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- 3

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7 Abstract

8 The elimination of a certain number of leaves around bunches before veraison is a common practice in 9 vineyards to increase berries sunlight exposure, which, if acting in synergy with temperature increase, may 10 affect grape anthocyanin and flavonol composition and give rise to contradictory results. The aim of this study was to analyze the effect over two years of leaf removal on anthocyanin and flavonol composition at 11 harvest in four red Vitis vinifera L. varieties: Cabernet Sauvignon, Nero d'Avola, Raboso Piave and 12 13 Sangiovese, characterized by different anthocyanin and flavonol profiles. The concentration of total anthocyanins in berries did not vary among control and defoliated vines in all varieties in both vintages, 14 15 while total flavonols strongly increased after the treatment. Our results showed a genotype-dependent 16 response to leaf removal that may induce a strong enhancement of the di-substituted branch of the flavonoid 17 pathway, with consequences on anthocyanins and flavonols profile.

**18** Keywords: grapevine;defoliation;anthocyanins;flavonols;sun exposure; temperature.

19 1. Introduction

Defoliation is a common crop management practice on grapevine in many viticultural regions. The 20 21 elimination of a certain number of basal leaves conventionally applied in the fruiting zone from berry set to 22 veraison, enhances air circulation, berries sunlight exposure and increases berry temperature, while reduces Botrytis bunch rot infection and increases fungicide spray penetration (English et al., 1989; Stapleton and 23 24 Grant, 1992; Zoecklein et al., 1992). Especially the effects of veraison defoliation on grape composition have 25 been shown to be strongly influenced by intensity of treatment, genotype and climatic conditions (Downey et 26 al., 2006; Guidoni et al., 2007; Hunter et al., 1991, Matus et al., 2009). Leaf removal applied at veraison has 27 a strong impact on bunch microclimate and a limited impact on the vine source-sink balance due to the 28 lower photosynthetic activity of basal leaves compared to the intermediate and apical leaves at that stage

29 (Poni et al., 1994). In general after leaf removal, bunches are subjected to synergistic effects due to increase of light and temperature that, depending on the seasonal and climatic conditions, may affect grape 30 composition. Several authors, mainly reporting the effects of shading on grape color, agreed that low light 31 32 reduces anthocyanin and other flavonoid concentrations, while increasing light increases the flavonoid content of grapes (Crippen and Morrison, 1986 a, b; Dokoozlian and Kliewer, 1996; Hale and Buttrose, 33 34 1974; Hunter et al., 1991; Iland, 1988; Kliewer and Lider, 1968; Kliewer, 1970; Matus et al., 2009; 35 Zoecklein et al., 1992). Further investigations into the effects of increasing light exposure on grape color 36 gave rise to contradictory results. Some studies reported that high light levels resulted in decreased 37 anthocyanin levels (Bergqvist et al., 2001; Pastore et al., 2013; Spayd et al., 2002), while in other cases no 38 change was observed in total anthocyanin concentration (Downey et al., 2004; Haselgrove et al., 2000; Price 39 et al., 1995; Spayd et al., 2002). When exposure to sunlight is associated with excessive berry temperature, 40 as occurs in warm conditions, this may often lead to berry sunburn that has a negative impact on the color of 41 some red berry grapevine varieties (Kliewer and Torres, 1972; Mori et al., 2005; Mori et al., 2007). It has 42 been pointed out that the lower anthocyanin content in berries under high temperature reflects the combined 43 impact of reduced biosynthesis and increased degradation in which the role of peroxidase enzymes in 44 anthocyanin catabolism is probably involved (Movahed et al., 2016). The modification of bunch light 45 exposure around veraison can also affect anthocyanin composition. As is well-known, grape anthocyanins are based on cyanidin, peonidin, delphinidin, petunidin and malvidin that are glycosylated at the third 46 47 position of the B ring. The glucoside portion can be esterified with acetyl and coumaroyl compounds, giving origin to the different anthocyanins commonly found in V. vinifera varieties (Mazza, 1995). Several 48 49 researches have shown shifts in anthocyanin composition after bunches microclimatic variation, with an 50 increase in the di-substituted anthocyanin concentration (cyanidin and peonidin) in shaded bunches giving 51 rise to an increased di-substituted to tri-substituted anthocyanins (delphinidin, petunidin and malvidin) ratio 52 (Downey et al., 2004; Ristic et al., 2007; Spayd et al., 2002). Although other authors showed opposite results 53 since bunch light exposure increased the proportion of di- respect to tri-substituted anthocyanins (Chorty et 54 al., 2010; Guidoni et al., 2008; Tarara et al., 2008), there is agreement in the literature that greater bunch shading results in a shift toward acylated anthocyanins (Downey et al., 2004; Le Guan et al, 2016). These 55 56 contradictory results also in terms of composition may be probably ascribe again to both light and temperature effects, which frequently coexist, playing a conflicting role especially in warm climatic conditions. Cabernet Sauvignon berries under high temperature showed an anthocyanin shift with a decreased proportion of di-substituted anthocyanins (Mori et al., 2005; Mori et al., 2007; Tarara et al., 2008), which are considered less stable than tri-substituted ones at high temperature. In Sangiovese these results are only slightly confirmed. In fact, berries ripened under high temperature showed similar profiles at harvest with respect to control berries, but the proportional depletion of malvidin 3-glucoside was the lowest compared to all the other glycosylate anthocyanin forms (Pastore et al., 2013).

