatment interaction was divided only in the case of *F*-test significance. The comparison of treatments in 2014, to quantify potential carry-over effects of earlier growing seasons, was performed by means separation calculated applying the Student–Newman–Keuls test at $P \le 0.05$ and 0.01 levels.

Results

Weather conditions

Over the 5 years of the trial, differences in average seasonal temperature were evident, with 2009, 2011 and 2012 being warmer, and 2010 and 2013 cooler. The accumulation of GDD (base 10°C) between budburst and harvest was similar from 2009 to 2012, and lower in 2013 (Table 1). In the warmest seasons, 2009, 2011 and 2012, only 82, 104 and 65 mm of precipitation fell from June to August, respectively, the period encompassing fruitset, veraison and berry ripening. In September 2012, 20 days before harvest, there was substantial rainfall. The regular distribution of rainfall in 2010 likely led to consistent and adequate water availability in the soil throughout the growing season, improving growth and yield, while in 2013 a large amount of rain fell in May (Table 1), just after budburst, enhancing early shoot growth. Weather conditions in the 2009 and 2011 seasons

Table 1. Weather variables on a monthly basis, from budburst (April) to harvest (September/October) in Montepulciano vines.

Year	April	May	June	July	August	September	October	Budburst–harvest†
GDD‡								
2009	132	341	366	487	498	370	188	2123
2010	148	262	405	495	443	304	161	2120
2011	184	287	398	452	520	425	201	2099
2012	129	247	448	545	526	345	244	2274
2013	153	229	350	481	474	333	208	1989
Precipitation (mm)								
2009	64	20	61	5	3	13	88	160
2010	60	96	102	35	50	78	82	408
2011	25	13	25	59	0	30	51	153
2012	17	44	11	16	38	205	66	339
2013	29	128	61	24	18	60	81	347
***T* > 30°C (*N*, days)**								
2009	0	5	2	11	13	0	0	31
2010	0	0	1	10	6	0	0	17
2011	0	1	1	8	13	10	0	33
2012	0	0	8	18	15	2	0	42
2013	0	0	4	8	9	0	0	21

†Data are from day of the year (DOY) 103 to 271 in 2009, from DOY 105 to 288 in 2010, from DOY 101 to 269 in 2011, from DOY 109 to 281 in 2012 and from DOY 117 to 287 in 2013. ‡GDD, growing degree days, daily temperature base 10°C.

caused water stress, and symptoms of photoinhibition, indicated by chlorosis, and of necrosis were observed in basal leaves.

Shoot growth

Shoot number per vine ranged between 21 and 24 (Table 2), exceeding the quantity of nodes left during winter pruning (14 buds arising from seven spurs of two nodes each). This was due to bursting and subsequent development of shoots from dormant buds. Prior to St, total leaf area (TLA) per vine ranged from 1.86 to 2.05 m^2, and the inflorescence number per vine and fruitfulness (ratio of inflorescence number to shoots) was highest in St + Dpa and St + Dpv + Bt-treated vines. The St treatments, applied prior to anthesis, removed an average of seven to nine shoots with TLA ranging from 0.26 to 0.36 m^2. During the 5 years, St + Dpv + Bt vines developed canes weighing 19–27% more than those from other treatments. In addition, the St treatments removed on average 0.30 m^2 of leaf area whereas in 2013 leaf area removed was just 0.17 m^2 (Table 2). As the process of St removed unfruitful shoots, fruitfulness was increased compared to the Wp Control vines (Table 2).

Leaf layer number

The reduction in leaf layers in the fruiting zone began with the St treatment, where LLN declined from 2.98 to 2.50, and from 2.25 to 2.00 at 20 and 40 cm from the cordon, respectively, compared to that of Wp vines. With the added Dpa treatment, the LLN in the fruit zone (LLNfz) dropped substantially to 0.05 and 0.85 at 20 and 40 cm from the cordon, respectively, and remained lower than that of the Control vines until full canopy development (Figure 1). The Dpv

Table 2. Effect of shoot thinning on the vegetative and fruit characteristics of Montepulciano vines.

	Pre-shoot thinning†				Post-shoot thinning			
	Shoots/ vine (No.)	TLA/ vine (m^2)	Inflorescences/ vine (No.)	Fruitfulness‡	Shoots/ vine (No.)	TLA/ vine (m^2)	Inflorescences/ vine (No.)	Fruitfulness
Treatment (T)								
Wp	24a	1.86	21b	0.89c	24a	1.86a	21a	0.89c
St	23ab	1.90	21b	0.94bc	14b	1.64bc	17b	1.22b
St + Dpa	21b	2.05	23a	1.11a	14b	1.72ab	19ab	1.37a
St + Dpv	22b	1.90	21b	0.99b	14b	1.54c	17b	1.22b
St + Dpv + Bt	22b	1.98	23a	1.07a	14b	1.68bc	19ab	1.35a
Sig.	*	NS	*	**	**	**	**	**
Year (Y)								
2009	22bc	2.45a	19c	0.87b	15b	2.16a	14c	0.95c
2010	24a	2.51a	25a	1.06a	16a	2.21a	20a	1.28a
2011	20c	2.57a	22a	1.12a	15b	2.26a	21a	1.41a
2012	22bc	1.35b	23a	1.06a	16a	1.14b	20a	1.29a
2013	23ab	0.87c	20b	0.89b	17a	0.70c	17b	1.11b
Sig.	**	*	**	**	**	*	**	**
T × Y§	**	NS	*	*	**	NS	**	**

Within columns, different letters indicate a significant difference between means (Duncan's multiple range test). *, **, significant at $P \leq 0.05$ and $P < 0.01$, respectively. †Shoots were thinned on day of the year (DOY) 149 (26 May 2009), 155 (4 June 2010), 157 (6 June 2011), 151 (30 May 2012) and 149 (29 May 2013). ‡Fruitfulness is the ratio of number of inflorescences to number of shoots. §The year effect is also shown as seasonal data averaged over all treatments (n = 40). NS, not significant; Sig., significance; St, winter pruning and shoot thinning; St + Dpa, winter pruning, shoot thinning and pre-anthesis defoliation; St + Dpv, winter pruning, shoot thinning and pre-veraison defoliation; St + Dpv + Bt, winter pruning, shoot thinning, pre-veraison defoliation, and bunch thinning; TLA, total leaf area; Wp, winter pruning.

had a lighter effect, lowering LLNfz at 20 and 40 cm from the cordon to 1.06 and 2.50, respectively, results similar to those of the St + Dpa treatment. There was little impact of treatments on the LLN at 60 cm and beyond from the cordon (Figure 1).

