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Effects of antitranspirant Di-1-*p*-Menthene sprayed in post-veraison on berry ripening of ‘Sangiovese’ grapevines with different crop load

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Short title: Antitranspirant and crop load effects on grapes

Abstract

Background and Aims. The effects of global warming on grape ripening has led to the identification of strategies for controlling sugar accumulation. The effectiveness of the application of the film-forming antitranspirant Vapor Gard® (VG, di-1-p-menthene) was assessed on the berry ripening of vines with different crop loads.

Methods and Results. Over the 2 years, the Sangiovese vines with high (H) or medium (M) crop load (leaving about 1 bunch every 2 shoots) were sprayed (T) or not (C) with VG antitranspirant film-forming spray in post-veraison. The VG-treatment, inhibited leaf photosynthesis during bunch ripening for about 30 days, limiting berry sugar accumulation. The relationship between yield per vine and total soluble solids (TSS) was confirmed, with VG-treatment producing greater effects: increments of 1 kg of yield corresponded to a reduction of about 0.67 °Brix in the control vines and of 0.79 °Brix in the treated vines.

Conclusions. The temporary reduction of photosynthesis reduced berry TSS with a potential effect on wine alcohol content. In high crop load conditions, the VG-treatment delayed the grape's technological maturity more than the effect exerted by the crop load.

Significance of the Study. The VG antitranspirant sprayed during post-veraison hindered berry sugar to a greater extent than high crop load.

Key words: film-forming, must composition, sugar accumulation, photosynthesis, vine yield.

Introduction

Climate change is exerting a great influence on grape and wine composition. The increase in air temperature has impacted wine growing areas almost worldwide, leading to shorter growth seasons and advanced phenological phases with repercussions on the berry ripening and harvest dates (Palliotti et al. 2014).

In order to evaluate the ripening needs of the vine in relation to a defined climate, climatic indexes are widely used. In a recent study on cv. Montepulciano, bioclimatic indexes were used to estimate the bud break and the harvest date over the period 1974–2013. The results suggested a significant reduction in the growth cycle of the cv. Montepulciano due to earlier harvest date, considering that there were no changes in the estimated dates of bud break. Furthermore, the correlations indicated that earlier harvest dates are associated with the increase of heat accumulation during the first period of vegetative growth corresponding to the months from March to June in the Northern hemisphere (Di Lena et al. 2019). The grape's sugar concentration at harvest, instead, is influenced by the hot temperatures that occurred from July to September in the Northern hemisphere (Lanari et al. 2014). The excessive sugar content at harvest, one of the most important outcomes of global warming, is associated with the rapid degradation of organic acids and the lessening of aromatic components, which in the resulting wine leads to a high alcohol content, low acidity and aromatic and phenolic imbalances (Palliotti et al. 2014). Consequently, vineyard yield and berry ripening management are very important, considering also that the increase in sugar in the berries depends on other environmental factors such as the increase of CO_2 concentration in the atmosphere (Schultz 2000).

In recent years, research has been undertaken to identify and develop techniques able to reduce the sugar accumulation rate and increase titratable acidity and phenolic substances, by controlling the efficiency of the canopy (Lanari et al. 2013, Gatti et al. 2015, Zenoni et al. 2017) or through the delay of the phenological phases (Friend and Trought 2007, Gatti et al. 2016, Palliotti et al. 2017, Silvestroni et al. 2018).

In high-yielding cultivars, such as Sangiovese grapevines, the high crop load could be used as a strategy to slow the sugar accumulation and raise the acidity and anthocyanin concentration. However, reducing the yield, through bunch thinning operations typically performed manually, is often necessary in vineyards that are managed for premium quality wine. These techniques, widely used in combination with winter pruning to control yield and decrease crop load (Silvestroni et al. 2019), generally allow the improvement of grape composition and sugar concentration and increase the ratio between leaf area and yield.

The time in which the bunch thinning is carried out is very important. If the intervention is performed during veraison, it is possible to obtain increases in the must sugar concentration and in phenolic substances such as tannins and anthocyanins. The increase in the phenolic concentration of the skin, after bunch thinning, is also strongly influenced by seasonal trends (Silvestroni et al. 2016).

Some studies on different crop species (Iriti et al. 2009; Del Amor et al. 2010; Francini et al. 2011) showed that the use of antitranspirants limited photosynthetic activity and could be another strategy to slow sugar accumulation in vines (Palliotti et al. 2010 and 2013).

Interesting results were obtained on the temporary reduction of leaf transpiration and photosynthetic capacity in grapevines, after foliar spraying, with the application of the antitranspirant Vapor Gard® (VG). The VG antitranspirant, once sprayed onto leaves, forms a semipermeable film that constitutes a physical barrier that limits water vapour loss, improving water use efficiency (WUE), but also limiting the entry of CO_2, with the direct consequence of a lowering in the net photosynthetic rate (Palliotti et al. 2010). The VG antitranspirant, also known as pinolene, is generated by pine resin and is characterised by the active ingredient di-1-*p*-menthene.

In a previous study on Sangiovese grapevines grown in a field, carried out by Palliotti et al. (2013), the reduction of moisture loss and the inhibitory effect on photosynthetic capacity and stomatal conductance was shown, due to the film formed by the VG application on the leaves. The significant reduction in net photosynthesis and transpiration rate began in early August, when the canopies were sprayed, resulting in low concentrations of soluble solids at harvest, due to a slowdown in the sugar accumulation during berry ripening, and an increase in the anthocyanin concentration, but without changes on the titratable acidity. The VG antitranspirant, due to its characteristics, is used in organic farms and is added to plant protection products. A previous study reported that the formation of the film from natural products can also provide a protective role in the penetration sites of some pathogens, in both crop species and vines (Garde-Cerdàn et al. 2017).

This study was based on the use of the VG antitranspirant, applied just after the beginning of veraison, on the canopies of Sangiovese vines with different crop loads, to assess the combined effect of the crop load and the antitranspirant on both sugar accumulation in the berries and must composition at harvest.

Material and Methods

Plant material, experimental conditions and experimental design

The trial was carried out over two consecutive seasons, namely 2010 and 2011, in a 7-year-old hillside vineyard (~5% slope) situated near the city of Ancona in the Marche region of east-central Italy (latitude: 43°32’N; longitude: 13°22’E; elevation 203 m above sea level).

The vines were planted in 2004 with certified virus-free cuttings of cv. Sangiovese (clone R24) grafted onto Kober 5BB rootstock, oriented north-north east to south-south west and planted at 1.20 m vine spacing and 2.75 m row spacing resulting in a density of 3030 vines/ha. Grapevines were cordon trained, vertically shoot positioned and hand pruned in winter, leaving seven spurs of two nodes per vine.

Cordons were set at 0.8 m aboveground with two pairs of catch wires providing trellising extending 0.9 m above the cordons. Pest and disease management programs were carried out according to local practices determined by field scouting, experience and weather conditions. During the two years of trial, usually at mid-June, shoots were mechanically trimmed when their growth exceeded the top wires.

