American Journal of Enology and Viticulture (AJEV). doi: 10.5344/ajev.2013.13015
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Postveraison Application of Antitranspirant Di-1-*p*-Menthene to Control Sugar Accumulation in Sangiovese Grapevines

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Acknowledgments: This research was partially funded by the Italian Ministry for University (PRIN 2009 Grant) and Biogard Division (Grassobbio, BG, Itlay). The authors are grateful to Dr. Fabrizio Leoni, Dr. Riccardo Cini, and Dr. Massimo Benuzzi for critical appraisal and helpful discussion.

Manuscript submitted Jan 2013, revised Apr 2013, accepted May 2013

Abstract: The effectiveness of a postveraison application of the film-forming antitranspirant Vapor Gard® (VG, a.i. di-1-*p*-menthene) was investigated as a technique to delay grape ripening and reduce sugar accumulation in the berry. The study was carried out over the 2010-2011 seasons in a non-irrigated vineyard of cv. Sangiovese in central Italy. VG was applied at 2% concentration to the upper two-thirds of the canopy (most functional leaves) and it significantly lowered leaf assimilation and transpiration rates and increased intrinsic water use efficiency. The Fv/Fm ratio was not modified emphasizing that photoinhibition did not occur at the PSII complex, whereas the reduction of pool size of plastoquinone matched well with reduced CO_2 fixation found in VG-treated vines. In both years VG treatment reduced both the pace of sugar accumulation in the berry as compared to control vines, scoring a -1.2 Brix at harvest and wine alcohol content at -1% without compromising the recovery of concentrations of carbohydrates

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and total nitrogen in canes and roots. Concurrently, organic acids, pH and phenolic richness of grapes and wines were unaffected, whereas a lowering in anthocyanin content in the berry (-19% compared to control vines) and in the wine (-15% compared to control vines) were found. The application of VG at post-veraison above the cluster zone is an effective and easy-to-do viable technique to hinder berry sugaring and obtain less alcoholic wines. To be effective it is advised to perform the spraying at around 14-15 Brix making sure that the lower leaf epidermis is fully wetted by the chemical.

Key words: berry composition, vine yield, reserve storage, photosynthesis, chlorophyll fluorescence, wine composition

Introduction

The specific climate is crucial to establish the overall style of a wine produced from well-defined areas. Reaching complete grape maturation is critical to determining the best cultivar to be grown, while climate variability determines year-to-year differences in the grape and wine quality (Jones and Hellman 2003). In particular, temperature and irradiance are considered critical because of their direct effect upon numerous outcomes including: the length of growing season; vine and berry phenological stages; vine yield by means of flower and berry abscission; berry growth; and the synthesis and accumulation of sugars, organic acids, polyphenols and aromatic compounds in the berries (Gladstones 1992). A steady trend of increased warming, beginning more than 20 years ago, is pushing traditional areas of grape growing toward accelerated ripening (Jones 2005) leading, in turn, to excessive sugar accumulation in the fruit and high alcohol in the wine. Yet, climate change and increased variability are thought to contribute to only 50% of the increase in alcohol levels in wines (Jones 2007) leaving the

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balance to other sources. The rising sugar content in grape and alcohol in wines are dependent upon other environmental traits and technical choices and among these are: a) higher potential canopy photosynthesis due to the steady increase of CO_2 concentration in the atmosphere (Schultz 2000); b) improvements in vineyard management and in control strategies of pests and insects; c) law-enforced yield constraints in several Appellation areas; d) greater use of cultivars genetically characterized by low productivity due to reduced cluster weight and/or grafted on low vigor rootstocks; and e) improved sanitary status of propagation material.

Improving sugar accumulation in berries has long been one of the main objectives of research in viticulture; yet, the role of sugar concentration has recently undergone a strong change. Today, an increasing number of consumers prefer wines with more moderate alcohol content (Seccia and Maggi 2011), an attitude linked to more severe controls on vehicle drivers, as well as to mouthfeel sensations. Regarding the latter, it has been shown that ethanol can enhance the perception of sweetness and bitterness, while reducing that of acid, saltiness and sourness (Martin and Pangborn 1970, Fisher and Noble 1994).

The limitation in grape sugar concentration achieved in the vineyard is also useful to minimise costly interventions in the winery aimed at dealcoholizing wines up to -2% vol., such as membrane techniques, supercritical fluid extraction, vacuum distillation, etc. These techniques have recently been made legal throughout the European Union (Council Regulation n. 606/2009).

Moreover, one of the negative consequences of a premature Brix development is that in several viticultural areas this process occurs during the hottest part of the season (Jones et al. 2005) when both the color and aroma profile can be adversely affected (Lacey et al. 1991,

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Reynolds and Wardle 1993, Mori et al. 2007). Under these conditions, grapes often combine an excessively low acidity and high pH, thus requiring additional cellar costs to balance the must. This action typically involves the addition of tartaric acid before fermentation in order to avoid microbiological instability and improve mouth-feel (Keller 2010).