Defoliation and leaf area

The Dpa treatment removed an average of 0.83 m^2 leaves per vine, reducing the TLA compared to that of Wp and St vines by 52 and 47%, respectively. The TLA in Dpa vines remained lower than that of Wp and St vines until harvest. During the 5-year period, Dpa treatments removed the lowest amount of leaf area in 2012, with only 0.07 m^2; 69% less than the average of other years (Table 3). Over the five seasons, pre-veraison defoliation decreased the TLA in St + Dpv and St + Dpv + Bt vines by an average of 0.56 m^2 (−23%) and 0.69 m^2 (−10%), respectively, compared with Wp vines (Table 3).

Photosynthesis and stomatal conductance

Over the five seasons, only in 2011 did the St + Dpa leaves show a significantly higher photosynthetic capacity (P_n) and stomatal conductance (g_s) than those of the St vines (Table 4), from approximately 15 days after the treatments were applied through to their highest photosynthetic capacity in both July and September (Table S1). In August of the 2012 season, both the St and St + Dpa leaves showed low P_n and g_s values of below 4 μmol $CO_2/(m^2·s)$ and 40 mmol $H_2O/(m^2·s)$, respectively. The St + Dpa vines, however, began to show compensation effects in September through higher P_n and g_s values in distal leaves (Table S2).

Yield

With the Montepulciano vines, we found a significant year × treatment interaction for the number of bunches per vine, yield per vine, bunch mass and berry number per bunch (Table 5). Removal of seven to nine shoots with three to four bunches per vine (Table 2) lowered the bunch count in St vines, but failed to reduce the yield per vine, except in the second year (Tables 5,6). Compared to Wp vines, the lowest number of bunches in St vines was associated with the highest bunch mass (+18%), due to an increase in the number of berries (+13%) (Table 5). In all 5 years, the St + Dpa and St + Dpv + Bt treatments had the lowest yield per vine, −27 and −21%, respectively, compared to that of Wp vines. Interestingly, the St + Dpa vines had a similar bunch number to the Wp vines, but were lighter (−19%) due to fewer berries (−25%). In contrast, the reduced yield in St + Dpv + Bt vines was mainly due to fewer bunches (−33%) achieved via bunch thinning (Tables 5, 7). The pre-veraison defoliation treatment did not substantially affect yield per vine compared to that of Wp. In 2011, a summer drought characterised the ripening period, and reduced yield to the lowest of any year, averaging 2 kg/vine, due to fewer berries (Table 6). In contrast, rainfall was evenly distributed during the summer of 2010, thus favouring fruit growth and leading to the highest yield, averaging about 6 kg/vine with bigger bunches with larger berries (Table 7).

Bunch architecture and Botrytis

The St + Dpa and St + Dpv + Bt treatments affected bunch architecture through a minor shortening of the first rachis branch compared to that of the other treatments (Table 8).

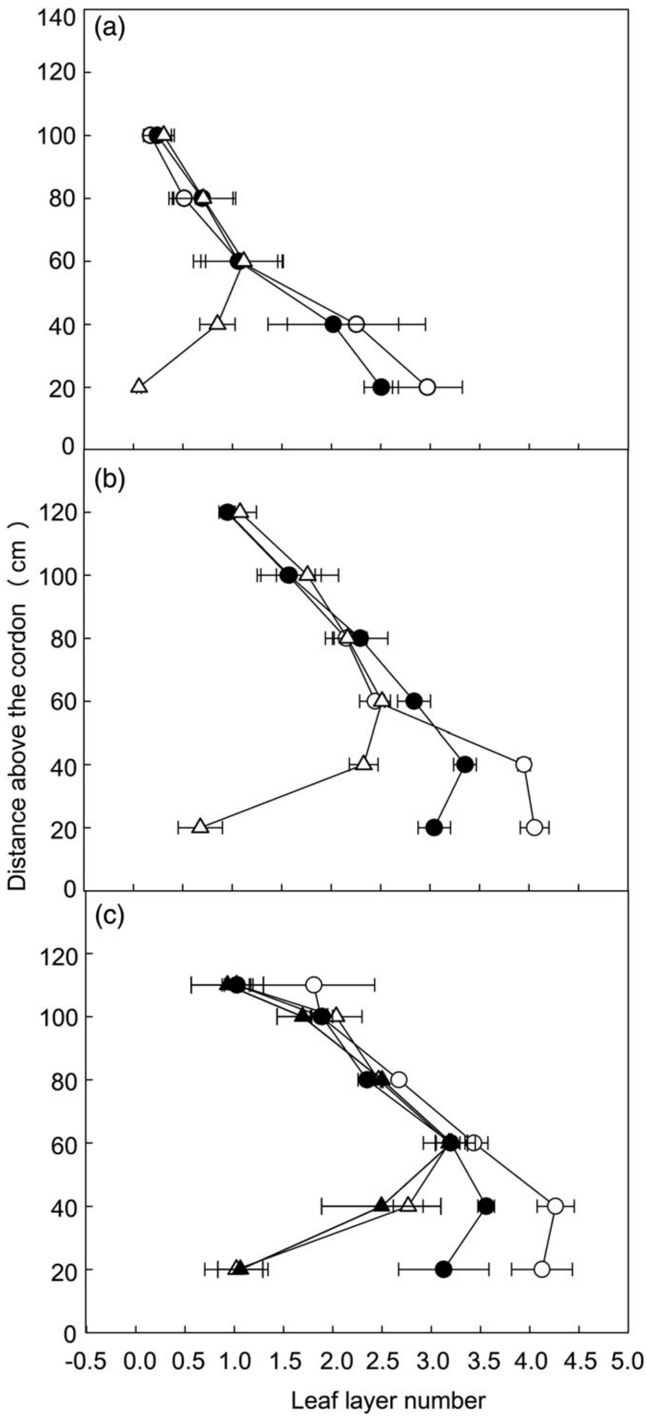

Figure 1. Seasonal evolution of leaf layer number recorded in (a) May, (b) June and (c) July from 2009 to 2013 in Montepulciano vines subjected to the canopy management treatments: winter pruning (Wp) (○); winter pruning and shoot thinning (St) (●); winter pruning, shoot thinning and pre-anthesis defoliation (St + Dpa) (Δ); and winter pruning, shoot thinning and pre-veraison defoliation (St + Dpv) (▲). Mean ± SE, n = 8 vines per treatment.