Sunlight is known to enhance flavonol accumulation in berries (Downey et al., 2006) and recent papers focused on the effects of solar UV radiation, suggest a strong positive correlation between illumination and flavonol levels, reflecting their role as UV protectants (Carbonell-Bejerano et al., 2014, Price et al., 1995; Spayd et al., 2002). High accumulation of flavonols was also observed in different varieties subjected to leaf removal compared to controls (Lemut et al., 2013; Pereira et al., 2006) and this was also supported by an increase in flavonol synthase gene expression in the berries (Pastore et al., 2013).

Although in Sangiovese berries a shift in flavonol composition was registered after veraison defoliation due to higher accumulation of quercetin and kaempferol than myricetin compared to control berries (Pastore et al., 2013), studies on other cultivars have shown that the abundance of all flavonol compounds increases with the same intensity following defoliation (Spayd et al., 2002).

Considering that the profile of anthocyanins (Mattivi et al., 2006) and flavonols (Downey et al., 2003) in each variety are relatively stable over seasons and that distinctive varietal responses to light and temperature may be observed in flavonol and anthocyanin accumulation and composition in berry skin (Mattivi et al., 2006), the aim of this study was to analyze anthocyanin and flavonol composition of berries at harvest by describing the response of four red varieties, characterized by different anthocyanin and flavonol profiles, to veraison leaf removal over two years.

80 **2.** Material and methods

2. Material and methods

The trial was conducted in 2008 and 2009 on adult *Vitis vinifera L*. Cabernet Sauvignon, Nero d'Avola, Raboso Piave and Sangiovese vines grafted to SO4, in a vineyard with no irrigation system located in Bologna, Italy (44°30'N, 11°24'E), with north–south oriented rows. Vine spacing was 1.0 m x 3.0 m and the training system was a vertical shoot positioned spur pruned cordon (12 buds per vine), with cordon height at 1.0 m above the ground and canopy height of about 1.3-1.4 m. Pest management followed local practices in the Emilia Romagna Region. Each vine in the trial was uniformed for bud load and bunch number at flowering. Nine vines per treatment in three blocks were selected in a single uniform row and each vine was randomly assigned to the following treatments: a) control (C), no treatment; b) veraison defoliation (D), hand defoliation of six basal leaves at veraison. In the defoliation treatments, any laterals growing in the 6 basal node of the main shoot were also removed.

91 Defoliation treatments and harvest were performed according to the berry ripening trend in each cultivar and92 year as reported in Table 1.

Weather data (mean daily air temperature and rainfall) were recorded from April to September in both years,by a meteorological station located close to the experimental site.

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# 2.1. Agronomic parameters at harvest

At harvest the number and weight of bunches per vine were measured. For each bunch we determined the
surface areas infected by Botrytis and damaged by sunburn. During winter, the wood pruned from each vine
was weighed.

# 99 **2.2. Temperature monitoring**

Berry skin temperature was monitored in 2008 and 2009 in four selected bunches on control and defoliated 100 vines of each tested variety. For each treatment, temperature data were collected from stage 33 (beginning of 101 102 bunch closure, berries touching, according to Lorenz et al., (1995) until harvest and this fluctuated for each 103 cv: Cabernet Sauvignon and Nero d'Avola from JD 226 to 276 in 2008 and from JD 217 to 271 in 2009; Raboso Piave from JD 226 to 287 in 2008 and from JD 225 to 281 in 2009; Sangiovese from JD 211 to 265 104 105 in 2008 and from JD 210 to 261 in 2009. Eight T-type thermocouples (RS components, MI, Italy) were 106 positioned in the sub-cuticular tissues of the berry skin. Four were positioned on two different bunches, two 107 on the east side and two on the west side of the cordon. For each side, one thermocouple was inserted in a 108 berry located in the external part of the bunch and the other in the internal part. Each probe was then 109 connected to a CR10X data logger (Campbell Scientific Ltd., Leicestershire, UK) that registered temperature data every 15 minutes. In three days during August in 2008 and in 2009 for each bunch, the percentage of 110 bunch exposure was visually estimated in three moments of the day: in the morning (9.00-9.30 a.m.), when 111 the sun position is at its Zenith (1.30- 2.00 p.m.) and in late afternoon (5.30-6.00 p.m.). 112

## 2.3 Biochemical analysis

For each treatment, we collected 40 berries from each of the three vines in each block at harvest. The samples were divided into two parts. Twenty berries were weighed and immediately tested for ripening by crushing and filtering the must through a strainer for the evaluation of °Brix, titratable acidity and pH. The remaining 20 berries were used to extract anthocyanins and flavonols for HPLC analysis according to Mattivi et al. (2006).