Among the canopy management techniques which have been tested to regulate sugar accumulation in the berries and/or modulate an accelerated or unbalanced ripening, application of antitranspirant compounds have proven to be interesting for their low cost and ease of application (Palliotti et al. 2012). Antitranspirants have been widely used to counteract drought events since, once applied to leaves, they significantly reduce water loss and heat stress (Gale and Poljakoff-Mayber 1967, Rosati 2007). Depending on the mode of action, the following two types of antitranspirant have been classified: a) film-forming polymers sprayed on leaf surfaces (Gale and Poljakoff-Mayber 1967); and b) stomata-closing compounds (Zelitch 1969). The second group includes alkenilsuccinic acids, phenylmercuric acetate, abscisic acid and a new formulation called chitosan (B-1,4-D-glucosamine), a deacetylated chitin derivative. The latter compound has been recently proved to be effective in protecting bean leaves from ozone damage (Francini et al. 2011), in reducing powdery mildew incidence in grapevine leaves and improving total polyphenols and antioxidant activity in grapes and wine of Montepulciano (Iriti et al. 2011). The film-forming polymer kaolin, an inert clay mineral, was effective at controlling heat stress in several species by increasing canopy reflectance of infrared and ultraviolet radiations, thereby reducing leaf and fruit tissue temperature (Rosati 2007). The effects of kaolin on leaf photosynthesis provide contrasting results, due also to counteractive effects of the antitranspirant

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on stomatal aperture and gas-exchange especially under water deficit conditions (Davenport et al. 1972, Rosati 2007).

Recently, it was demonstrated that source limitation during the post-veraison stage, through mechanical leaf removal apical to the cluster zone, was able to reduce sugar accumulation in the berry and delay grape Brix accumulation without delaying increases in pigment and phenolic ripening (Palliotti et al. 2013). It is conceivable that source limitation might also be imposed through the use of antitranspirant compounds (Gale and Poljakoff-Mayber 1967), which have already shown efficacy to reduce gas-exchange in different crop species (Iriti et al. 2009, Francini et al. 2011) including the grapevine (Palliotti et al. 2010).

Using field-grown Sangiovese vines, a two-year study was conducted to test: 1) the effectiveness of a post-veraison application of an organic film-forming antitranspirant at delaying sugar accumulation in the berries, and 2) evaluate its effects on vine physiology, wine quality and replenishment of the storage of reserves in cane wood and roots.

Materials and Methods

Plant material and experimental layout. The study was carried out over the 2010 and 2011 seasons in a non-irrigated commercial vineyard sited in central Italy near Deruta (Perugia, Umbria region, 42°59’ N, 12°25’ E, elevation 405 m asl, loamy soil type). The vineyard was a 12-year-old planting of *Vitis vinifera* L. cv. Sangiovese (clone VCR30 grafted onto 420A rootstock) planted at 2.5 m × 1.0 m inter- and intra-row and trained to a vertically shoot-positioned, spur-pruned cordon trellis with a bud-load of about 10 nodes per meter of row length. The cordon was trained 0.9 m aboveground with three pairs of foliage wires on a canopy wall

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extending 1.2 m above the cordon. Pest management was carried out according to local standard practice and shoots were mechanically trimmed when most started to outgrow the top wire.

Four adjacent rows of 60 vines each, were selected to create a completely randomized-block design with each row as a block. Half of the vines of each block were randomly assigned to antitranspirant Vapor Gard® treatment (VG) and the vines of the other half were used as an unsprayed control (C). In 2010, due to heavy rain occurring one week after the first treatment, VP was applied twice, on August 10 and 27 respectively, whereas in 2011 it was sprayed once, on August 18. The antitranspirant Vapor Gard® (Intrachem Bio Italia, Grassobbio, BG, Italy) is a water emulsifiable organic concentrate for use on plants designed to reduce transpiration by forming a clear, soft and flexible film that reduces normal moisture loss. Its active ingredient is di-1-*p*-menthene ($C_{20}H_{34}$), a therpenic polymer, also known as pinolene, which is produced from resins of conifers by a distillation process. Each year, VG was prepared at 2% concentration in water, stirred slowly to form an emulsion, and all the leaves of the canopy located above the cluster area were sprayed using a portable pump. During treatment were wet well the abaxial surfaces of the leaves in order to cover the stomatal pores.

Leaf gas-exchange and chlorophyll fluorescence. In 2010, beginning one week before spraying, single leaf gas exchange readings were taken on VG and C vines at varying intervals until harvest in the morning hours (1000-1100 hr) of clear days using a portable, open system, LCA-3 infrared gas analyzer (ADC Bio Scientific Ltd, Herts, UK). The system featured a broad leaf chamber having a 6.25 cm^2 window and all readings were taken at ambient relative humidity with an air flow adjusted to 350 mL min^{-1}. Twelve primary leaves per treatment (three replicates per block) were chosen at nodes 8-10 above the distal bunch and sampled under saturating light

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138 photosynthetic active radiation (PAR > 1400 µmol photons m^{-2} s^{-1}). Assimilation rate (A),
139 transpiration rate (E), stomatal conductance (g_s) and substomatal CO_2 concentration (Ci) were
140 calculated from inlet and outlet CO_2 and H_2O concentrations. Intrinsic water use efficiency
141 (WUEi) was then derived as the A to g_s ratio**.** On the same leaves used for the gas-exchange
142 readings, temperature was also measured using an infrared thermometer (Mod. TM909L9, Assi-
143 control, Italy).