The measure of bunch compactness was expressed as mass/length, and was also classified according to the OIV system. The values were significantly lower in St + Dpa vines, enhancing air movement within the bunch, and lowering the incidence of *B. cinerea* compared to other treatments. The wetter seasons (2010 and 2013) created a favourable environment for the growth and diffusion of *B. cinerea*, and at harvest, increasing the number of bunches that were

Table 3. Effect of defoliation before and after pre-anthesis, pre-veraison and at harvest on the total leaf area in Montepulciano vines.

	Total leaf area (m^2)				
	Rapid shoot elongation phase: anthesis		Full canopy vine: veraison		
	Pre-defoliation	Post-defoliation	Pre-defoliation	Post-defoliation	Harvest
Treatment (T)					
Wp	1.86a	1.86a	4.18a	4.18a	3.93a
St	1.64bc	1.64b	3.49b	3.49a	3.20b
St + Dpa	1.72ab	0.89c	3.06c	3.06b	2.84c
St + Dpv	1.54c	1.54b	3.79ab	3.23b	2.79c
St + Dpv + Bt	1.68bc	1.68b	4.47a	3.78ab	3.50ab
Sig.	**	*	**	*	**
Year (Y)					
2009	2.16a	1.92a	2.96d	2.86c	3.96a
2010	2.21a	1.97a	4.76a	4.43a	3.45b
2011	2.26a	2.02a	3.92b	3.58b	2.55c
2012	1.14b	1.07b	3.82b	3.46b	3.46b
2013	0.70c	0.62c	3.53c	3.40b	2.85c
Sig.	*	*	*	*	*
T × Y†	**	NS	*	NS	*

Within columns, different letters indicate a significant difference between means (Duncan's multiple range test). *, **, significant at $P \leq 0.05$ and $P < 0.01$, respectively. †The year effect is also shown as seasonal data averaged over all treatments (n = 40). NS, not significant; Sig., significance; St, winter pruning and shoot thinning; St + Dpa, winter pruning, shoot thinning and pre-anthesis defoliation; St + Dpv, winter pruning, shoot thinning and pre-veraison defoliation; St + Dpv + Bt, winter pruning, shoot thinning, pre-veraison defoliation, and bunch thinning; Wp, winter pruning. In 2009, 2010, 2011, 2012 and 2013, Dpa was applied on day of the year (DOY) 149 (26 May 2009), 155 (4 June 2010), 159 (8 June 2011), 151 (30 May 2012) and 149 (29 May 2013); Dpv was applied on DOY 198 (17 July 2009), 201 (20 July 2010), 209 (27 July 2011), 208 (27 July 2012) and 207 (26 July 2013); harvest took place on DOY 271 (28 September 2009), DOY 288 (15 October 2010), DOY 269 (26 September 2011), DOY 282 (8 October 2012) and DOY 287 (14 October 2013).

Table 4. Net photosynthesis and stomatal conductance during the full canopy development (June–September) from 2009 to 2012 in Montepulciano vines subjected to winter pruning and shoot thinning and to pruning, shoot thinning and pre-anthesis defoliation.

	Net photosynthesis [μmol CO_2/(m^2·s)]				Stomatal conductance [mmol H_2O/(m^2·s)]			
Treatment (T)	2009	2010	2011	2012	2009	2010	2011	2012
St	6.3a	6.6a	10.9b[c]	9.1a	75a	98a	131b	103a
St + Dpa	6.6a	6.6a	12.5a	8.6a	83a	95a	167a	113a
Sig.	NS	NS	**	NS	NS	NS	**	NS

Within columns, different letters indicate a significant difference between means (Duncan's multiple range test). **, significant at $P < 0.01$; n = 8. NS, not significant; Sig., significance; St, winter pruning and shoot thinning; St + Dpa, winter pruning, shoot thinning and pre-anthesis defoliation.

infected with a high proportion of rot, thus affecting composition, and probably lowering yield (not measured). During the 5-year period, incidence of sunburn was judged low irrespective of treatment (Table 8).

Berry components

Generally, none of the year × treatment interactions produced significant changes in berry growth components (Table 9). Despite there being no differences in berry

Table 5. Effect of different canopy management treatments on the yield components, bunch and berry characteristics, recorded from 2009 to 2013 in Montepulciano vines.

	Yield/vine (kg)	Bunches/vine (No.)	Bunch mass (g)	Berry mass (g)	Berries/bunch (No.)
Treatment (T)					
Wp	4.8a	21a	200c	2.4b	83b
St	4.6a	17b	236b	2.5b	94a
St + Dpa	3.5b	19ab	162d	2.6ab	62c
St + Dpv	4.7a	17b	245b	2.6ab	94a
St + Dpv + Bt	3.8b	14c	278a	2.8a	99a
Sig.	*	*	*	*	*
Year (Y)					
2009	4.9b	18b	268b	2.9b	92a
2010	6.2a	21a	299a	3.2a	93a
2011	2.0d	19ab	115d	1.9c	61c
2012	3.1c	20a	159c	1.9c	84b
2013	5.2a	19ab	280b	2.8b	100a
Sig.	*	**	*	*	*
T × Y†	*	*	*	NS	*

Within columns, different letters indicate a significant difference between means (Duncan's multiple range test). *, **, significant at $P \leq 0.05$ and $P < 0.01$, respectively. †The year effect is also shown as seasonal data averaged over all treatments (n = 40). NS, not significant; Sig., significance; St, winter pruning and shoot thinning; St + Dpa, winter pruning, shoot thinning and pre-anthesis defoliation; St + Dpv, winter pruning, shoot thinning and pre-veraison defoliation; St + Dpv + Bt, winter pruning, shoot thinning, pre-veraison defoliation, and bunch thinning; Wp, winter pruning.