119 **2.4 Statistical analyses** 

120 Yield components and grape composition parameters were processed for each variety by analysis of variance 121 using the mixed procedure available in SAS v9.0 (SAS Institute, Inc., Cary, NC, USA). Treatment 122 comparisons were analyzed using the Tukey test with a cut-off at  $P \le 0.05$ .

To compare anthocyanin and flavonol composition in different varieties, treatments and years multivariate analysis was applied on the data of each compound. An exploratory principal component analysis was performed separately on anthocyanins and flavonols to point out differences and any gradients.

126 **3. Results** 

# 127 **3.1.** Climatic data and impact of defoliation on berry skin temperature

The weather during 2008 and 2009 was on the average of the area and total rainfall from April through September was very similar in the two seasons (320 mm and 317.4 mm respectively). Mean and maximum temperature (Figure 1) during the growing season in 2008 ((19.8 °C and 35.9 °C respectively) was lower than in 2009 (20.9 °C and 36.8° C respectively) and this reflected on total active heat summation calculated using base 10 °C days from April through September (1758 °C in 2008 and 2006 °C in 2009).

Sangiovese was the earliest variety for both veraison and harvest, while Raboso Piave was the latest. It should be noticed that the number of days between veraison and harvest was similar among varieties and ranged from 50 to 61.

We monitored the berry skin temperature from the application of leaf removal until harvest in the control and defoliated vines of each variety. The berries of all tested varieties in the control treatment were exposed to temperatures >30 °C for less time than in the defoliated samples with differences between the two treatments ranging from up to 70 hours to a minimum of 31 hours (for the same cv Sangiovese respectively in 2009 and 2008, Table 2). In both treatments, the number of hours with berry temperature above 30 °C was higher in 141 2009 than in 2008. The estimation of the percentage of bunch exposure after defoliation showed in both142 years an increase of around 20 % in the daily average (Table 2).

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- 144

# **3.2 Vegetative and productive traits**

There were only minor differences between the two years in vegetative and productive measurements at 145 harvest following the leaf removal in all tested varieties. Starting from a uniform bunch number per vine, no 146 147 differences were detected after defoliation in yield per vine or berry mass at harvest, for either variety or 148 year. Vintage had an influence on berry mass in all varieties with higher values in 2009 than 2008 and only 149 in Cabernet Sauvignon, an increase in yield per vine was registered in the second year (Table 3). In 2009 150 Raboso Piave and Sangiovese showed a significant increase in the percentage of sunburned bunches on defoliated compared with control vines, whereas only Nero d'Avola had significantly fewer bunches 151 attacked by Botrytis on defoliated vines in 2009 (Table 3). It should be noted that the untreated Nero d'Avola 152 153 was the most sensitive cultivar to *Botrytis*, showing the highest level of attack in 2009. Surprisingly, Sangiovese cv, despite a strong *Botrytis* incidence in 2009, did not respond to leaf removal with significant 154 155 rot reduction (Table 3). Sugar concentration in must at harvest was not affected by veraison defoliation, but differed in the two vintages, while total acidity and pH in Cabernet Sauvignon, Nero d'Avola and 156 Sangiovese were reduced and increased respectively by defoliation (Table 3). 157

158

# **3.3. Anthocyanins and flavonols**

# 159 **3.3.1. Univariate analyses**

The concentration of total anthocyanins in the berries (mg/g) did not vary among treatments at harvest in both vintages and in all varieties (Table 4). In Sangiovese, where the profile showed only traces of acetate and coumarate anthocyanins, the total concentration corresponded mainly to glycosylate anthocyanins. In Cabernet Sauvignon, Nero d'Avola and Raboso Piave the concentration of glycosylate, acetate and coumarate anthocyanins was not modified following leaf removal treatments compared to the control (Table 4).

The di-substituted to tri-substituted anthocyanins ratio significantly increased with defoliation in Nero d'
Avola and Sangiovese cultivars. Raboso Piave showed a similar tendency but without significant differences

between treatments, while Cabernet Sauvignon revealed an opposite trend in each year and a strong Year xTreatment interaction effect.

There were significant differences between vintages in Cabernet Sauvignon and Raboso Piave anthocyanin
concentrations, with the highest level recorded in 2008. Moreover, Raboso Piave showed a clear Year x
Treatment interaction for all measured compounds except acetate anthocyanins (Table 4).