144 To highlight a possible instability of the photochemical apparatus, chlorophyll fluorescence
145 was measured between 1100 and 1200 hr of August 16 (a day with very low assimilation rate in
146 VG treated vines) with a lightweight portable continuous excitation fluorometer (Handy-PEA,
147 Hansatech Inst. Ltd., Norfolk, UK). These measurements were performed on the same leaves
148 sampled for gas exchange with the addition of lateral leaves from the same shoots (twelve per
149 treatment, three replicates per block, taken in the middle part of lateral shoots). Dark adaptation
150 was achieved by covering the sample area to be analyzed with a small, lightweight leaf clip for at
151 least 30 minutes. The small shutter plate of the clip was then opened and the dark-adapted leaf
152 tissue exposed to an actinic light flash (wavelength of 650 nm, intensity > 3000 µmol m^{-2} s^{-1}).
153 The instrument provides the F_v/F_m ratio, which is a widely accepted indicator of the maximum
154 photochemical efficiency of photosystem II (PSII), where F_m is the fluorescence maximum over
155 the induction curve. F_v, termed variable fluorescence, was calculated as the difference between
156 F_m and F_o, where F_o is the ground fluorescence (Strasser *et al.* 1995). The area above the
157 fluorescence curve between F_o and F_m (Area), which indicates the pool size of plastoquinone on
158 the reducing size of PSII, was also automatically calculated.

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Yield component and grape composition. In 2010 and 2011, beginning from the VG treatment until harvest, total soluble solids (Brix) was periodically assessed on 180-berry samples (four samples of 45 berries per treatment and measurement date, one replicate per block) using a temperature-compensating refractometer (RX-5000 Atago-Co Ltd, Tokyo, Japan). The rate of Brix accumulation/day, was also calculated.

Harvest dates were 27 September in 2010 and 14 September in 2011. Grapes from 50 experimental vines per treatment were individually picked and the number of clusters per vine and the crop weight were recorded, and the average cluster weight calculated. Each year, four samples of 300 berries per treatment (one replicate per block) were randomly collected and their fresh weight was recorded. After crushing, Brix, titratable acidity and pH for each sample were analysed. Titratable acidity was measured with a Titrex Universal Potentiometric Titrator (Steroglass S.r.l., Perugia, Italy), titrating with 0.1 N NaOH to an end point of pH 8.2. Results were expressed as g/L of tartaric acid equivalent. Must pH was measured using a PHM82 standard pHmeter (Radiometer, Copenhagen, Denmark). Anthocyanin and phenolic contents (expressed as mg/cm^2 skin) were determined on berry skins according to Ough and Amerine (1980) and Slinkard and Singleton (1977), respectively. From each treatment, twenty 10-mm diameter disks of the grape skin (five replicates per each block) were cut and carefully separated from the flesh. Disks were taken from the external, mid portion of well exposed clusters**.** Each skin disk (0.785 cm^2) was macerated in 25 mL of methanol containing 0.1% HCl (v/v) at pH 1 and incubated at room temperature (about 25 °C) for 24 h in the dark with periodic shaking. The total anthocyanin content was determined by measuring the absorbance at 520 nm, without filtration or centrifugation and with no correction for background absorbance, at pH 1 using an

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extinction coefficient (molar absorbance value) of 28,000 and molecular weight of 529 (typical of malvidin-3-glucoside). Total soluble phenols were assayed as follows: to each 0.2 mL sample, 1.8 mL of distilled water (diluted to contain 0 to 250 mg/L gallic acid equivalent) was added and then followed by 10 mL of 10% aqueous Folin-Ciocalteau reagent (Sigma) and 8 mL of 7.5% (w/v) aqueous Na_2CO_3. The mixture was held at 24 °C and after 2 h the absorbance was read at 750 nm and compared to a gallic acid standard curve. Yeast assimilable nitrogen (YAN) content, including ammonium salts and *a*-amino acids, was estimated according to Masneuf and Dubourdieu (1999). This method is based on the reaction of formaldehyde with amino functions.