The study was conducted on vines sprayed (T) with the antitranspirant VG (a.i. di-1-*p*-menthene ($C_{20}H_{34}$)) at 2% concentration, Intrachem Bio Italia, Grassobbio, BG, Italy) and compared with unsprayed control vines (C). In addition, vines were characterised based on the % of bunches removed (60%) during bunch thinning. At the beginning of veraison, 3 August 2010 and 18 July 2011 (with approximately 40-70% of berries coloured), bunch thinning was performed to obtain medium crop loads (M) on vines, leaving about 1 bunch every 2 shoots, and high crop loads (H) on vines not subjected to bunch removal. The experimental design consisted of two blocks with 12 Sangiovese vines each chosen from within one row of 50 vines. Each block was divided into 4 plots of three vines each, which were assigned the same treatment, for a total of six replicates per treatment. Specifically, each block consisted of: three vines treated with antitranspirant and characterised by high crop load (TH), three vines treated with antitranspirant and characterised by medium crop load (TM), three control vines with high crop load (CH) and three control vines with medium crop load (CM).

In our study, the VG antitranspirant was carefully sprayed on the leaves above the bunch zone with a portable pump, not on the bunches, as it has recently been shown that applying VG to bunches can have an important effect on water loss and consequently on sugar concentration (Fahey and Rogiers, 2019). In 2010, after a first treatment with VG, carried out on 9 August, there was a heavy rainfall and it was necessary to repeat the treatment on 27 August. In 2011 the vines were treated only on 18 August.

During the 2-year period, the mean and maximum daily temperature and rainfall data were recorded at the Agenzia Servizi al Settore Agroalimentare delle Marche site, which has a meteorological station 1 km from the vineyard. Growing degree-days (GDD, base 10 °C) accumulated from 1 April to harvest were calculated.

Vine growth and canopy measurements

In each year of the trial, the annual vine growth was assessed by counting and weighing the canes on all vines, and the Ravaz index, commonly used to evaluate the balance between vine growth and yield, was calculated as the ratio of yield to pruning mass.

In each year, after recording the bunch number and weight per vine, the total leaf area (TLA) and canopy density (expressed as leaf layer number, LLN) were determined via Point Quadrat Analysis (Smart and Robinson 1991). On 8 and 13 October, respectively in 2010 and 2011, the TLA and LLN were estimated using 100–120 insertions, according to the full height of the canopy, at 10 cm intervals with a thin metal rod following a sampling grid. The metal rod simulates the ray of sunlight, and each contact with a canopy component represents the sunlight interceptions.

Gas exchange measurements

During the two-year trial, the single leaf gas exchange readings of both T and C vines were taken twice (1 and 13 September in 2010 and 24 August and 5 September in 2011) after spraying with the VG antitranspirant (27 August in 2010 and 18 August in 2011), to evaluate the effects on the photosynthetic capacity of the leaves.

Six fully expanded leaves in both T and C vines, chosen among those inserted at nodes 6–10 on a main shoot, were measured under saturating light [photosynthetically active radiation (PAR) > 1200 μmol photons/ (m^2 s)]. Measurements were carried out in the morning (0930–1130) on clear days using a portable, open-system LCA3 infrared gas analyser (ADC BioScientific, Hoddesdon, England). The system featured a broad leaf chamber with a 6.25 cm^2 window and all measurements of net photosynthesis (Pn), stomatal conductance (gs) and transpiration rate (E), were made at ambient relative humidity with airflow adjusted to 350 mL/min. The Pn, E and gs were calculated from inlet and outlet CO_2 and H_2O relative concentrations. The extrinsic water-use efficiency (WUEe) was then derived as the Pn to E ratio, while the intrinsic water-use efficiency (WUEi) was calculated by the Pn to gs ratio.

Vine yield and grape composition

For each year (2010 and 2011) of the study, after the VG treatment, the total soluble solids [TSS (°Brix)] were periodically assessed on 100 berries, until the harvest. Harvest dates were on 28 September 2010 (DOY 271) and 14 September 2011 (DOY 257), when the TSS began to level off, as measured in grapes sampled from representative positions in bunches. Berries were sampled per treatment and per plot three times during September.

Grapes were individually picked and the total number of bunches per vine was counted and weighed. Mean individual bunch weight was calculated as the ratio of total bunch weight per vine (yield) and the total number of bunches per vine.

At harvest, 100 berries per vine were collected and weighed to determine the fresh berry weight. The berries were crushed, and the juice was used to determine levels of TSS, pH, titratable acidity (TA), tartaric and malic acid. The TSS were measured using a temperature-compensating Maselli LR-01 digital refractometer (Maselli Misure, Parma, Italy). Must pH was analysed with a Crison two decimal pH meter (Crison Instruments, Barcelona, Spain) by a glass electrode, TA with a Crison Titrator (Crison Instruments) using 0.25 N NaOH to a pH 7.00 endpoint, expressed as g/L of tartaric acid equivalent. The tartaric acid concentration was measured by the ‘colorimetric method’ (Rebelein, 1973), based on the reaction between tartaric and vanadium acid which produces an orange colour, measured by spectrophotometry at 500 nm. Malic acid concentration was measured with an enzymatic kit (Enzyplus-Raisio, Raisio, Finland).

The concentration of anthocyanin and phenolic substances was determined according to Mattivi (2004) using the same berry sample analysed for the must features. The berries were further pressed to obtain dried samples (skins and seeds only), which were placed in a jar containing an extractive buffer solution of hydrochloric acid (15 mL in 1 L of water), homogenised with an Ultra-Turrax T25 (Janke & Kunkel, IKA-Werke, Staufen, Germany) for 1 minute at 10 000 rpm. A subsample of homogenate was, subsequently, transferred to a centrifuge tube and centrifuged (model ALC 4218, International, Cologno Monzese, Milano, Italy) for 10 minutes at 3257 *g*. The liquid phases were collected in dark glass bottles (25 mL) and used for the anthocyanin determination. Initially, the liquid phase was diluted with ethanol hydrochloric acid and analysed in a 10 mm cuvette on a spectrophotometer (UV-1601, Shimadzu Corporation, Kyoto, Japan) at 520 nm. The anthocyanin concentration was calculated as malvidin 3-glucoside chloride equivalents (mg/kg of grape).

To determine the concentration of phenolic substances, the liquid phase was diluted with water. Then, 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 minutes. Absorbance was then registered at 700 nm on the spectrophotometer using a 10 mm cuvette. The concentration was determined using a calibration curve and expressed as (+)–catechin, mg/kg of grape.

Statistical analysis

The results were tested with Statistica version 4.3 (StatSoft, Tulsa, OK, USA) for homogeneity of variance and subjected to ANOVA. The graphical and regression representations were obtained using the Sigma Plot version 10 (SPSS, Chicago, IL, USA). In each year and between years, data of TLA and LLN, grape composition at harvest and yield components were tested using means separation calculated by applying the Student–Newman–Keuls test at $P \leq 0.05$.