The concentration of total flavonols at harvest increased significantly in defoliated berries of all varietiescompared to controls in both years (Table 4).

Each variety showed a characteristic composition in control berries as quercetin is the main component in Sangiovese, myricetin is in Nero d'Avola, while Raboso Piave and Cabernet Sauvignon showed similar proportions of quercetin and myricetin. The total flavonols increase was quite similar in all varieties but each flavonol compound showed a different increment following leaf removal. The highest proportional increase concerned quercetin in Raboso Piave (Table 5).

# 180 **3.3.2. Multivariate quantitative data**

Comprehensive analysis of the total data set of anthocyanin (Figure 2) and flavonol (Figure 3) concentrations in mg per gram of berry skin of the varieties Cabernet Sauvignon, Nero D'Avola, Raboso Piave and Sangiovese in 2008 and 2009, was conducted, applying an exploratory principal component analysis separately on anthocyanins and flavonols to evaluate the distribution of single observations and rank the data.

As presented in Fig. 2, 90% of the variability due to anthocyanin concentration is accounted for the two discriminant functions. The first one accounts for 55% of the information and is mainly correlated with the concentration of cyanidin 3-glucoside and peonidin 3-glucoside on one side and malvidin 3-glucoside on the other. Sangiovese and Raboso Piave are close to each other and clearly separated from Nero d'Avola, which is near Cabernet Sauvignon, according to the first component (PC1), by bunching at positive and negative PC1 values, respectively (Figure 1). The second function (PC2) accounts for 35% of the variability and seems to be responsible for the differences between treatments and years.

Raboso Piave shows high variability and treatments are not clearly separated, while it is possible to identify aseparation in Sangiovese between defoliated and control vines independently of the season. In Cabernet

Sauvignon the two years appear grouped and in Nero d'Avola the two treatments are distinguished mainlyaccording to the second component (PC2).

197 The same approach was applied for flavonol concentration and the results are reported in Figure 3 where the 198 two discriminant functions account for more than 99% of the variability. The PC1 accounts for 70.9% of the 199 variability mainly linked to the variation in quercetin. For all varieties, it is possible to separate the control 200 from defoliated vines according to the PC1.

The second function (PC2), which accounts for 28.8% of the variability, is dependent mainly on myricetin. According to this function, the observations allow genotype separation with Nero d'Avola and Cabernet Sauvignon mainly matched with positive values, while Sangiovese and Raboso Piave with the negative values of PC2 (Figure 3).

205 **4. Discussion** 

# **4.1. Vegetative and productive traits**

Leaves removal around bunches at veraison, implying modification in light and temperature exposure, is a powerful and widely-used strategy to improve berry bunch microclimate and to reduce rot susceptibility. The responses in berries anthocyanin and flavonol accumulation and composition following veraison defoliation could be very different and dependent on several factors including climatic conditions, leaf removal intensity, temperature increase and genotype (Bergvist et al., 2001; Spayd et al., 2002).

The four varieties included in this research, Sangiovese, Cabernet Sauvignon, Nero d'Avola and Raboso Piave, as expected did not modify vegetative and yield traits as a result of veraison leaf removal. In fact veraison defoliation, with the elimination of already senescent basal leaves, may have a limited effect on the vine source-sink balance and on berries sugar accumulation (Bledsoe et al., 1988; Pastore et al., 2013; Percival et al., 1994).

On the other hand, veraison defoliation usually had strong impact on bunches microclimatic conditions. In our study actually we estimated an average daily increase of 20% of bunch exposure in defoliated compared to control vines, in both years, while the berry temperature difference between the treatments within all cultivars and years, expressed as number of hours in which the berries overcome 30°C from veraison to harvest, never exceeded 70 hours. Moreover, during the two seasons the maximum air temperature was around 36.5 °C.

223 Although we did not measure the individual malic and tartaric acid fractions, we could argue that the 224 decrease in total acidity registered following defoliation in three of the four varieties, Cabernet Sauvignon, 225 Nero d'Avola and Sangiovese, independently of sugar concentration, is correlated to the thermal increase 226 due to higher bunch exposure to light, since light is not known to influence malic and tartaric acid accumulation in grape tissues (Crippen and Morrison, 1986 a; Kliewer and Lider, 1968). On the contrary, 227 temperature has been known for some time to have significant effects on berry acidity, accelerating the 228 229 breakdown of malic acid (Lakso and Kliewer, 1975; Kliewer and Schultz, 1964). This hypothesis is 230 supported by the significant differences registered in the total acidity between 2008 and 2009 in these 231 varieties. As previously described, the temperature during the 2009 season was higher than in 2008 and 232 consequently the acidity was lower in the second year. The fact that the acidity concentration in Raboso 233 Piave did not decrease as a result of defoliation treatment, suggests a cultivar-dependent thermal response of 234 acidity, as previously reported on different cultivars subjected to increased temperature regime (Bergqvist et 235 al., 2001; Sadras et al., 2013).