Microvinification and wine analysis. In 2010 and 2011, wines were made using microvinification techniques. At harvest, grapes from 120 VG treated and 120 C vines were harvested manually and transported to the experimental winery in 20-kg plastic boxes. For each treatment, the total harvested grape mass was divided into two lots, each weighing about 150 kg. Each lot was mechanically crushed, destemmed, transferred to 100-L stainless-steel fermentation containers, sulfited with 35 mg/L of SO_2, and inoculated with 35 mg/L of a commercial yeast strain (Lalvin EC-1118, Lallemand Inc., Ontario, Canada). Wines were fermented for 16 to 18 days on the skin and punched down twice daily, with the fermentation temperature ranging from 20 to 27°C. After alcoholic fermentation, the wines were pressed at 0 Brix and inoculated with 30 mg/L of *Oenococcus oenii* (Lalvin Elios 1 MBR, Lallemand Inc., Ontario, Canada). After completion of malolactic fermentation, the samples were racked and transferred to 60-L steel containers and 25 mg/L of SO_2 was added. Two months later, the wines were racked again, bottled into 750-mL bottles then closed with cork stoppers. After eight months, the wines were analyzed for alcohol, titratable acidity and pH (Iland et al. 1993). Wine color intensity

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(OD_{420}+OD_{520}), color hue (OD_{420}/OD_{520}) and total phenol and anthocyanin concentrations were determined with a spectrophotometer. Total phenols were quantified according to Ribéreau-Gayon (1970) by measuring the absorbance at 280 nm of wine diluted 1:100 with distilled water. Anthocyanins were analysed as reported by Ribéreau-Gayon and Stonestreet (1965). All determinations were carried out in duplicate, yielding four replicates per treatment.

Carbohydrates and nitrogen storage in permanent vine organs. At the end of December 2010 and 2011 the soluble sugars and starch concentrations in canes (node 3) and roots (fine brown with 1.5 ± 0.2 mm diameter taken at 10 to 20 cm soil depths) were determined on six replicates per treatment according to a colorimetric method (Loewus 1952) using the anthrone reagent (Merck, Darmstadt, Germany). Absorbance readings at 620 nm wavelength were performed on a Jasco V-630 spectrophotometer (Tokyo, Japan). On the same material, total nitrogen concentration was also determined using a Kjeldahl method.

Statistical analysis. Two-way analysis of variance (ANOVA) was used to assess treatment and year effects on yield components, grape and wine composition, and reserves storage in canes and roots using the SigmaStat 3.5 software package (Systat Software, Inc. San Jose, CA, USA). Mean separation was performed by Student-Newman-Keuls test ($P \leq 0.05$). Unless a significant year × VG treatment interaction occurred, values are presented as means pooled over years. Seasonal evolution of gas-exchange parameters, chlorophyll fluorescence and soluble solids are shown as means ± standard error.

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Results

Heat accumulation expressed as growing degree days (GDD, base 10 °C) from April 1st to September 30th was quite similar in 2010 and 2011, with 1770 and 1849 GDD, respectively. The rainfall summation over the same period was lower in 2011 (232 vs 366 mm in 2010). In both years, no visual symptoms of water stress or significant leaf yellowing were observed and no new leaves developed from neither primary nor lateral shoots after applying the treatment.

One week after VG treatment, the sprayed Sangiovese leaves showed a large reduction in leaf assimilation (A) and transpiration rate (E) (Figure 1B and 1C) followed by a rapid recovery of A and E to levels similar to those of C vines. The rapid recovery was probably the result of heavy rain recorded on 16 and 17 August (42 and 36 mm of rain, respectively) (Figure 1A). After the second VG application, A and E rates decreased again sharply demonstrating the effectiveness of VG in rapidly reducing stomatal opening upon treatment. Thereafter the capacity for carbon gain of VG treated leaves remained limited for a period of four weeks until harvest, when A again converged toward levels seen in C vines. Conversely, at harvest, sprayed leaves still had a significantly E than leaves of C vines (Figure 1B). The depression of E after VG application resulted in a significant increase of WUEi in sprayed relative to a C vines and was of similar duration, suggesting a lower loss of water in treated relative to C vines while both achieved a similar carbon gain (Figure 1C). Moreover, leaf temperature was not significantly modified by the VP treatment within the 1000 – 1100 hr time window (Figure 1B).

In regard to chlorophyll fluorescence parameters, Fv/Fm ratio measured in both primary and lateral leaves did not show any difference between treatments (Figure 2); whereas the area

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parameter, which defines the pool size of plastoquinone, the primary electron acceptor on the reducing side of PSII, showed a significant reduction in VG-treated primary and lateral leaves.

Regardless of year, VG applied post veraison above the cluster zone affected neither yield per vine nor average cluster and berry weight (Table 1). Similarly, no statistical difference was found in total acidity, must pH, total phenolics, and YAN between treatments, whereas in the VG-treated vines, Brix and the anthocyanin content were significantly reduced by about 1.2 Brix and 19%, respectively, as compared to C vines.

Dynamics of berry Brix showed that, regardless of season, accumulation slowed about 10 days after VG treatment (Figure 3). Berry fresh weight for VG treated vines did not change as compared to C vines in either year. The reduction in Brix found in VG-treated vines seems linked to impaired canopy photosynthetic capacity and/or limitation in sugar translocation from leaves to berries. Between VG application and harvest, the rate of Brix accumulation in the berries was, in fact, lowered from 0.31 Brix/day in C vines to 0.27 Brix/day in VG-treated vines in 2010 and from 0.29 Brix/day in the C vines to 0.23 Brix/day in VG-treated vines (Figure 3). At 2010 harvest, a reduction of 33 mg of soluble solids per berry was assessed in VG-treated vines compared to C vines, while in 2011 this limitation was equal to about 20 mg/berry.