In the figures, seasonal development of berry mass, TSS, must pH, TA and the concentration of anthocyanin and phenolic substances are shown as mean values SE.

Linear regression analysis was performed on yield per vine, TSS, anthocyanin concentration and TA, combining the years.

Results

Environmental conditions

The average temperature (Tmed) was slightly higher during the 2011 season than in 2010. The growing degree-days (GDD, base 10°C) accumulated from April to harvest were 2017 in 2010 and 2065 in 2011 (Table 1). A drought occurred during the summer of 2011, while rainfall was high and distributed throughout the growing season in 2010. Total rainfall from April to harvest was very low in 2011 (122 mm) and relatively high in 2010 (435 mm). In the 2010 season, in every month except July, a total rainfall of at least 70 mm of water occurred (Table 1). In May, just after budburst, a large amount of rain fell that favoured the early shoot growth phase. During the first half of August, a period that includes the beginning of the berry ripening phase, there were a series of rainfalls and 71 mm of water fell (Table 1), of which 34 mm fell in a single day. Furthermore, in September, 15 days before the harvest, a significant rainfall of 35 mm occurred in one day. The distribution of rainfall in 2010 probably led to constant and adequate water availability in the soil during the growing season, improving growth and yield.

In the drier season of 2011, only 84 mm of rainfall fell from June to August, the period encompassing fruit set, veraison and berry ripening. In the period between August and the harvest (14 Sept), during the summer of 2011, there were 21 days where there was a daily maximum air temperature higher than 30 °C and 43 consecutive days without rainfall (Table 1). Despite these conditions and the fact that the vines were not irrigated, no visual symptoms of water stress and significant leaf yellowing were observed in the basal leaves.

Leaf layer number and total leaf area

As expected, the canopy density, measured as LLN and TLA per vine at harvest, was not influenced by the VG foliar spraying during the berry ripening phase (Table 2).

The canopy development was strongly influenced by the seasonal meteorological evolution, in fact the high temperatures of the 2011 growing season led to a limited development of LLN and TLA, compared to the 2010 growing season (Table 2).

Gas exchange data

Due to the seasonal trend, in 2010, Pn and E values were higher than in 2011, in all vines regardless of the crop load (Figure 1). In any case, in both years, one week after VG treatment, precisely on 1 September 2010 and 24 August 2011, the sprayed leaves showed a reduction in E values compared to C vines. The E values in TH and TM vines were around 45% lower than the respective CH and CM vines (Figure 1).

In 2010, Pn values in C vines exceeded 10 [μmol CO_2/ (m^2 s)], while in T vines Pn values were around 6 [μmol CO_2/ (m^2 s)], showing a lower Pn capacity (about -40% in T vines compared to C vines). The gs did not show important variations between TM and CM vines, ranging from 52 to 57 [mmol H_2O/ (m^2 s)], however TH leaves showed a lower gs than those of CH vines. In 2011, the Pn values were low in all vines, due to the dry seasonal trend. The VG treatment further lowered the Pn values in the T vines by 43% in TH vines compared to CH vines, and 33% in TM vines compared to CM vines. In general, the gs values were low in all vines, with values ranging from 16 to 38 [mmol H_2O/ (m^2 s)] (Figure 1).

Two weeks after the VG treatment, E values were raised in all vines, but sprayed leaves still showed lower values (Figure 1).

In 2010, two weeks after the VG treatment, the C vines maintained a high Pn capacity with values around 10-11 [μmol $CO_2/(m^2\ s)$], while the T vines exhibited a Pn capacity that was lower than the C vines, with values between 8-9 [μmol $CO_2/(m^2\ s)$], indicating a partial recovery of the Pn capacity and showing a reduction of only 18-20% compared to C vines (Figure 1). In 2011, two weeks after treatment, in a period corresponding to 15 days before harvest, the TH vines revealed a partial recovery of Pn capacity with a reduction of only 24% compared to the CH vines, while TM vines showed a complete recovery compared to the CM vines. The increase in Pn capacity over two years was associated with the rise in gs values, ranging from 80 to 118 [mmol $H_2O/\ (m^2\ s)$] in 2010 and 33 to 41 [mmol $H_2O/\ (m^2\ s)$] in the 2011 season (Figure 1).

One week after VG treatment, in the 2010 season, the WUEe (Pn/E) values in T vines were slightly higher compared to C vines. In the 2011 season, there was an increase of 8% in WUEe in TH vines and an increase of 21% in TM vines compared to the respective C vines. After two weeks, in 2010, the WUEe values had declined to below 4 [μmol CO_2/mol H_2O] in C vines, while in T vines values were maintained near 5 [μmol CO_2/mol H_2O]. Conversely, in 2011 the WUEe did not increase and all vines showed values between 2.2 and 2.7 [μmol CO_2/mol H_2O] (Figure 1).

One week after VG treatment, the value of WUEi (Pn/gs) was statistically different between T and C vines, in both years, with values of T vines being consistently higher, especially in the 2010 season, reaching 23 [μmol CO_2/mol H_2O] (Figure 1). Despite the lower WUEi values in 2011 in comparison to 2010, T vines still showed WUEi values higher than C vines (Figure 1).

Two weeks after VG treatment, regardless of the crop load and treatment, over the two years the vines showed similarly low WUEi values of around 10 [µmol CO_2/mol H_2O] in 2010 and 12 [µmol CO_2/mol H_2O] in 2011 (Figure 1).

Berry ripening and grape composition at harvest

The VG applied in post-veraison above the bunch zone did not affect berry development that, in the last 20 days before harvest, showed berry masses of between 2.42 and 2.79 g in 2010, and ranged from 1.9 g to 2.3 g in 2011 (Figure 2).

The TSS accumulation in the berry was lower in 2010 than in 2011 (Table 3). The maximum value was reached at harvest time by the CM vines in both years (24.93 °Brix in 2010 and 27.36 °Brix in 2011). Compared to the berries of C vines, those of TH vines showed a delay in sugar accumulation during both seasons. This delay remained until the harvest, when TH vines presented values of 2.7 and 1.6 °Brix lower than CH vines, in 2010 and 2011 respectively. Even TM vines, during the last berry ripening phase, accumulated sugar more slowly compared to CM vines, showing a sugar concentration at harvest lower than CM (-1.0 °Brix in 2010 and -1.6 °Brix in 2011), but without significant differences (Figure 2).

Similarly, the VG treatment affected berry pH in TH vines, that was significantly lower at harvest than in all other vines (Figure 3). Only in the 2010 season was the TA evolution in TH berries delayed, but by the time of harvest it was similar to CH berries (Table 3). Over the two years, at harvest, no significant differences were observed in the levels of tartaric acid and malic acid between T and C vines. Within the year, the TA resulted higher in the cooler 2010 season, characterised by the tartaric acidic values being lower and malic acid values being higher than the warmer 2011 season (Table 3).