Table 6. Effect of different canopy management treatments on yield and bunches per vine recorded at harvest from 2009 to 2013 in Montepulciano vines.

	Yield/vine (kg)					Bunches/vine (No.)				
Treatment	2009	2010	2011	2012	2013	2009	2010	2011	2012	2013
Wp	5.2a	8.5a	2.1	3.2	5.0	21a	28a	21ab	24a	23a
St	5.6a	6.8bc	2.2	3.2	5.2	20a	20b	19b	18bc	20a
St + Dpa	3.1b	4.5c	1.8	2.6	5.3	17ab	23b	25a	21a	22a
St + Dpv	5.3a	7.2ab	2.3	3.1	5.4	18a	20bc	17b	19b	19a
St + Dpv + Bt	5.2a	3.7c	1.9	3.2	4.8	15b	13c	12c	16c	12b
Sig.	**	**	NS	NS	NS	**	**	**	**	**

Within columns, different letters indicate a significant difference between means (Student–Newman–Keuls test). **, significant at $P \le 0.01$. NS, not significant; Sig., significance; St, winter pruning and shoot thinning; St + Dpa, winter pruning, shoot thinning and pre-anthesis defoliation; St + Dpv, winter pruning, shoot thinning and pre-veraison defoliation; St + Dpv + Bt, winter pruning, shoot thinning, pre-veraison defoliation and bunch thinning; Wp, winter pruning. Harvest dates were day of the year (DOY) 271 (28 September 2009), DOY 288 (15 October 2010), DOY 269 (26 September 2011), DOY 282 (8 October 2012) and DOY 287 (14 October 2013).

Table 7. Effect of different canopy management treatments on bunch mass and berries per bunch recorded at harvest from 2009 to 2013 in Montepulciano vines.

	Bunch mass (g)					Berries/bunch (No.)				
Treatment	2009	2010	2011	2012	2013	2009	2010	2011	2012	2013
Wp	251b	303ab	101bc	128bc	215c	90b	98ab	54b	70b	84c
St	284b	346a	111bc	183a	256bc	98ab	111a	57b	99a	92bc
St + Dpa	182c	198d	73c	120c	236c	63c	60c	40c	61b	80c
St + Dpv	284b	361b	128ab	169ab	285b	97ab	108a	64ab	88a	102b
St + Dpv + Bt	341a	287c	160a	194a	407a	111a	87b	78a	90a	128a
Sig.	**	**	**	**	**	**	**	**	**	**

Within columns, different letters indicate significant differences between means (Student–Newman–Keuls test). **, significant at $P \le 0.01$; $n = 8$. Sig., significance; St, winter pruning and shoot thinning; St + Dpa, winter pruning, shoot thinning and pre anthesis defoliation; St + Dpv, winter pruning, shoot thinning and pre veraison defoliation; St + Dpv + Bt, winter pruning, shoot thinning, pre veraison defoliation and bunch thinning; Wp, winter pruning. Harvest dates in 2009, 2010, 2011, 2012 and 2013 were day of the year (DOY) 271 (28 September), DOY 288 (15 October), DOY 269 (26 September), DOY 282 (8 October), and DOY 287 (14 October), respectively.

mass, skin mass or skin-to-flesh ratio between all treatments, the St + Dpa and St + Dpv + Bt produced the greatest flesh mass compared to that of Wp and St. The number of seeds per berry remained unchanged, while the seed mass differed between treatments; St + Dpa vines also produced berries with the heaviest seeds. In 2013, plentiful early rainfall and moderate temperature throughout the season produced heavier berries with the highest flesh mass and the lowest skin-to-flesh ratio (Table 9).

Table 8. Effect of different canopy management treatments on bunch morphology and incidence of rot or sunburn recorded from 2009 to 2013 in Montepulciano vines.

			Bunch compactness		*Botrytis cinerea* (%)		Sunburn (%)	
	Bunch length (mm)	Branching length (mm)	Mass/ length (g/mm)	OIV rating†	Diff.	Inc.	Diff.	Inc.
Treatment (T)								
Wp	129ab[c]	51a	1.6b	6.5a	58.3a	11.0a	5.5b	2.2
St	135a	54a	1.7b	6.7a	54.8a	10.7a	5.2b	1.9
St + Dpa	125b	41c	1.3c	5.5b	40.8b	4.8b	3.1b	0.4
St + Dpv	133ab	49ab	1.8b	6.5a	48.4a	7.5a	10.3a	2.0
St + Dpv + Bt	137a	43bc	2.0a	6.8a	52.2a	10.4a	3.3b	0.5
Sig.	*	*	**	**	*	*	*	NS
Year (Y)								
2009	110c	54b	2.4a	8.4a	0.0d	0.0c	5.6b	1.8
2010	142a	76a	2.1b	5.1d	65.4b	4.1b	11.3a	1.3
2012	131b	36c	1.2d	5.6c	52.0c	6.7b	3.0b	1.8
2013	144a	24d	1.9c	6.6b	86.3a	24.8a	2.0b	0.7
Sig	**	**	**	**	**	**	**	NS
T × Y‡	NS	**	**	NS	NS	*	NS	NS

Within columns, different letters indicate a significant difference between means (Duncan's multiple range test). *, **, significant at $P \le 0.05$ and $P < 0.01$, respectively. †OIV, bunch compactness visually estimated using OIV code 204 (Organisation Internationale de la Vigne et du Vin 1983**). ‡The year effect is also shown as seasonal data averaged over all treatments ($n = 32$. Diff., *Botrytis cinerea* and sunburn diffusion on bunches; Inc., *B. cinerea* and sunburn incidence on bunches. NS, not significant; OIV, Organisation Internationale de la Vigne et du Vin; St, winter pruning and shoot thinning; St + Dpa, winter pruning, shoot thinning and pre-anthesis defoliation; St + Dpv, winter pruning, shoot thinning and pre-veraison defoliation; St + Dpv + Bt, winter pruning, shoot thinning, pre-veraison defoliation, pre-veraison defoliation and bunch thinning; Wp, winter pruning.