Wines made from grapes of VG-treated vines after one year of aging had a 1% lower alcohol content than wines made from grapes of C vines, while total acidity, pH, total dry extract and phenolics including tannins were similar (Table 2). The concentration of anthocyanin was instead significantly reduced in the VG-treated wines (-15%), consequently the chromatic intensity of the wines was lowered, but without measurable variation in the color hue.

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Samples taken at the end of December, and analyzed for the alcohol-soluble sugars, starch and total nitrogen stored in the stems and roots, showed no concentration differences between treatments and years (Table 3).

Discussion

The antitranspirant VG applied at post veraison on the most functional leaves, namely fully expanded median and apical leaves from either primary and lateral shoots located above the cluster zone, significantly lowered the leaf assimilation and transpiration rates and optimized WUEi. Though, in 2010, heavy rain occurring soon after the first treatment likely caused premature wash off of the chemical and the spray had to be repeated 10 days later. As the reduction of stomatal conductance (g_s), A and E rates following VG spraying was accompanied by a marked reduction (from 60% to 70% as compared to leaves of C vines) of substomatal CO_2 concentration (182 to 218 ppm in control leaves versus values ranging from 112 to 165 ppm in VG treated leaves), it is apparent that this behavior was linked to some physical impairment of stomatal opening and function. The fact that the film-forming VG exerts a physical barrier to gas exchange, thus hampering the CO_2 entering the stomata and the water vapor leaving the stomata, was found almost 40 years ago on *Vicia faba* by Davenport et al. (1972), who also noted that under the transparent film the stomata were more open. Scanning electron micrographs on bean plants (Iriti et al. 2009) confirmed these results. Moreover, in peach, Davenport has reported that midday leaf water potential increased after an antitrasnpirant application as compared to unsprayed plants. Thus, maintenance of high moisture of the leaf tissue in conjunction with possible effects of light reflectance might explain why treated leaves did not heat up significantly

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in agreement with what it was found in a tropical plant using the same compound (Moftah and Al-Humaid 2005). It has to be pointed out that, in terms of light reflectance, VG behaves differently as compared to kaolin-based foliar reflectants which have proven to cause a significant reduction of leaf and/or berry temperature (Moftah and Al-Humaid 2005, Rosati 2007, Shellie and King, 2013) especially under limiting water supply.

At the same time, the Fv/Fm ratio was not modified emphasizing that photoinhibition did not occur at the PSII complex, while the observed reduction of the plastoquinone pool size complements a parallel reduction of the capacity of VG-treated vines to fix CO_2.

The significant improvement of intrinsic WUEi, extending from the time of VG application until the final stage of ripening, indicates a lower water loss through stomata for a similar carbon gain. This behavior occurred because the limitation in stomatal conductance of H_2O was proportionally higher than the depression of its assimilation rate.

A significant source limitation following VG spraying has been previously assessed in different species (Iriti et al. 2009, Francini et al. 2011) including the grapevine (Palliotti et al. 2010) and, quite remarkably, the above source limitation is reached without modifying neither the vine leaf-to-fruit ratio nor the cluster microclimate during ripening. This strategy of canopy management, applied late in the season, has been effective in reducing the pace of sugar accumulation in the berry, as compared to control vines, scoring a -1.2 Brix at harvest and lowering the alcohol content in the resulting wines by -1% vol. It can be recommended as a valuable cultural practice in viticultural areas where berry ripening takes place early during the hottest part of the season. In such a context, maturation is often associated with hot periods leading to an accelerated ripening process; pH and sugar concentration rise too high, yet doing so

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with a still unfinished or atypical phenolic and aromatic profile requiring grapes to hang longer on the canopy. For red grape cultivars, a premature harvest cannot, obviously, be proposed. Poor phenolic and aromatic maturity would increase the likelihood of higher extractability of proanthocyanidins from seeds, which, in turn, would lead to wines with excessive grassy and bitter tastes. In the absence of atypical phenolics, grassy flavours, bitter tastes, and unusual aromatic compounds in berries and wines we can state that the VG treatment does not produce effects similar to those of "premature harvest".

The removal of basal leaves is a common practice used to improve grape composition and health. In fact, Hunter et al. (1991) reported improvements of anthocyanin content in the berry and in wine quality after late defoliation. While high temperatures tend to accelerate grape ripening, too much heat leads to symptoms of berry shrivelling and sunburn, through excessive water loss and protein denaturation, respectively, as well as impairment of grape and wine color, and aromatic intensity (Lacey et al. 1991, Reynolds and Wardle 1993, Spayd et al. 2002, Mori et al. 2007). Therefore, in all areas where an increase of temperature during ripening is now likely, basal leaf removal cannot be applied without serious risk of lowering the quality of the grapes, including the aromatic potential due to a reduction of methoxypyrazine accumulation (Lacey et al. 1991, Scheiner et al. 2010), as well as the accumulation of terpenes (Belancic et al. 1997).