The anthocyanin synthesis was delayed during the berry ripening phase especially in TH vines (Figure 4) and, at harvest, they showed a concentration significantly lower than that of CH vines in the 2010 season, with a difference of -31% (Table 3). Similarly, the concentration of phenolic substances at harvest, was reduced in TH berries by 11% in 2010 and 8% in 2011. In 2010, all vines had a higher concentration of anthocyanins and phenolic substances compared to that of 2011 (Table 3).

The yield per vine, recorded in both T and C vines, showed a strong relationship with TSS, showing a negative trend line and an R^2 equal to 0.79 and 0.88 respectively (Figure 5). The relationship between yield and anthocyanin concentration was higher (R^2 of 0.46) in T vines than in C ones, as was the relationship between yield per vine and TA (R^2 of 0.42) (Figure 5).

Vine yield and vine size

The yield was reduced by the bunch thinning treatment, which reduced the bunch number from 28-31 to 10-11 per vine in 2010 and from 14-15 to 9-10 per vine in the 2011 season. In the hot 2011 season, the yield was significantly lower than in 2010, in all the vines regardless of treatment (Table 4). In TM vines, compared to TH, bunch thinning reduced the yield by 63% in 2010 and 54% in 2011. Compared to CH vines, in CM vines yield was lower by 59% in 2010 and 48% in 2011. The bunch and berry mass were unaffected by bunch thinning and by the application of VG post-veraison, as was the berry number per bunch (Table 4).

Over the two years, regardless of treatment, the vines with a medium crop load (leaving about 1 bunch every 2 shoots) had the highest ratio between leaf area and yield (Table 5), mainly due to the low yield induced by bunch thinning. Within the year, cane numbers per vine were similar between vines, as was the pruning mass. When the pruning mass was combined with yields, in the TM and CM vines, it was lower, leading to significantly lower RI values. Comparing between the years, RI was at its highest during the cold 2010 season (Table 5).

Discussion

In comparison to the hot 2011 season, the regular distribution of rainfall in 2010 led to a consistent and adequate water availability in the soil throughout the growing period, improving both the vegetative growth and yield of the vines.

The effectiveness of the VG treatment, in reducing the stomatal opening on Sangiovese leaves, is shown by the significant reduction of the Pn and E rates and by the increase in WUE (intrinsic and extrinsic). The effect of the VG antitranspirant, sprayed onto the leaves in the post-veraison phase above the fruit zone, was shown by the Pn and gs values recorded one week after the treatment, that confirmed previous results observed on grapevines (Palliotti et al. 2013) and other species such as apples (Weller and Ferree 1978), beans (Iriti et al. 2009) and peppers (Del Amor et al. 2010).

The low permeability of film-forming polymers to both H_2O and CO_2 has long been known (Woolley 1967). Moreover, the effectiveness of the antitranspirant as an assessment tool for ozone damage in the field has been recently demonstrated, also suggesting a protective action for the leaves (Francini et al. 2011). This permeability of the film-forming caused a lowering of assimilated carbon, which influenced the sugar accumulation in the berries, as occurred in our trial.

Two weeks after the VG-treatment, the initial Pn rates in the leaves of T vines increased, showing a partial or complete recovery compared to the respective C vines. Thus, the effect of VG-treatment in reducing Pn was temporary and extended for at least

21 days from the VG application. Therefore, considering the period between the VG application and harvest, corresponding to 33 (27 August and 28 September) and 28 (18 August and 14 September) days respectively in 2010 and 2011, the reduction in Pn capacity of the T leaves resulted in a limitation in storing carbon until just before harvesting, when the Pn increased to values similar to the C vines. At harvest, however, the leaves of T vines still had a lower E than C vines. The depression of E was accompanied by an increase in WUEe until the final berry ripening phase (Figure 1), in T leaves compared to C leaves.

The lower sugar concentration in the berries of the T vines, is a consequence of the antitranspirant's capacity to reduce the leaf Pn through the transparent film formed on the leaf surface. The lower values of TSS determined in the T vines were in agreement with the results reported by Palliotti et al. (2013).

In our study, over two years the lowest values of TSS, observed in high yielding TH vines during the berry ripening phase, was due to the cumulative effects of the low Pn capacity of the canopies and the high crop load. The vines that underwent a drastic reduction in the bunch number, both in the control (CM) and treated vines (TM), presented an increase in sugar concentration at harvest, with more marked differences in the 2010 season (+3.9 ºBrix in TM vines compared to TH vines and +1.6 ºBrix in CM vines compared to CH vines). In the hottest season of 2011, the difference in TSS between the vines decreased to +0.9 ºBrix and +0.3 ºBrix respectively in the grape must of both TH and CH.

The must pH showed differences between treatments of the vines, with the lowest values being recorded in TH vines, as for the sugar concentration, denoting a delay in the berry ripening phase in the vines subjected to the VG-treatment. No effect was observed in the values of TA, tartaric and malic acids due to the VG-treatment and/or the crop load of the vines. These parameters were, instead, influenced by seasonal trends. The TA values were lower in 2011 due to the greater degradation of malic acid.

The concentration of anthocyanins and phenolic substances increased in the Sangiovese TH vines in 2011 and this is an important aspect, as it is a variety which is characterised by a low ability to develop and accumulate colour compared to other black-berried grapevine cultivars (Palliotti et al. 2013). This improvement is not attributable to the bunch thinning or the VG spraying, but instead is due to the seasonal trend in 2011, which probably favored the synthesis of anthocyanins.

The relationship between yield per vine and TSS suggests that the VG-treatment can act as a strategy to contain the sugar concentration of the berries, with a more marked effect when combined with a high crop load. The data obtained from the T vines are positioned mainly below those found by the C vines. The variability of the data shows the combined effect of the VG-treatment and the high crop load in TH vines with lower TSS values also when compared to CM vines. Increments of 1 kg of yield corresponds to a reduction of about 0.79 °Brix in T vines and of 0.67 °Brix in C vines, with a corresponding increase of 0.10 and 0.06 g/L of total acidity and a loss of anthocyanin concentration of 23.62 mg/kg and 14.15 mg/kg respectively in both T and C vines. The relationship between yield per vine and anthocyanin concentration and TA suggests the possibility of using the combination of VG-treatment and crop load as an indicator of anthocyanin concentration and TA.

In our trial, the berry size was not significantly altered, because the bunch thinning and the VG-treatment were carried out at veraison, a phase in which the berry growth tends not to double in weight and volume. The effect of the VG antitranspirant above the bunch zone is different depending on the phenological phase in which it is sprayed on the canopies. If the application of VG is carried out during post-veraison is effective in reducing gs and Pn, obtaining a minor sugar accumulation and ultimately less alcoholic wine. Instead, applications at the beginning of the season, such as in the pre-bloom phase, led to a limitation of the leaf function, which led to a reduction in the yield and the bunch compactness. From bloom to veraison, the VG-treatment inhibits Pn and E rates leading to smaller berry size at harvest (Palliotti et al., 2010).