Table 9. Effect of different canopy management treatments on berry characteristics recorded in 2012 and 2013 in Montepulciano vines.

	Berry mass (g)	Skin mass (mg)	Flesh mass (g)	Seeds mass (mg)	Seeds/ berry (No.)	Skin-to-flesh ratio (%)
Treatment (T)						
Wp	2.4	291	2.06b	70b	1.9	14.1
St	2.5	276	2.08b	74ab	1.9	13.3
St + Dpa	2.6	298	2.27a	84a	2.1	13.1
St + Dpv	2.6	302	2.19ab	72b	2.0	13.8
St + Dpv + Bt	2.8	311	2.47a	79ab	2.1	12.6
Sig.	NS	NS	**	**	NS	NS
Year (Y)						
2012	1.9b	311	1.66b	74	1.9	18.7a
2013	2.8a	280	2.77a	78	2.1	10.1b
Sig.	**	NS	**	NS	NS	*
T × Y†	NS	NS	NS	NS	NS	NS

Within columns, different letters indicate a significant difference between means (Duncan's multiple range test). *, **, significant at $P \le 0.05$ and $P < 0.01$, respectively. †The year effect is also shown as seasonal data averaged over all treatments ($n = 40$). NS, not significant; St, winter pruning and shoot thinning; St + Dpa, winter pruning, shoot thinning and pre-anthesis defoliation; St + Dpv, winter pruning, shoot thinning and pre-veraison defoliation; St + Dpv + Bt, winter pruning, shoot thinning, pre-veraison defoliation and bunch thinning; Wp, winter pruning.

Grape composition

The St treatment did not affect grape composition, as TSS, pH, TA, YAN, as well as the concentration of both phenolic substances and anthocyanin in St vines was similar to that of Wp vines. The TSS in grapes of the St + Dpa and St + Dpv + Bt treatments was 24.4°Brix. These treatments also recorded the highest concentration of anthocyanin and phenolic substances. Despite no significant difference among treatments for TA, the St + Dpv + Bt vines showed a higher concentration of malic acid and tartaric acid compared to that of St + Dpa vines. A significant year × treatment interaction was identified for TSS, pH and YAN. At harvest, TSS was highest in 2011, the warmest season (Tables 10,11), when fruit also recorded the highest tartaric acid concentration and greatest degradation of malic acid. Between treatments, a significant difference in pH and YAN concentration was found in 2010 and 2011 (Table 11). None of the canopy management treatments had a significant influence on the leaf area-to-yield ratio (Table 12), whereas pre-veraison defoliation and bunch thinning treatments reduced yield, which decreased the RI (Table 12).

Potential carry-over effects

In 2014, all vines were winter pruned, leaving seven spurs of two nodes each per vine; no other treatment was applied. After budburst, the vines showed an average of 23–28 shoots per vine, and the fruitfulness ranging between 0.81 and 1.13 for treatments St + Dpa and St + Dpv + Bt, respectively (Table 13). The Dpa treatment, repeated for 5 years, lowered the fruitfulness per vine by 27% compared to the initial value recorded in 2009. Carry-over effects were found in yield per vine, bunch mass and number of berries per bunch only in St + Dpa-treated vines. It is noteworthy that, in 2014, vines previously subjected to Dpa continued to produce the lowest yield (−29% vs Wp vines and −35% vs St vines) with the smallest bunches (−21% vs Wp vines and −28% vs St vines) consisting of the fewest number of berries (−21% vs Wp vines and −27% vs St vines), when compared to other treatments (Table 13). In 2014, the reduction in yield components is related to the reduction of several bunch components (Table 14). In particular, all the defoliation treatments reduced significantly the bunch and branching length when compared to the winter pruning and St treatments (Table 14). Moreover, no carry-over

Table 10. Effect of different canopy management treatments on grape composition at harvest recorded from 2009 to 2013 in Montepulciano vines.

	TSS (°Brix)	pH	TA (g/L)	Tartaric acid (g/L)	Malic acid (g/L)	Anthocyanin (mg/kg)	Phenolic substances (mg/kg)	YAN† (mg/L)
Treatment (T)								
Wp	22.9c[c]	3.39b	6.0	7.90a	0.86b	1281b	2504b	92a
St	23.0c	3.40b	5.9	7.43ab	0.93b	1283b	2470b	91a
St + Dpa	24.4a	3.40b	6.0	7.92a	0.95b	1431a	2785a	81a
St + Dpv	23.7b	3.42ab	5.8	7.37ab	0.91b	1266b	2440b	86a
St + Dpv + Ct	24.4a	3.46a	5.9	7.08b	1.19a	1238b	2372b	93a
Sig.	**	**	NS	*	**	*	*	NS
Year (Y)								
2009	22.8d	3.33c	5.7b	7.83b	0.84c	1211b	1732d	74c
2010	23.6c	3.46b	5.8b	7.31b	1.32a	1006c	2040c	103b
2011	25.5a	3.48b	5.9b	9.29a	0.75c	1560a	3763a	131a
2012	24.9b	3.54a	5.0c	5.76c	0.92bc	1645a	3452b	75c
2013	21.7e	3.24d	7.1a	7.51b	1.02b	1077c	1584d	61d
Sig.	**	*	*	**	**	**	**	**
T × Y†	**	**	NS	NS	NS	NS	NS	**

Within columns, different letters indicate a significant difference between means (Duncan's multiple range test). *, **, significant at $P \le 0.05$ and $P < 0.01$, respectively. †The year effect is also shown as seasonal data averaged over all treatments ($n = 40$). NS, not significant; Sig., significance; St, winter pruning and shoot thinning; St + Dpa, winter pruning, shoot thinning and pre-anthesis defoliation; St + Dpv, winter pruning, shoot thinning and pre-veraison defoliation; St + Dpv + Bt, winter pruning, shoot thinning, pre-veraison defoliation, and bunch thinning; Wp, winter pruning; YAN, yeast assimilable nitrogen.

Table 11. Effect of different canopy management treatments on the TSS, pH and yeast assimilable nitrogen, recorded at harvest in Montepulciano vines.