The late season source limitation induced by VG treatment proved to be effective, regardless of season, at delaying Brix accumulation in the berries without compromising the replenishment of the concentration of reserves in storage organs. We speculate that the photosynthesis recovery from just before until after harvest, has probably been sufficient to replenish the cane and root

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331 reserves of soluble sugars and starch. Depending on weather conditions, in central Italy, leaves
332 can retain a good photosynthetic rate up to 60-70 days after harvest.

333 Notably, the reduction of Brix accumulation in the berry achieved with VG treatment took
334 place without significant detriment to the accumulation of phenolic compounds, while berry
335 pigmentation was lowered. Regarding the latter, usually anthocyanins are negatively influenced
336 by high temperature and over-heating (Spayd et al. 2002). A recent paper from Kotseridis et al.
337 (2012) has shown that, in cv. Sangiovese, color accumulation was least when full leaf removal
338 was applied, while it improved when some leaf cover around the clusters was maintained. Our
339 experimental approach did not alter the microclimate around the fruiting zone since no leaves
340 were removed. Consequently, the reduction of color may be linked to a down-regulation of the
341 expression of genes involved in the synthesis of phenylalanine-ammonia-lyase (PAL), a key
342 enzyme engaged in phenylpropanoid and flavonoid biosynthetic pathways, following a strong
343 reduction of the source:sink ratio after VG application. Recently, Pastore et al. (2011) found that
344 this enzyme as well as the galactinol synthase, an important regulator of carbon partitioning,
345 were strongly up-regulated after applying a cluster thinning treatment, which caused a sharp
346 increase in the source:sink balance. On the other hand, Pirie and Mullins (1974) found that in red
347 grapes the sugar content could regulate the synthesis and accumulation of anthocyanins and,
348 likewise, Roubelakis-Angelakis and Kliewer (1986) and Vitrac et al. (2000) reported an increase
349 of PAL activity and accumulation of anthocyanins after treatments with sucrose and other sugars.

350 Moreover, since the stomata under the film formed by VG application remain open
351 (Davenport et al. 1972, Iriti et al. 2009), it is conceivable that the turgor of fruit cells remains
352 high and this may cause a decrease in sugar influx and ABA, which, in turn, could deactivate the

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353 expression of sugar transporters and anthocyanin pathway genes. In grapes it has been
354 demonstrated that exogenous ABA application increased the expression of genes coding for
355 anthocyanin synthesizing enzymes (Jeong et al. 2004) and activated invertase enhancing the
356 accumulation of glucose and fructose (Pan et al. 2005). Iriti et al. (2009) reported a drastic
357 reduction of ABA in VG treated as compared to untreated bean leaves (0.058 vs 0.218 mg/g).

358 The reduction of anthocyanins assessed in VG-treated vines is certainly undesirable if the
359 wines are intended for aging, but would be acceptable for young wines, rosé wines, or base
360 wines to be used for blending with dark colored wines. Indeed, Sangiovese is used to produce top
361 wines such as Brunello di Montalcino, Nobile di Montepulciano and Chianti, but is also widely
362 used for the production of light table wines, where a loss of 15-20% grape anthocyanins is not a
363 problem. In cultivar naturally rich in extractable anthocyanins (> 1 g/kg), such as Teroldego,
364 Lagrein, Enantio, Rebo, Marzemino, Croatina, Syrah, Merlot, Montepulciano, etc., a 15-20%
365 loss of anthocyanins in quite sustainable.

366
367 ## Conclusion

368 The application of the organic film-forming antitranspirant, Vapor Gard, to cv. Sangiovese
369 vines post veraison and above the cluster zone is a suitable strategy to delay ripening in the berry
370 as compared to non-treated vines. The technique proved to be effective and easy to apply method
371 to hinder berry sugaring and to obtain lower alcohol wines. Concurrently, apart from the 15-20%
372 loss of anthocyanins, this technique had no other negative impact on phenolic compounds,
373 organic acids, or pH in grape and wines, nor on the replenishment of the concentration of
374 carbohydrates in canes and roots under the conditions of this trial. To be effective in reducing the
375 accumulation of total soluble solids in the berries, the Vapor Gard emulsion should be applied

above the cluster zone at approximately 14-15 Brix and should completely wet the lower leaf surface where stomata are located.

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American Journal of Enology and Viticulture (AJEV). doi: 10.5344/ajev.2013.13015
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Table 1 Yield components and grape composition recorded at harvest in Sangiovese vines treated in post veraison with antitranspirant Vapor Gard (VG) or control (C). Data averaged over treatments and years in the absence of significant interactions.