The overall increase in berry mass registered in all four varieties in 2009 could be linked to the higher rainfall recorded in July of that year compared to the same period in 2008, which may have conditioned the berry cell division stage of growth and final berry mass.

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#### 4.2. Anthocyanins and Flavonols

The concentration of total anthocyanins in the berries did not vary among treatments at harvest in both vintages in all varieties, so it could be assumed that light conditions were appropriate for anthocyanin biosynthesis in control vines and no improvement arose from bunch light exposure at veraison. At the same time in the current study, the temperature increase following leaf removal recorded in both years did not induce a negative impact on the berry color. On the contrary, anthocyanins reduction in berries under temperature rise is reported in several articles (Downey et al., 2006; Kliewer and Torres, 1972; Mohvaed et al., 2016; Mori et al., 2005; Mori et al., 2007).

The mechanism that suppresses anthocyanin accumulation in berry skins under high-temperature ripening conditions is not completely clear, but recent evidence suggests that the low anthocyanin content in berries ripened at high temperature reflects the combined impact of reduced biosynthesis and increased degradation verified in Sangiovese (Movahed et al., 2016) and in other varieties (Yamane et al., 2006; Mori et al., 2007). 251 At least for Sangiovese cv which usually shows great sensitivity to thermic condition variation, we may 252 ascribe the lack of response in terms of anthocyanins concentrations to several reasons: first in our study the 253 temperature condition of control berries already reached a high level of heat accumulation, corresponding to 254 more than 250 hours over 30°C degree during the ripening period, secondly the temperature differences between control and defoliated berries were quite low with a maximum of 70 hours over 30 °C. In fact in a 255 previous research a strong anthocyanins reduction, in Sangiovese berries ripened at more than 140 hours 256 257 over 30 °C in comparison to control, was found (Mohaved et al., 2016). The multivariate approach applied 258 on the complete anthocyanin concentration data sets allowed the varieties to be differentiated independently 259 of treatments and seasons. The association of Sangiovese and Raboso Piave and their separation from 260 Cabernet Sauvignon and Nero d'Avola is mainly driven by their typical anthocyanin profile, featuring a 261 higher concentration of peonidin 3-glucoside and cyanidin 3-glucoside and a lower concentration of malvidin 262 3-glucoside in comparison to the other two varieties. In Sangiovese, the effect of veraison defoliation on 263 anthocyanin concentration was stable between the two vintages, causing a clear separation between control 264 and defoliated vines due to the increase in the di-substituted to tri-substituted ratio. Instead, in Raboso Piave 265 the effect of veraison defoliation on total anthocyanin concentration seems to be vintage dependent, with 266 opposite behavior in each year, but with di/tri ratio followed a general tendency to increase under defoliation 267 treatment.

268 Cabernet Sauvignon and Nero d'Avola share a similar anthocyanin profile characterized by a high 269 concentration of the three forms of malvidin present in grapevine and low level of di/tri ratio and showed a 270 general higher stability to treatments and seasons compared to Sangiovese and Raboso Piave. Despite this, 271 the Nero d'Avola response to veraison defoliation showed an increasing trend of the di/tri ratio as verified in 272 Sangiovese, while not steady effects were registered in Cabernet Sauvignon according to multivariate 273 analyses. In fact, it showed a more stable behavior under the defoliation treatments but revealed slight 274 variations according to season with lower anthocyanins concentration. This last aspect is likely due to the 275 connection between sugar accumulation in berry flesh and anthocyanin concentration in the skin, previously 276 pointed out in several papers regarding in vivo and in vitro experiments (Gollop et al., 2002; Pirie and 277 Mullins, 1976; Roubelakis-Angelakis and Kliewer, 1986). In fact Cabernet Sauvignon showed a general delay in sugar accumulation in 2009 clearly linked with the higher yield level, which may be responsible forthe lower anthocyanins level recorded in that year.

On the contrary in Raboso Piave, a late ripening variety, since yield level was similar in the two seasons, the lower anthocyanin concentration at harvest in 2009 may be attributed to a strong sensitivity to temperature as shown by the high level of sunburned berries registered in defoliated vines in the season with highest air temperature (Table 3). Although we did not sample sunburned berries, which often exhibit poor color development (Krasnow et al., 2010), we may argue that the same conditions of higher irradiance and temperature that induced the sunburn may be responsible for a decrease in anthocyanins as previously reported in several red berry varieties (Pastore et al., 2013; Spayd et al., 2002).