Over the two years, regardless of treatment, the ratio between the leaf area and the yield, exclusively related to changes in grape yield, was higher in both TM and CM vines (where about 1 bunch every 2 shoots remained), which showed lower RI values. In the cold 2010 season, the RI values in medium yielding vines, regardless of the VG application, were lower than in high yielding vines, indicating a good balance between yield (kg of grapes per vine) and vine vigor (annual pruning mass) in TM and CM vines, and suggesting an imbalance of the canopy due to a high yield in TH and CH vines. The warm 2011 season influenced the pruning characteristics of all vines, leading the RI to drop below values of 3 kg/kg.

Conclusions

Managing the crop load of vines and treating the canopies with VG were effective measures in delaying grape ripening and led to obtaining musts with reduced sugar content, without any impact on fresh weight and yield capacity.

The reduction in the sugar concentration at harvest is a consequence of the VG antitranspirant film-forming on the leaves, that temporarily limits not only the transpiration, but also the photosynthetic activity, thus reducing the amount of assimilates which can be translocated into berries during ripening, causing the reduction in the must sugar concentration. The data on sugar accumulation suggests that in all conditions of high crop load, the VG-treatment could cause a delay of the grape’s technological maturity, even higher than that exerted by the crop load.

In the close relationship between yield per vine and TSS, the effect of the VG-treatment is evident, and becomes more pronounced when combined with the high crop load. Finally, also the anthocyanin concentration and TA were positively related to the yield per vine in T vines, with a more marked effect in vines with a high crop load.

The use of the VG antitranspirant could, therefore, be an appropriate technical strategy to mitigate the accumulation of sugars in the berries, nowadays in excessive concentration, and to condition the must composition according to the crop load.

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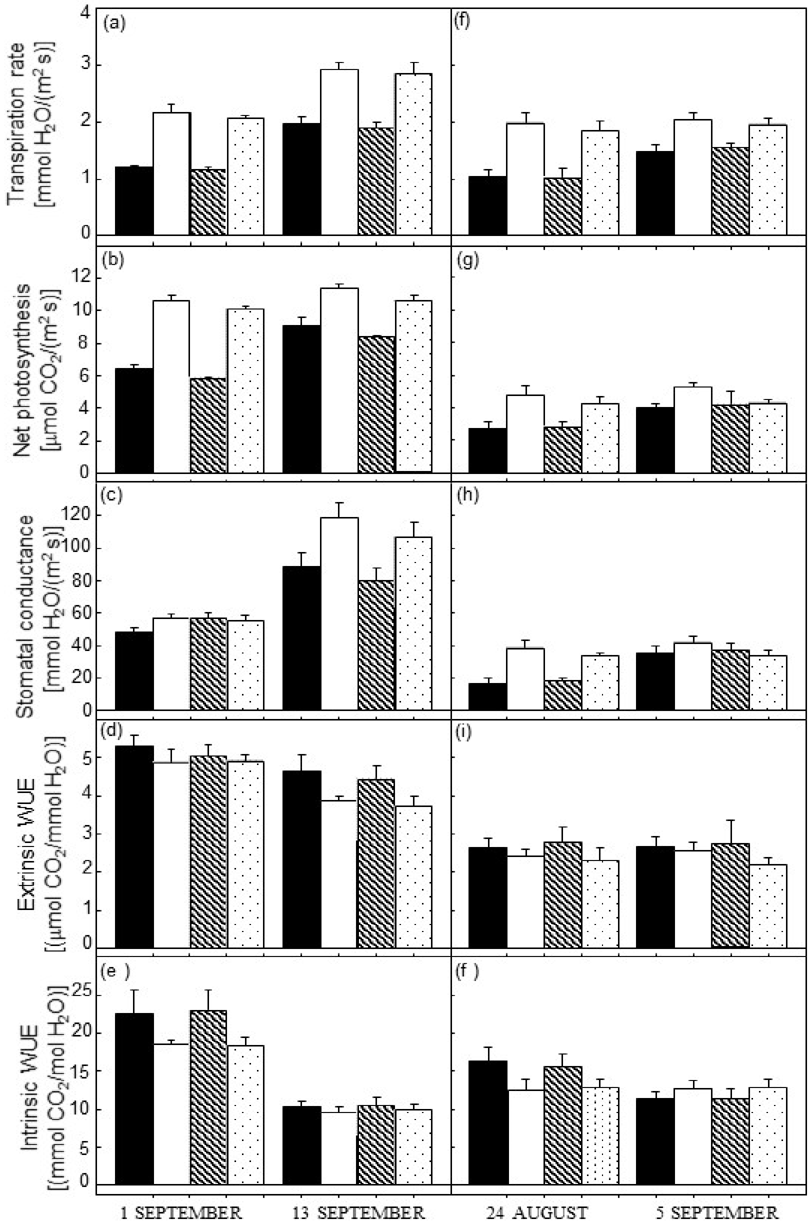

Figure 1. Transpiration rate (a, f), net photosynthesis (b, g), stomatal conductance (c, h), extrinsic water use efficiency (d, i) and intrinsic water use efficiency (e, l) in 2010 (a, b, c, d, e) and 2011 (f, g, h, i, l), recorded in two time of seasons on Sangiovese VG-treated vines with high (TH) (black) and medium (TM) (red) crop load and control vines with high (CH) (white) and medium (CM) (blue) crop load. (mean SE, n = 6 vine per treatment).