	Treatment			Year		
Parameter	**C**	**VG**	**Sig.[a]**	**2010**	**2011**	**Sig.[a]**
Nodes retained (n°/vine)	9.3	9.9	ns	9.6	9.5	ns
Clusters (n°/vine)	10.0	10.5	ns	11.0	9.8	ns
Yield/vine (kg)	3.21	3.16	ns	3.34	3.12	ns
Cluster weight (g)	324	305	ns	306	318	ns
Berry weight (g)	2.32	2.29	ns	2.19	2.37	ns
Total soluble solids (°Brix)	24.0 a	22.8 b	*	22.8	23.3	ns
Titratable acidity (g/L)	6.5	6.2	ns	6.5	6.3	ns
Must pH	3.37	3.34	ns	3.28	3.36	ns
Anthocyanins (mg/cm^2 skin)	0.381a	0.308 b	*	0.345	0.325	ns
Total phenols (mg/cm^2 skin)	0.775	0.698	ns	0.751	0.714	ns
YAN (mg/L)[b]	124	123	ns	138	109	ns

[a]Means within rows denoted by different superscript letters are significantly different by the Student-Newman-Keuls test. *, ns indicate significance at $P \leq 0.05$ or not significant, respectively.
[b]Yeast-assimilable nitrogen content including ammonium salts and α-amino acids.

Table 2 Wine composition recorded over 2010-2011 vintages in Sangiovese vines treated with antitranspirant Vapor Gard in post veraison (VG) or control (C). Data averaged over treatments and years in the absence of significant interactions. Wines were analyzed one year after alcoholic fermentation in both years.

	Treatment			Year		
Parameter	**C**	**VG**	**Sig.[a]**	**2010**	**2011**	**Sig.[a]**
Alcohol (% v/v)	14.3 a	13.3 b	*	13.0	13.4	ns
Titratable acidity (g/L)	6.05	5.60	ns	6.12	6.01	ns
pH	3.47	3.56	ns	3.40	3.52	ns
Total dry extract (g/L)	22.8	21.6	ns	21.6	22.5	ns
Anthocyanins (g/L)	0.218 a	0.185 b	*	0.194	0.215	ns
Total phenolics (g/L)	1.53	1.42	ns	1.51	1.48	ns
Total tannins (g/L)	1.04	1.01	ns	1.11	1.15	ns
Color intensity ($OD_{420nm} + OD_{520nm}$)	9.2 a	6.1 b	*	8.1	7.9	ns
Color hue (OD_{420nm}/OD_{520nm})	0.67	0.73	ns	0.68	0.71	ns

[a]Means within rows denoted by different superscript letters are significantly different by the Student-Newman-Keuls test. *, ns indicate significance at $P \leq 0.05$ or not significant, respectively.

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Table 3 Cane wood and root reserves recorded in Sangiovese vines treated with antitranspirant in post veraison (VG) or control (C). Data averaged over treatment and year in the absence of significant interactions.

	Treatment			Year		
Parameter	**C**	**V G**	**Sig.[a]**	**2010**	**2011**	**Sig.[a]**
Cane wood						
Total nitrogen (% dw)	0.48	0.53	ns	0.59	0.42	ns
Alcohol-soluble sugars (mg/g dw)	229.0	213.9	ns	209.7	243.0	ns
Starch (mg/g dw)	59.0	55.8	ns	53.8	61.0	ns
Root						
Total nitrogen (% dw)	0.78	0.80	ns	0.88	0.71	ns
Alcohol-soluble sugars (mg/g dw)	120.7	132.4	ns	120.7	132.5	ns
Starch (mg/g dw)	193.2	177.8	ns	178.6	192.0	ns

[a]Means within rows denoted by different superscript letters are significantly different by the Student-Newman-Keuls test. ns indicates not significant. dw indicates dry weight.

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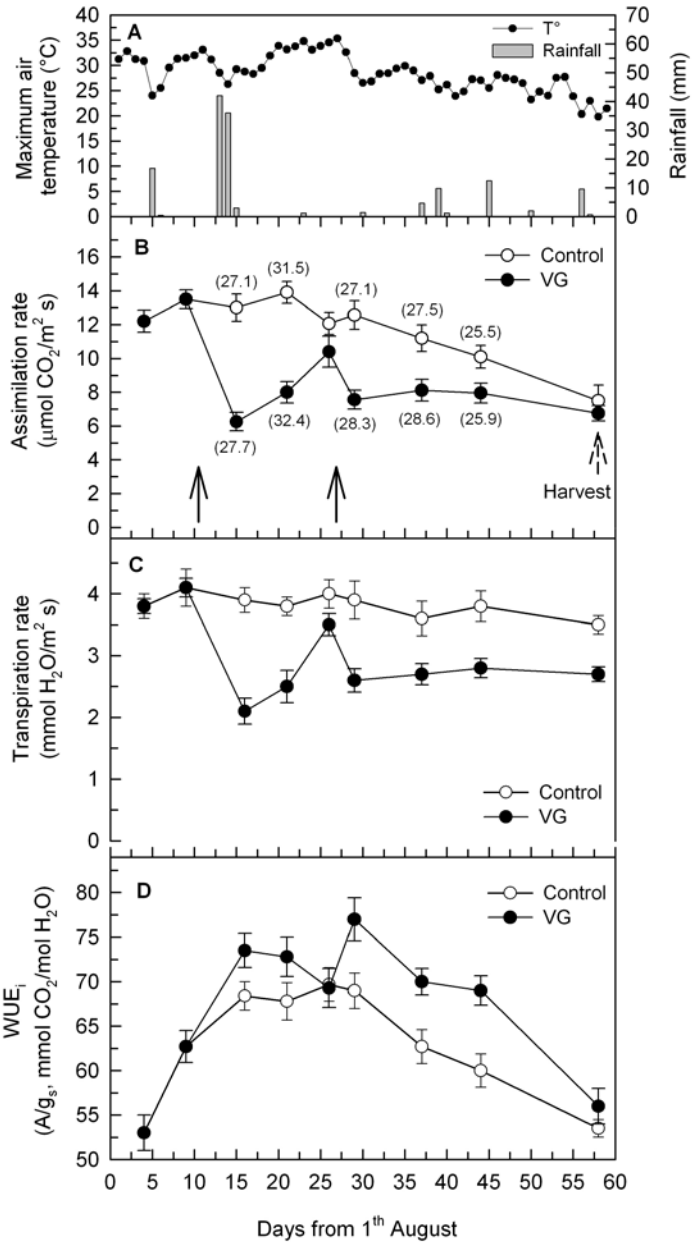