287 The increase of di/tri ratio after defoliation in Nero d'Avola, Sangiovese and partially in Raboso Piave 288 cultivars seems to disagree with previous findings referring to both light and temperature increases effects 289 (Mori et al., 2005, Tarara et al, 2008), or with other research reported that light exclusion induces an increase 290 of the di/tri ratio compared to control bunches (Downey et al., 2004). It should be considered that in our 291 experimental vineyard, bunches of control vines were naturally shaded and that conditions were not 292 comparable to the one obtained through the light exclusion imposed in the cited research. Moreover, the 293 increase of di-substituted anthocyanins we registered is not in agreement with their supposed lower stability 294 at high temperature due to the chemical degradation hypothesis reported by several authors (Cohen et al, 295 2012; Mori et al., 2007). Anyway our biochemical results were supported by other researches in Sangiovese 296 (Pastore et al., 2013) and in Nebbiolo (Guidoni et al., 2008). Moreover the hypothesis that climate variables, 297 such as light or temperature, could repress or enhance the biosynthesis of di-substituted or tri-substituted 298 anthocyanins is confirmed by molecular studies on Sangiovese and Kyoho grapes, in which specific 299 responses were recorded on main genes at the split-up point of the biosynthesis of di- and tri-substituted 300 anthocyanins (F3'H and F3'5'H) under light exposure or high temperature (Azuma et al., 2012; Movahed et 301 al., 2016; Pastore et al., 2013). Since we did not separate the effect of temperature and light, it is not clear 302 which of them could be responsible.

303 Despite the total flavonol concentration appeared very variable among the four cultivars in the study, it was 304 very different between control and defoliated vines in all varieties in both vintages. The higher bunch 305 exposure induced by leaf removal in comparison to control berries resulted in an increase of total flavonols 306 in all varieties, and this effect was more evident in 2009 than in 2008. Sunlight is known to enhance flavonol 307 accumulation in berries (Downey et al., 2006) and there is a strong positive correlation between illumination 308 and flavonol levels, reflecting their role as UV protectants (Pastore et al., 2013; Price et al., 1995; Spayd et 309 al., 2002). Moreover, coherently with our results, several papers reported that the level of flavonols in berries was almost negligible when they had not been exposed to light and that the subsequent exposure of those 310 tissues to sunlight determined the rise of flavonol accumulation after the increase in the expression of the 311 312 gene encoding flavonol synthase (Downey et al., 2004; Pastore et al., 2013). Previous research on Sangiovese showed that in similar light conditions, temperature increase caused strong flavonol 313 314 concentration reduction, suggesting a negative effect of high temperature on flavonol synthase (Mohaved et 315 al., 2016). In our research, the temperature rise was associated with an increase in light exposure and flavonol concentration, revealing that the influence of light is dominant on the synthesis of these compounds 316 compared to the thermal effect, at least under the observed temperature range. 317

As previously described the total content and pattern of flavonols is highly variable across genotypes and our results confirm that red grape varieties like Sangiovese synthesize mainly di-substituted derivatives like quercetin (Flamini et al., 2013). In control vines, Cabernet Sauvignon and Raboso Piave have similar proportions of myricetin and quercetin, while Nero d'Avola exhibits a high concentration of myricetin. Kaempferol is present in no or low concentration in all the varieties included in this study.

The multivariate approach applied on the complete flavonol concentration data sets separated the control from defoliated vines due to the significant increase in the latter, mainly driven by the rise of quercetin which appears the compound more responsive to light, as previously reported by other authors on Tempranillo (Carbonell-Bejerano et al., 2014). In our experimental conditions, this response drives towards a reduction in the differences between the original flavonol profiles of the four varieties.

## 328 5. Conclusion

In our conditions, where control berries were naturally shaded and subjected to quite high level of temperature which overcome 30° C for several hours, the response of four varieties to veraison defoliation in terms of anthocyanins accumulation remain unclear. We could not exclude that the similar anthocyanin content between treatments in all varieties is caused by the higher berry temperature on defoliated vines, which may have reduced anthocyanin concentration counterbalancing the supposed enhancement due to lightexposure increase.

335 The strong increase in flavonol concentration in all varieties under defoliation suggests that the influence of light is dominant on the synthesis of these compounds compared to the thermal effect and that they may 336 represent a marker of berries sun exposure. Furthermore, the stimulation of the synthesis of quercetin, 337 derived from the di-substituted branch of the flavonoids pathway, also triggers the production of cyanidin, 338 339 suggesting that defoliation may induce, according to genotypes, a specific response at the split-up point of 340 the biosynthesis of di- and tri-substituted flavonoids with consequences on the profile of both anthocyanins 341 and flavonols. Based on the overall results obtained from univariate and multivariate analyses it appears that 342 the relationship between anthocyanin and flavonols and veraison defoliation is very complex and depends on many factors including genotype and the synergistic or antagonistic effect of different levels and extent of 343 344 both temperature and light intensity experienced by the berries.