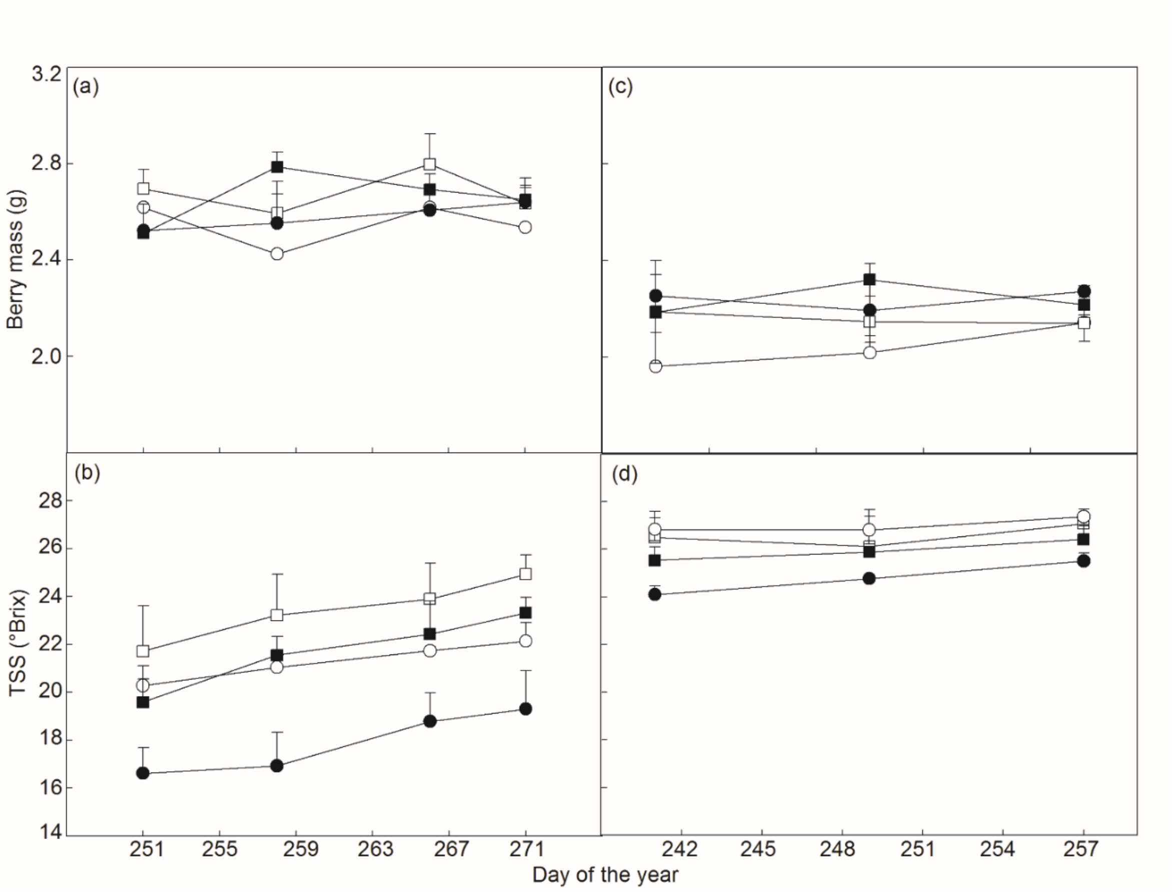

Figure 2. Evolution of berry mass (a, c) and TSS (b, d) in 2010 (a, b) and 2011 (c, d), recorded in Sangiovese VG-treated vines with high (TH) (●) and medium (TM) (■) crop load and control vines with high (CH) (○) and medium (CM) (□) crop load. (mean SE, n = 100 berries per treatment, at harvest n = 100 berries per vine).

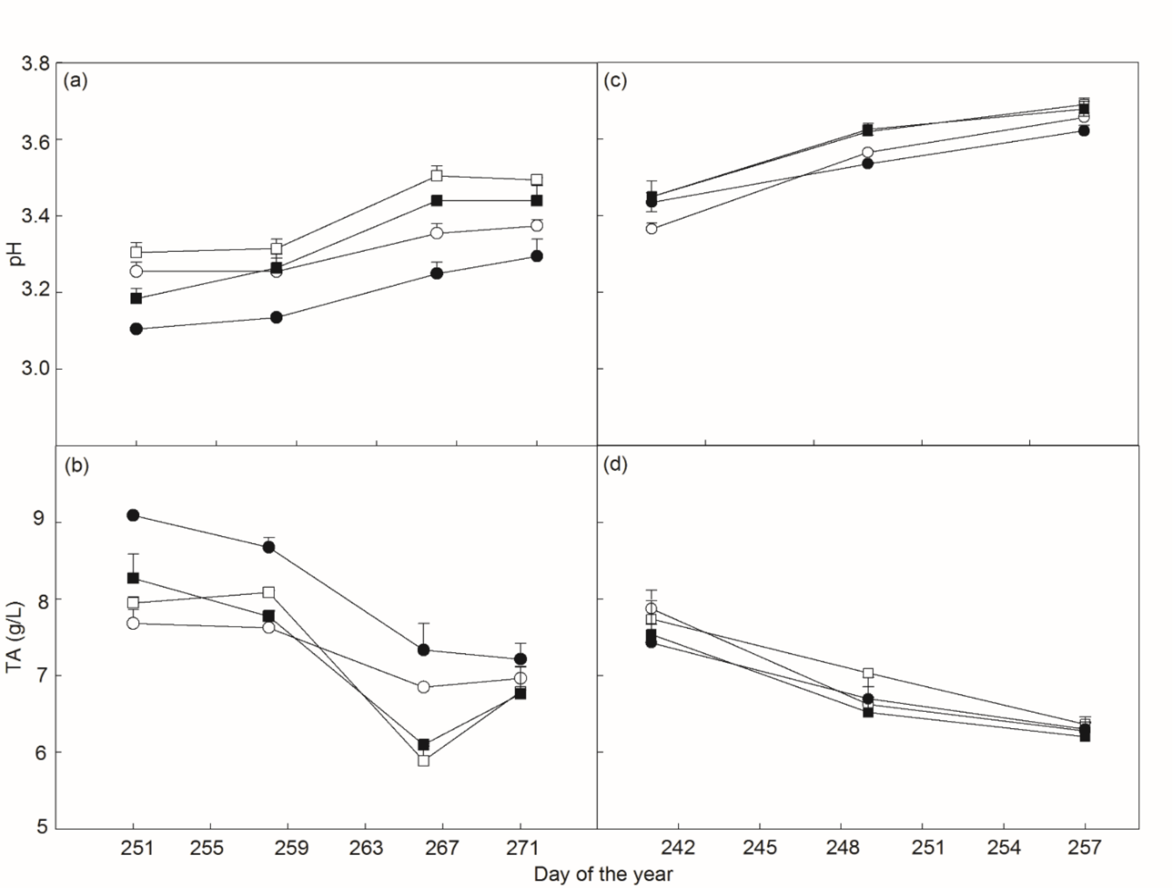

Figure 3. Evolution of pH (a, c) and TA (b, d) in 2010 (a, b) and 2011 (c, d), recorded in Sangiovese VG-treated vines with high (TH) (●) and medium (TM) (■) crop load and control vines with high (CH) (○) and medium (CM) (□) crop load. (mean SE, n = 100 berries per treatment, at harvest n = 100 berries per vine).