Treatment	TSS (°Brix)					pH					YAN (mg/L)				
	2009	2010	2011	2012	2013	2009	2010	2011	2012	2013	2009	2010	2011	2012	2013
Wp	22.1b	21.8c	24.6c	24.5	21.2b	3.29	3.36b	3.48ab	3.58	3.22	74	80b	158a	86	61
St	22.0b	23.2b	24.3c	24.6	21.0b	3.29	3.44b	3.48ab	3.53	3.24	61	97b	155ab	77	63
St + Dpa	24.2a	24.3b	26.1a	24.9	22.6a	3.38	3.46b	3.41b	3.50	3.25	83	109ab	94d	67	51
St + Dpv	22.8b	23.2b	25.5bc	25.2	21.5ab	3.35	3.43b	3.48ab	3.56	3.27	68	95b	127bc	70	70
St + Dpv + Bt	22.6b	25.5a	27.0a	25.2	22.0ab	3.36	3.62a	3.54a	3.55	3.24	82	133a	120cd	74	58
Sig.	**	**	**	NS	**	NS	**	**	NS	NS	NS	**	**	NS	NS

Within columns, different letters indicate a significant difference between means (Student–Newman–Keuls test). **, significant at $P \leq 0.01$. NS, not significant; Sig., significance; St, winter pruning and shoot thinning; St + Dpa, winter pruning, shoot thinning and pre-anthesis defoliation; St + Dpv, winter pruning, shoot thinning and pre-veraison defoliation; St + Dpv + Bt, winter pruning, shoot thinning, pre-veraison defoliation and bunch thinning; Wp, winter pruning; YAN, yeast assimilable nitrogen. Harvest dates were day of the year (DOY) 271 (28 September 2009), DOY 288 (15 October 2010), 269 (26 September 2011), DOY 282 (8 October 2012) and DOY 287 (14 October 2013).

effects on must components were observed, with all treatments recording similar values for TSS, pH, TA, YAN and concentration of phenolic substances and anthocyanin (Table 15).

Discussion

In 2011, low rainfall during August and high temperature in September led to stomatal closure and reduced P_n, which indicates moderate water stress (Cifre et al. 2005). This led to lower vegetative growth and the development of smaller bunches with a higher tartaric acid concentration (Kliewer 1977, Crippen and Morrison 1986, DeBolt et al. 2008). In addition, the increased exposure to sunlight probably induced an increase in malic acid degradation (DeBolt et al. 2008). Fruit in the 2011 season also had the highest TSS probably due to it being the warmest year (Lanari et al. 2014). This year was also characterised by low rainfall in July and the absence of rain in August. In 2012, low rainfall from mid-May through July, and high temperature in the last 10 days of June and most of July also led to severe water stress, as suggested by low g_s [below 40 mmol H_2O/(m^2·s)] recorded at the beginning of August. Under these field conditions (high air temperature, high light radiation and scarce water availability in the soil), Montepulciano confirmed its isohydric behaviour by closing stomata to limit water loss, lowering the g_s and P_n, as previously observed by Silvestroni et al. (2005), and more recently by Palliotti et al. (2015). When comparing the five growing seasons, bunch mass and berry mass were higher in 2010. The final berry mass across all five seasons was associated with precipitation during the month of June, when cell division occurs. Although the five seasons differed in terms of weather, treatment effects on leaf-area-to fruit ratios did not differ.

Shoot thinning

Shoot thinning pre-anthesis led to a reduction in shoot number, from 33 to 39%, and significantly influenced vine vigour during the growing season. No compensation was seen in the length of remaining shoots or the number and/or length of laterals – as previously observed by Bravetti et al. (2012) and Silvestroni et al. (2016) on Sangiovese. Instead, Myers et al. (2008) indicated that the compensation effect following St in Sangiovese vines was reflected in a greater leaf area, an effect seen in this study through a 62% greater leaf area increase in St by the time hedging was done. For St vines, there was a significant increase in fruitfulness due to the removal of unfruitful shoots (Table 2). Despite their being fewer bunches per vine, each bunch contained more berries, which led to the higher bunch mass. As a result, St failed to reduce yield, which is in agreement with previous studies (Bravetti et al. 2012, Silvestroni et al. 2016). Also St had no effect on the RI, a finding that contrasts with that of Naor et al. (2002) and Reynolds et al. (1994), who found that St tended to decrease RI. This may be explained by the studies of Freeman et al. (1979) and Myers et al. (2008) who found that pruning mass was not always affected by shoot density due to the ability of the vine to redirect energy into fewer shoots, thus increasing the individual shoot mass. In our study, St led to a more open canopy, observed primarily in the fruit zone (LLNfz), between 20 and 40 cm above the cordon, where the canopy density was lower in St treatments than in Wp vines. Despite this, no increase in anthocyanin or phenolic substances was observed in contrast to some other studies

Table 12. Effect of different canopy management treatments on the vegetative and pruning characteristics recorded from 2009 to 2013 in Montepulciano vines.

	Leaf area/yield (m^2/kg)	Canes/vine (No.)	Cane mass/vine (g)	Ravaz index (kg/kg)
Treatment (T)				
Wp	0.87b	17ab	46.8b	6.15a
St	0.76c	13b	56.1b	6.13a
St + Dpa	0.87b	13b	54.3b	5.00b
St + Dpv	0.69c	13b	56.0b	6.27a
St + Dpv + Bt	0.99a	14b	68.5a	3.96c
Sig.	**	**	**	**
Year (Y)				
2009	0.58c	14b	54.6b	6.36b
2010	0.71c	14b	59.9a	7.75a
2011	1.79a	15a	44.0c	3.13c
2012	1.17b	14b	58.4b	3.73c
2013	0.65c	14b	64.8a	5.77b
Sig.	**	**	**	**
T × Y†	**	**	**	**

Within columns, different letters indicate a significant difference between means (Duncan's multiple range test). ** significant at $P < 0.01$**. †The year effect is also shown as seasonal data averaged over all treatments ($n = 40$). St, winter pruning and shoot thinning; St + Dpa, winter pruning, shoot thinning and pre-anthesis defoliation; St + Dpv, winter pruning, shoot thinning and pre-veraison defoliation; St + Dpv + Bt, winter pruning, shoot thinning, pre-veraison defoliation and bunch thinning; Wp, winter pruning. Harvest dates were day of the year (DOY) 271 (28 September 2009), DOY 288 (15 October 2010), DOY 269 (26 September 2011), DOY 282 (8 October 2012) and DOY 287 (14 October 2013).