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## **Figure Captions:**

Figure 1. Seasonal trends (1 April–30 September) of diurnal air mean, maximum and minimum temperature recorded close to the trial site in (A) 2008 and (B) 2009. Vertical bars indicate daily rainfall. The Degree Days and total rainfall from 1 April to 30 September were, respectively, 1768 and 332 mm in 2008 and 2006 and 317 mm in 2009.

Figure 2. Principal component analysis of the total data set of anthocyanin concentrations (mg per 351 gram of berry skin) of control (red) and defoliated (green) of Cabernet Sauvignon, Nero D'Avola, 352 353 Raboso Piave and Sangiovese in 2008 (empty) and 2009 (full). The name of single anthocyanin compound responsible of cultivars, treatments and seasons scattering, are represented with arrows 354 and asterisks. In particular each name correspond to: Malv-3-G, malvidin 3-glucoside; Malv3-G ac, 355 356 malvidin-3-acetyl-glucoside; Malv 3-G coum, malvidin 3-coumaroyl glucoside; Del 3-G, delphinidin 3-Glucoside; Peo3-G, peonidin 3-glucoside; Peo3-G coum, peonidin 3-coumaroyl 357 glucoside; Cyan 3-G, cyanidin 3-Glucoside. 358

359	Figure 3. Principal component analysis of the total data set of flavonols concentrations (mg per
360	gram of berry skin) of control (red) and defoliated (green) of Cabernet Sauvignon, Nero D'Avola,
361	Raboso Piave and Sangiovese in 2008 (empty) and 2009 (full). The name of single flavonol
362	compound (myricetin, kaempherol and quercetin) responsible of cultivars, treatments and seasons
363	scattering, are represented with arrows and asterisks.
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374	This work was partially supported by the University of Bologna PhD grant
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377	References
378	Azuma, A., Yakushiji, H., Koshita, Y., Kobayashi, S., 2012. Flavonoid biosynthesis-related genes
379	in grape skin are differentially regulated by temperature and light conditions. Planta. 236(4),1067-
380	1080.
381	Bergqvist, J., Dokoozlian, N., Ebisuda, N., 2001. Sunlight exposure and temperature effects on
382	berry growth and composition of Cabernet Sauvignon and Grenache in the Central San Joaquin
383	Valley of California. Am. J. Enol. Vitic. 52 (1), 1-7.
384	Bledsoe, A.M., Kliewer, W.M., Marois, J.J., 1988. Effects of timing and severity of leaf removal on
385	yield and fruit composition of Sauvignon blanc grapevines. Am. J. Enol. Vitic. 39, 49-54. 14

- Carbonell-Bejerano, P., Diago, M. P., Martínez-Abaigar, J., Martínez-Zapater, J. M., Tardáguila, J.,
  Núñez-Olivera, E., 2014. Solar ultraviolet radiation is necessary to enhance grapevine fruit ripening
  transcriptional and phenolic responses. BMC plant biology, 14(1), 1.
- Chorty, E., Guidoni, S., Ferrandino, A., Novello, V., 2010. Effect of different bunch sunlight
  exposure levels on ripening and anthocyanin accumulation in Nebbiolo grapes. Am. J. Enol. Vitic.
  61(1), 23-30.
- Cohen, S.D., Tarara, J.M., Gambetta, G.A., Matthews, M.A., Kennedy, J.A., 2012. Impact of
  diurnal temperature variation on grape berry development, proanthocyanidin accumulation, and the
  expression of flavonoid pathway genes. J. Exp. Bot. 63(7), 2655-2665.
- Crippen, D.D., Morrison, J.C., 1986 a. The effect of sun exposure on the compositional
  development of Cabernet Sauvignon. Am. J. Enol. Vitic. 37, 235-242.
- Crippen, D.D., Morrison, J.C., 1986 b. The effect of sun exposure on the phenolic content of
  Cabernet Sauvignon berries during development. Am. J. Enol. Vitic. 37, 243-247.
- Dokoozlian, N.K., Kliewer, W.M., 1996. Influence of light on grape berry growth and composition
  varies during fruit development. J. Amer. Soc. Hort. Sci. 121(5), 869–874.
- 401 Downey, M.O., Dokoozlian, N.K., Krstic, M.P., 2006. Cultural practice and environmental impacts
- 402 on the flavonoid composition of grapes and wine: a review of recent research. Am. J. Enol. Vitic.
  403 57, 257–268.
- 404 Downey, M.O., Harvey, J.S., Robinson, S.P., 2003. Synthesis of flavonols and expression of
- 405 flavonol synthase genes in the developing grape berries of Shiraz and Chardonnay (Vitis Vinifera
- 406 L.). Aust. J. Grape Wine Res. 9, 110-121.
- 407 Downey, M.O., Harvey, J.S., Robinson, S.P., 2004. The effect of bunch shading on berry 408 development and flavonoid accumulation in Shiraz grapes. Aust. J. Grape Wine Res. 10, 55-73.
- 409 English, J.T., Thomas, C.S., Marois, J.J., Gubler, W.D., 1989. Microclimates of grapevine canopies
- 410 associated with leaf removal and control of Botrytis bunch rot. Phytopathology. 79, 395-401.