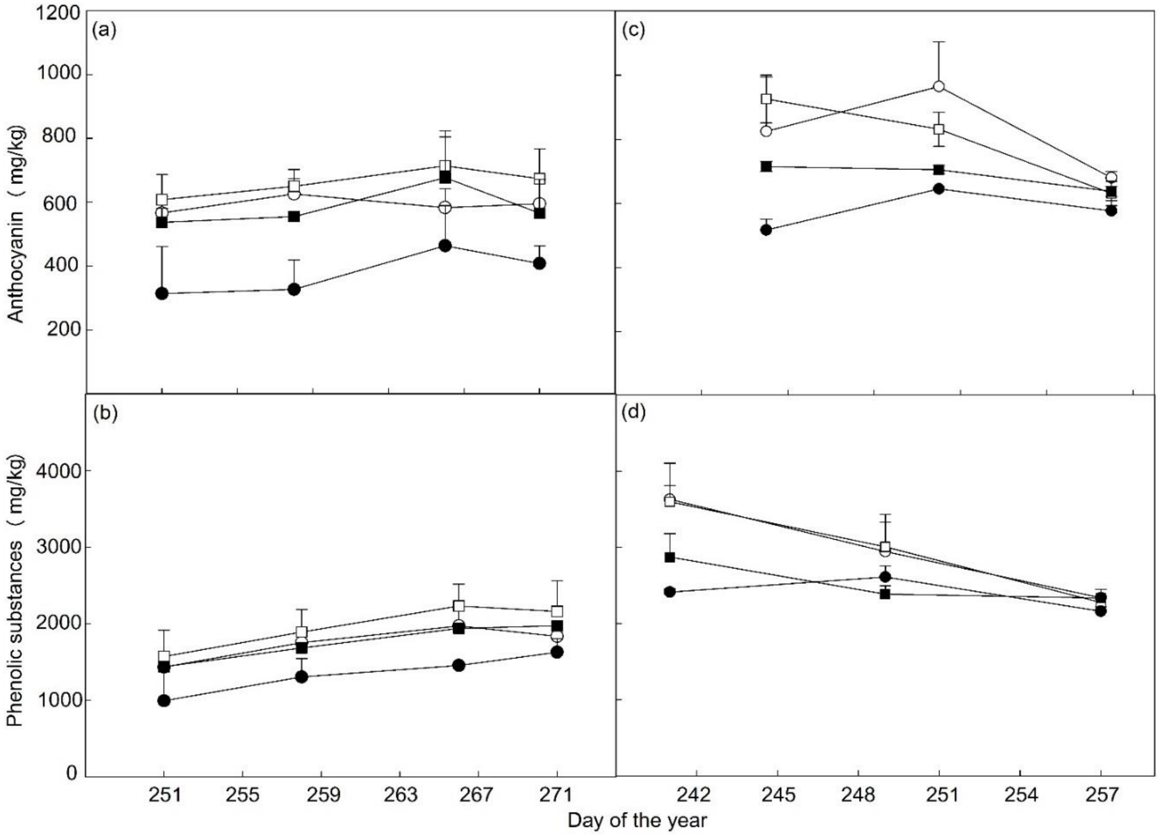

Figure 4. Evolution of the concentration of anthocyanin (a, c) and phenolic substances (b, d) in 2010 (a, b) and 2011 (c, d) recorded in Sangiovese VG-treated vines with high (TH) (●) and medium (TM) (■) crop load and control vines with high (CH) (○) and medium (CM) (□) crop load. (mean SE, n = 100 berries per treatment, at harvest n = 100 berries per vine).

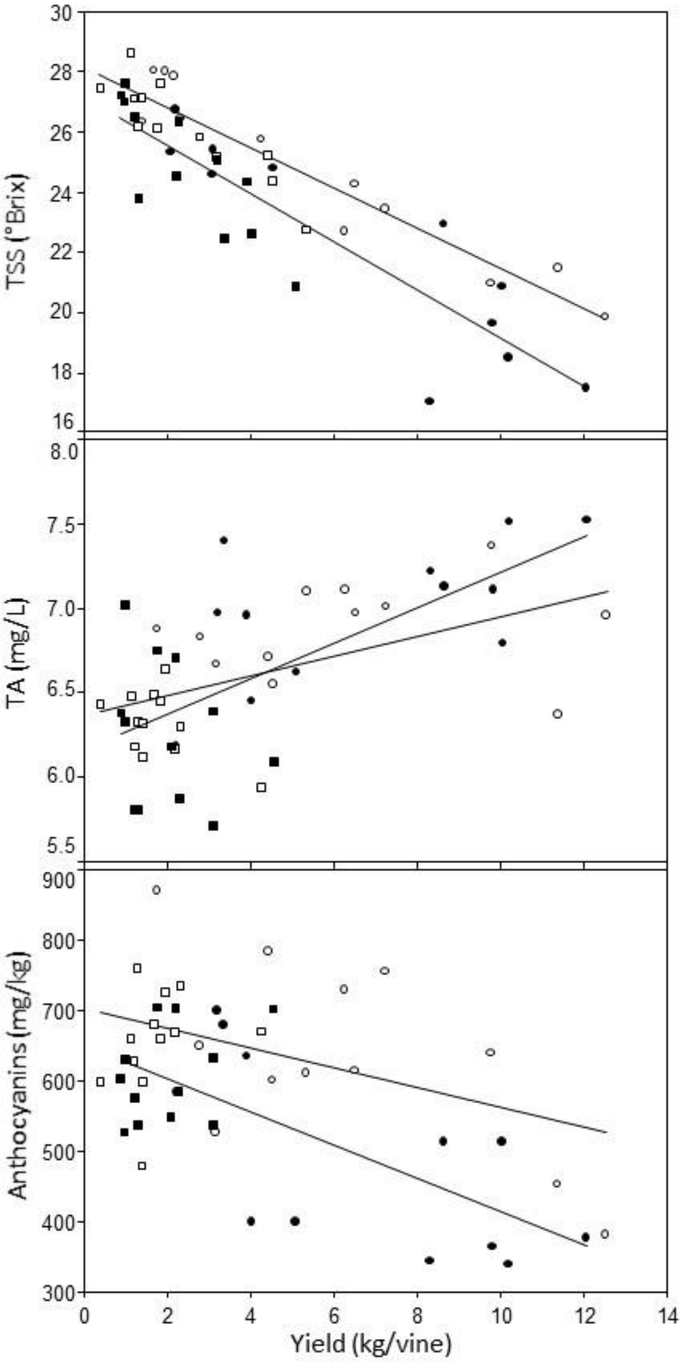

Figure 5. Regression relationships between yield per vine and TSS (a) in Sangiovese VG-treated vines with high (TH) (●) and medium (TM) (■) crop load (n = 12 plants, R^2 = 0.79, Y= -0.80 x + 27.18) and control vines with high (CH) (○) and medium (CM) (□) crop load (n = 12 plants, R^2 = 0.88, Y= -0.67 x + 28.08); yield per vine and TA (b) in Sangiovese VG-treated vines with high (TH) (●) and

medium (TM) (■) crop load (n = 12 plants, $R^2 = 0.42$, Y= 0.105 x + 6.16) and control vines with high (CH) (○) and medium (CM) (□) crop load (*n* = 12 plants, $R^2 = 0.29$, Y= 0.058 x + 6,36); yield per vine and concentration of anthocyanin (c) in Sangiovese VG-treated vines with high (TH) (●) and medium (TM) (■) crop load (n = 12 plants, $R^2 = 0.46$, Y= -23.62 x + 650.78) and control vines with high (CH) (○) and medium (CM) (□) crop load (n = 12 plants, $R^2 = 0.88$, Y= 14.148 x + 701.97).

Table 1. Weather variables on monthly basis, from April to September and growing degree-days from April to harvest in Sangiovese vines.