Table 13. Vegetative and yield components recorded in 2014 in Montepulciano vines subjected only to winter pruning.

Treatment								
Season 2014	Seasons 2009–2013	Shoots/ vine	Fruitfulness	Bunches/ vine (*N*)	Yield/ vine (kg)	Bunch mass (g)	Berry mass (g)	Berries/ bunch (No.)
Wp	Wp	28a	0.82b	23a	5.1a	222a	3.6	62a
Wp	St	24b	0.97ab	23a	5.5a	246a	3.7	67a
Wp	St + Dpa	25ab	0.81b	20b	3.6b	176b	3.6	49b
Wp	St + Dpv	23b	1.01ab	22ab	5.1a	227a	3.7	61a
Wp	St + Dpv + Bt	23b	1.13a	26a	6.3a	247a	3.7	67a
Sig.		**	**	**	**	**	NS	**

Within columns, different letters indicate a significant difference between means (Student–Newman–Keuls test). **, significant at $P \le 0.01$; $n = 8$. NS, not significant; Sig., significance; St, winter pruning and shoot thinning; St + Dpa, winter pruning, shoot thinning and pre-anthesis defoliation; St + Dpv, winter pruning, shoot thinning and pre-veraison defoliation; St + Dpv + Ct, winter pruning, shoot thinning, pre-veraison defoliation and bunch thinning; Wp, winter pruning.

(Spayd et al. 2002, Bernizzoni et al. 2011). In this study, TSS was not changed as a result of thinning, indicating that the response may be cultivar dependent, as the proportion of shoots reportedly thinned by Bernizzoni et al. (2011) was similar that described here.

Shoot thinning and pre-anthesis defoliation

After St, the added defoliation of six basal leaves at the pre-anthesis phenological stage led to a 50% reduction in the TLA, which remained significantly less that of St vines until harvest. This finding is similar to previous studies with

Table 14. Bunch morphology and incidence of rot recorded in 2014 in Montepulciano vines subjected only to winter pruning.

Treatment				Bunch compactness		*Botrytis cinerea* (%)	
Season 2014	Seasons 2009–2013	Bunch length (mm)	Branching length (mm)	Mass/ length (g/mm)	OIV rating†	Diff.	Inc.
Wp	Wp	143ac	80a	1.55a	6.1a	86.0	25.5
Wp	St	147a	74a	1.67a	5.9ab	88.8	20.5
Wp	St + Dpa	123b	57b	1.43b	5.2b	93.8	23.8
Wp	St + Dpv	139a	70a	1.63a	5.8ab	88.8	15.2
Wp	St + Dpv + Bt	143a	75a	1.72a	6.3a	91.3	24.2
Sig.		**	**	**	**	NS	NS

Within columns, different letters indicate a significant difference between means (Student Newman Keuls test). **, significant at $P \le 0.01$; $n = 8$. †OIV, bunch compactness visually estimated using OIV code 204 (Organisation Internationale de la Vigne et du Vin 1983). Diff., *Botrytis cinerea* and sunburn diffusion on bunches; Inc., *B. cinerea* and sunburn incidence on bunches; St, winter pruning and shoot thinning; NS, not significant; OIV, Organisation Internationale de la Vigne et du Vin; Sig., significance; St + Dpa, winter pruning, shoot thinning and pre-anthesis defoliation; St + Dpv, winter pruning, shoot thinning and pre-veraison defoliation; St + Dpv + Bt, winter pruning, shoot thinning, pre-veraison defoliation and bunch thinning; Wp, winter pruning.

Table 15. Grape composition at harvest recorded in 2014 in Montepulciano vines subjected to winter pruning only.

Treatment							
Season 2014	Seasons 2009–2013	TSS (°Brix)	pH	TA (g/L)	Anthocyanin (mg/kg)	Phenolic substances (mg/kg)	YAN (mg/L)
Wp	Wp	21.6	3.30	6.8	1032	2703	105
Wp	St	21.1	3.29	6.6	977	2683	93
Wp	St + Dpa	21.5	3.33	6.9	961	2566	115
Wp	St + Dpv	21.9	3.32	6.7	1067	2673	94
Wp	St + Dpv + Bt	21.0	3.28	7.0	819	2541	120
Sig.		NS	NS	NS	NS	NS	NS

Within columns, different letters indicate a significant difference between means (Student–Newman–Keuls test). NS, not significant, n = 8; St, winter pruning and shoot thinning; St + Dpa, winter pruning, shoot thinning and pre-anthesis defoliation; St + Dpv, winter pruning, shoot thinning and pre-veraison defoliation; St + Dpv + Bt, winter pruning, shoot thinning, pre-veraison defoliation and bunch thinning; Wp, winter pruning; YAN, yeast assimilable nitrogen.