Figure 1 Seasonal trends of maximum air temperature and rainfall (A), assimilation rate (B), transpiration rate (C) and intrinsic water use efficiency (D) (WUEi calculated as assimilation/stomatal conductance ratio) recorded in 2010 on fully expanded median Sangiovese primary leaves sprayed twice with antitranspirant Vapor Gard (VG) at 2% or untreated. Bold arrows indicate the time of VG application. Data are mean ± SE (n = 12). In frame B, values between brackets are mean leaf temperatures recorded with an infrared thermometer concurrently with gas-exchange readings.

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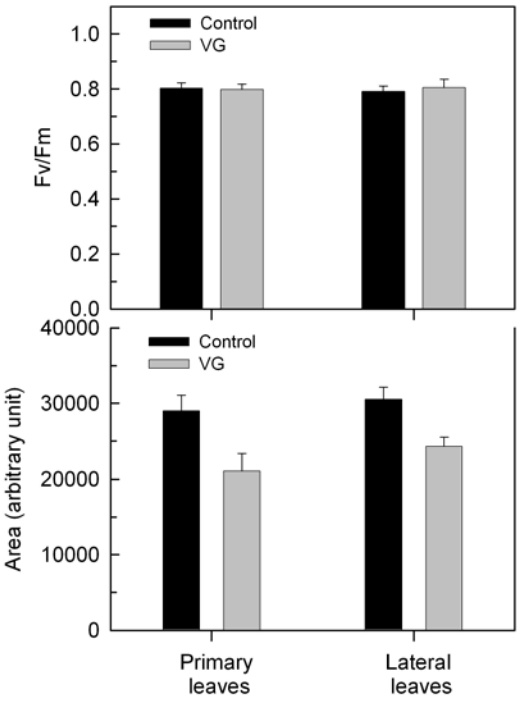

Figure 2 Maximal photochemical efficiency of PSII (Fv/Fm) and the pool size of plastoquinone on reducing size of PSII (Area) recorded in 2010 on median primary and lateral leaves of Sangiovese vines sprayed twice with antitranspirant Vapor Gard (VG) at 2% or untreated. Data are mean ± SE (n = 20).

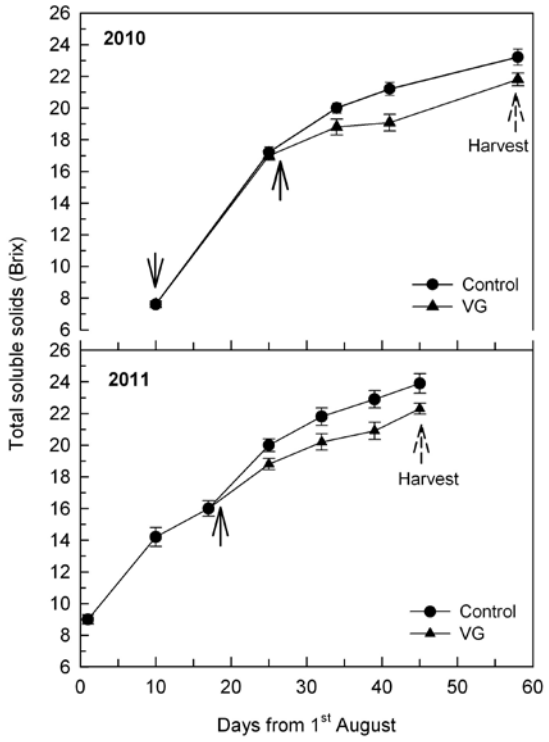

Figure 3 Seasonal trends of total soluble solids content recorded in 2010 and 2011 on Sangiovese vines treated in post-veraison with antitranspirant Vapor Gard (VG) at 2% or untreated. Data are mean ± SE (n = 6).