	April	May	June	July	August	September	GDD April-Harvest
Tmed							
2010	14	18	22	26	25	21	2017
2011	16	19	23	25	27	26	2065
T>30°C (days n°)							
2010	0	0	0	10	6	0	16
2011	0	1	2	8	15	6	36
Precipitation (mm)							
2010	70	97	107	14	71	76	435
2011	25	13	25	59	0	0	122

Daily average and maximum temperature and precipitation data were taken from the site of Agenzia Servizi al Settore Agroalimentare delle Marche.

GDD, Growing degree-days (daily temperature base 10 °C).

Data are from 1 April to 28 September in 2010 and from 1 April to 14 September in 2011.

Table 2. Leaf layer number and total leaf area determined at harvest in Sangiovese control vines and subjected to subjected to the antitranspirant treatment, with different crop load.

	LLN¶ (N)			**TLA (m^2)**		
	2010	**2011**	Sig.	**2010**	**2011**	Sig.
TH	2.93A	2.41B	**	3.56A	2.95B	**
CH	3.17A	2.25B	**	4.07A	2.75B	**
TM	2.98A	2.42B	**	3.61A	2.96B	**
CM	2.87A	2.04B	**	3.48A	2.61B	**
Sig.	n.s.	n.s.		n.s.	n.s.	

Within column mean separation performed with Student–Newman–Keuls test and shown by lowercase letters. Within row mean separation performed with Student–Newman–Keuls test and shown by capital letters.

LLN, Leaf layer number, *TLA*, total leaf area

**, a significant difference between years at $P < 0.05$; n.s., not significant.

TH, VG-treated vines with high crop load, *CH*, control vines with high crop load, *TM*, VG-treated vines with medium crop load, *CM*, control vines with medium crop load.

Table 3. Must composition at harvest in Sangiovese control vines and subjected to subjected to the antitranspirant treatment, with different crop load.

	TSS (°Brix)			**pH**			**TA (g/L)**			**Tartaric acid (g/L)**			**Malic acid (g/L)**			**Anthocyanins (mg/kg)**			**Phenolic substances (mg/kg)**		
	2010	**2011**	**Sig.**	**2010**	**2011**	**Sig.**	**2010**	**2011**	**Sig.**	**2010**	**2011**	**Sig.**	**2010**	**2011**	**Sig.**	**2010**	**2011**	**Sig.**	**2010**	**2011**	**Sig.**
TH	19.4b B	25.5b A	**	3.30b	3.62b	n.s.	7.2a A	6.3 B	**	6.96 B	9.05 A	**	2.21 A	1.60 B	**	408b B	637a A	**	1630b B	2333 A	**
CH	22.1a B	27.1a A	**	3.38b	3.66a	n.s.	7.0a A	6.3 B	**	6.43 B	9.13 A	**	2.32 A	1.59 B	**	595a	680a	n.s.	1838a B	2338 A	**
TM	23.3b B	26.4b A	**	3.44a	3.68a	n.s.	6.8b A	6.2 B	**	6.54 B	9.90 A	**	2.04 A	1.54 B	**	566a	577b	n.s.	1974a	2162	n.s.
CM	24.9a B	27.4a A	**	3.50a	3.69a	n.s.	6.8b A	6.4 B	**	6.23 B	9.77 A	**	2.36 A	1.69 B	**	674a	631a	n.s.	2162a	2277	n.s.
Sig.	**	**		**	**		**	n.s.		n.s.	n.s.		n.s.	n.s.		**	**		**	n.s.	

Within column mean separation performed with Student–Newman–Keuls test and shown by lowercase letters. Within row mean separation performed with Student–Newman–Keuls test and shown by capital letters.

TSS, Total soluble solids, *TA*, Titratable acidity

**, a significant difference between years at $P < 0.05$; n.s., not significant.

TH, VG-treated vines with high crop load, *CH*, control vines with high crop load, *TM*, VG-treated vines with medium crop load, *CM*, control vines with medium crop load.

Table 4. Yield parameters at harvest in Sangiovese control vines and subjected to subjected to the antitranspirant treatment, with different crop load.

	Yield/vine (kg)			**Bunches/vine (N)**			**Bunch mass (g)**			**Berry mass (g)**			**Berries/Bunch (N)**		
	2010	**2011**	Sig.	**2010**	**2011**	Sig.	**2010**	**2011**	Sig.	**2010**	**2011**	Sig.	**2010**	**2011**	Sig.
TH	9.9a	2.8a		31a	14a		318	202		2.64	2.27		121	89	
	A	B	**	A	B	**	A	B	**	A	B	**	A	B	**
CH	9.0a	2.3a		28a	15a		324	156		2.53	2.14		128	73	
	A	B	**	A	B	**	A	B	**	A	B	**	A	B	**
TM	3.6b	1.3b		11b	10b		348	175		2.65	2.22		131	78	
	A	B	**			n.s.	A	B	**	A	B	**	A	B	**
CM	3.7b	1.2b		10b	9b		361	163		2.64	2.13		134	75	
	A	B	**			n.s.	A	B	**	A	B	**	A	B	**
Sig.	**	**		**	**		n.s.	n.s.		n.s.	n.s.		n.s.	n.s.	

Within column mean separation performed with Student–Newman–Keuls test and shown by lowercase letters. Within row mean separation performed with Student–Newman–Keuls test and shown by capital letters.

**, a significant difference between years at P < 0.05; n.s., not significant.

TH, VG-treated vines with high crop load, *CH*, control vines with high crop load, *TM*, VG-treated vines with medium crop load, *CM*, control vines with medium crop load.

Table 5. Vegetative and pruning characteristics recorded in Sangiovese control vines and subjected to the antitranspirant treatment, with different crop load.

	Leaf area/yield (m^2/kg)			**Canes (N/vine)**			**Pruning wt (kg/vine)**			**Ravaz index (kg/kg)**		
	2010	**2011**	Sig.	**2010**	**2011**	Sig.	**2010**	**2011**	Sig.	**2010**	**2011**	Sig.
TH	0.30b	0.86b		19	20		0.87	1.07		11.3a	2.6a	
	B	A	**			n.s.	B	A	**	A	B	**
CH	0.35b	0.98b		21	18		0.83	1.05		10.8a	2.2a	
	B	A	**			n.s.	B	A	**	A	B	**
TM	0.83a	1.86a		22	20		0.77	1.34		4.7b	0.9b	
	B	A	**			n.s.	B	A	**	A	B	**
CM	0.78a	1.70a		20	18		0.64	1.17		5.8b	1.0b	
	B	A	**			n.s.	B	A	**	A	B	**
Sig.	**	**		n.s.	n.s.		n.s.	n.s.		**	**	

Within column mean separation performed with Student–Newman–Keuls test and shown by lowercase letters. Within row mean separation performed with Student–Newman–Keuls test and shown by capital letters.

**, a significant difference between years at $P < 0.05$; n.s., not significant.

TH, VG-treated vines with high crop load, *CH*, control vines with high crop load, *TM*, VG-treated vines with medium crop load, *CM*, control vines with medium crop load.