Sangiovese, where pre-anthesis defoliation prevented the development of lateral leaves (Intrieri et al. 2008). This suggests that the thinned shoots, which contained fewer or no bunches, have the capacity to grow a greater lateral leaf area than those with bunches. In contrast to observations made in St + Dpa-treated Sangiovese vines reported by Silvestroni et al. (2016), the canopy of St + Dpa Montepulciano vines responded with a higher photosynthetic capacity to compensate for the loss of leaves in favourable conditions where g_s values were above 115 mmol $H_2O/(m^2 \cdot s)$. In 2011, however, where water stress conditions were present in September, P_n and g_s values were not different between St and St + Dpa (Table S1). Despite having a slightly higher number of bunches per vine compared to St, the St + Dpa treatment affected yield by reducing bunch mass. This was due to a reduced berry number rather than reduced berry mass, similar to a previous study on Montepulciano vines (Bravetti et al. 2012). The carbohydrate supply at anthesis is a principal factor influencing fruitset (Caspari and Lang 1996), and the decrease in assimilation during this phenological stage can lead to an increase in the number of aborted flowers (Poni et al. 2006, Intrieri et al. 2008). The fact that there was no change to berry mass suggests that Dpa did not affect assimilate availability to developing berries following defoliation in Montepulciano (Intrieri et al. 2008). In contrast, some previous investigations reported that berry growth was reduced following defoliation (Tardaguila et al. 2010, Silvestroni et al. 2016) in Graciano, Carignan and Sangiovese, indicating that this response is cultivar dependent. There is likely to be an environmental effect as well, highlighted by the observation that final berry mass across all five seasons can be correlated with precipitation during the month of June, when cell division is occurring. Although yield was reduced in every season, the final yield per vine varied with year due to environmental influence, and was significantly reduced only from St in one season. It is likely that additional practices such as bunch thinning might be required to lower yield in seasons when Dpa is inadequate. Among all treatments, bunch compactness was significantly lowered only by Dpa. This was due to reduced fruitset, and led to a significant decrease in *B. cinerea* diffusion and incidence, as previously reported (Acimovic et al. 2016). The additional shortening of both the bunch length and branch length may have been due to a reduced supply of assimilates (Gatti et al. 2015). No change in skin mass or the skin–flesh ratio was observed as a result of Dpa in 2012 or 2013. This could be related to the lack of difference in berry mass in Dpa, as skins were reported to be thicker in berries that had a higher mass as a result of pre-anthesis defoliation (Poni et al. 2006), whereas Silvestroni et al. (2016) observed thicker skins in berries that were significantly smaller than those of St. Despite this, metabolites important to grape composition (phenolic substances, anthocyanin) were present at significantly higher concentration, likely due to an improved microclimate, as increased radiation and temperature have been identified as enhancing the accumulation of phenolic substances and anthocyanin (Poni et al. 2006, Bravetti et al. 2012, Pastore et al. 2013).

Shoot thinning and pre-veraison defoliation

The St + Dpv treatment led to a 15% reduction in TLA with the lowest value among any of the treatments at harvest. Compared to Wp vines, the Dpv treatment also lowered the LLNfz, but did not reduce yield per vine or improve berry skin composition. In agreement with Palliotti et al. (2011), Bravetti et al. (2012) and Silvestroni et al. (2016), the significantly higher concentration of sugars in the berries compared to that of St shows a the classical source–sink relationship, probably due to a photosynthetic compensation in remaining leaves, as reported by Silvestroni et al. (2016) on Sangiovese. A negative consequence of this treatment was the significantly higher incidence of sunburn. Pastore et al. (2013) related the incidence of sunburn damage in Sangiovese fruit to skin thickness. Vines defoliated pre-flowering had berries that were more resistant to sunburn probably due to their thicker skins, while vines defoliated at veraison reported skin thickness no different to that of the non-defoliated Control and severe sunburn damage.

Shoot thinning, pre-veraison defoliation and bunch thinning

As for St + Dpv, the leaf area removed had a minor effect on the sink–source balance; however, the addition of Bt led to a significant increase in cane mass. Only St + Dpv + Bt vines exhibited increased berry mass compared to that of the Control vines. The additional increase in mass due to Bt may have been due to an increase in the sink–source balance, consistent with results reported by Bravetti et al. (2012) for Montepulciano, but in contrast to results reported by Silvestroni et al. (2016) with Sangiovese. As a result, bunches were significantly more compact, but no impact on *B. cinerea* was observed. As expected, bunch thinning reduced yield via bunch number per vine, and resulted in the lowest RI values at harvest. This treatment, however, differed from St + Dpv in one season, perhaps due to compensation in berry mass and bunch mass in most years.

Consequently, TSS was increased significantly compared to that of St + Dpv, while berry composition was not altered. The leaf area-to-fruit ratio did not differ among treatments. Authors have reported either higher leaf area-to-fruit ratios in early defoliated treatments (Poni et al. 2006), but few significant differences among treatments (Intrieri et al. 2008, Tardaguila et al. 2010, Silvestroni et al. 2016) probably due to the concomitant impact of early defoliation on reducing fruitset and increasing lateral growth, therefore impacting the leaf area-to-fruit ratios.

Carry-over effects in year one post-trial

In 2014, treatments were not applied so that carry-over effects could be evaluated. The number of shoots per vine was found to be lower in vines from St, St + Dpv and St + Dpv + Bv treatments applied in previous years, resulting in a significantly increased fruitfulness in St + Dpv + Bv. The treatment that led to the most severe carry-over effects was St + Dpa. In 2014, vines developed significantly smaller bunches due to fewer berries. In addition, vines had fewer bunches per vine, resulting in a significantly lower yield. Another residual effect was observed with the shorter primary branching length and thus, bunch compactness mirrored results over the previous 5 years. In 2014, grape must composition including both basic chemistry parameters (TSS, pH, TA) and secondary metabolites (phenolic substances, anthocyanin) were not affected by previous treatments suggesting that environmental conditions are a greater influence on the accumulation of phenolic substances and anthocyanin (Crippen and Morrison 1986, Spayd et al. 2002) than either alteration of the source–sink balance or a reduction in fruitset.

Conclusions

In Montepulciano grapevines, the St treatment improved canopy density, but did not reduce yield or improve fruit composition. Additional practices, St + Dpv and St + Dpv + Bt, both provided a more open fruit zone but did not affect yield or fruit composition, other than TSS at harvest. Similarly St + Dpv + Bt did not improve fruit composition, other than increasing TSS at harvest, which offset a significant reduction in yield observed in most seasons. The St + Dpa treatment was effective at further reducing canopy density, especially in the fruit zone. During the years of the trial, this treatment improved vine health (lower incidence and diffusion of *B. cinerea*) and enhanced fruit composition, with a higher concentration of phenolic substances and anthocyanin. The treatment St + Dpa also substantially decreased yield, in both the current season and the following season. The carry-over effect suggests that this treatment should only be applied in alternate years.

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Supporting information

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Table S1. Net photosynthesis and stomatal conductance measured in June, July and September of the 2011 season in Montepulciano vines subjected to winter pruning and shoot thinning (St) and winter pruning, shoot thinning and pre-anthesis defoliation (St + Dpa) (n = 8).

Table S2. Net photosynthesis and stomatal conductance in leaves developed in the basal, medial and distal shoot portion measured in May, August and September of the 2012 season in Montepulciano vines subjected to winter pruning and shoot thinning (St) and winter pruning, shoot thinning and pre-anthesis defoliation (St + Dpa) (n = 8).