- Flamini, R., Mattivi, F., Rosso, M.D., Arapitsas, P., Bavaresco, L., 2013. Advanced knowledge of
  three important classes of grape phenolics: anthocyanins, stilbenes and flavonols. Int. J. Mol. Sci.
  14(10), 19651-19669.
- Gollop, R., Even, S., Colova-Tsolova, V., Perl, A., 2002. Expression of the grape dihydroflavonol
  reductase gene and analysis of its promoter region. J Exp Bot. 53(373), 1397-1409.
- 416 Guan, L., Dai, Z., Wu, B.H., Wu, J., Merlin, I., Hilbert, G., Renaud, C., Gomès, E., Edwards, E.,
- 417 Li, S.H., Delrot, S., 2016. Anthocyanin biosynthesis is differentially regulated by light in the skin
- and flesh of white-fleshed and teinturier grape berries. Planta. 243(1), 23-41.
- Guidoni, S., Ferrandino, A., Novello, V., 2008. Effects of seasonal and agronomical practices on
  skin anthocyanin profile of Nebbiolo grapes. Am. J. Enol. Vitic. 59(1), 22-29.
- Guidoni, S., Ferrandino, A., Novello, V., 2008. Effects of seasonal and agronomical practices on
  skin anthocyanin profile of Nebbiolo grapes. Am. J. Enol. Vitic. 59 (1), 22-29.
- Hale, C.R., Buttrose, M.R., 1974. Effect of temperature on ontogeny of berries of *Vitis Vinifera* L.,
  cv. Cabernet Sauvignon. J. Amer. Soc. Hort. Sci. 99, 390-394.
- 425 Haselgrove, L., Botting, D., Van Heeswijck, R.V., Høi, P.B., Dry, P.R., Ford, C., Iland, P.G., 2000.
- 426 Canopy microclimate and berry composition: the effect of bunch exposure on the phenolic
- 427 composition of Vitis vinifera L. cv. Shiraz grape berries. Aust. J. Grape Wine Res. 6, 141-149.
- Hunter, J.J., De Villiers, O.T., Watts, J.E., 1991. The effect of partial defoliation on quality
  characteristics of Vitis vinifera L. cv. Cabernet Sauvignon grapes. II. Skin color, skin sugar, and
  wine quality. Am. J. Enol. Vitic. 42 (1), 13-18.
- Iland, P.O., 1988. Leaf removal effects on fruit composition. In Proceedings of the Second
  International Symposium for Cool Climate Viticulture and Oenology. RE Smart et al.(eds.). 2, 137138.
- 434 Kliewer, W.M., Schultz, H.B., 1964. Influence of environment on metabolism of organic acids and
- 435 carbohydrates in *Vitis vinifera*. II. Light. Am. J. Enol. Vitic. 15, 119–129.

- Kliewer, W.M., Lider, L.A., 1968. Influence of bunch exposure to the sun on the composition of
  Thompson Seedless fruit. Am. J. Enol. Vitic.19, 175-184.
- Kliewer, W.M., 1970. Effect of day temperature and light intensity on colouration of *Vitis vinifera*L. grapes. J. Amer. Soc. Hort. Sci. 95, 693-697.
- Kliewer, W.M., Torres, R.E., 1972. Effect of controlled day and night temperatures on grape
  coloration. Am. J. Enol. Vitic. 23 (2), 71-77.
- Krasnow, M., Matthews, M., Smith, R., Benz, J., Weber, E., Shackel, K., 2010. Distinctive
  symptoms differentiate four common types of berry shrivel disorder in grape. California
  Agriculture. 64(3), 155-159.
- Lakso, A.N., Kliewer, W.M., 1975. The influence of temperature on Malic acid metabolism in
  Grape berries. Plant Physiol. 56, 370-372.
- Lemut S.M., Trost, K., Sivilotti, P., Arapitsas, P., Vrhovsek, U., 2013. Early versus late leaf
  removal strategies for Pinot Noir (*Vitis vinifera* L.): effect on colour- related phenolics in young
  wines following alcoholic fermentation. J. Sci. Food Agric. 93(15), 3670-3681.
- 450 Lorenz, D. H., Eichhorn, K. W., Bleiholder, H., Klose, R., Meier, U., Weber, E., 1995. Growth
- 451 stages of the grapevine: phenological growth stages of the grapevine (Vitis vinifera L. ssp.
- vinifera)—codes and descriptions according to the extended BBCH scale. Aust. J. Grape Wine Res.
  1(2), 100-103.
- Mattivi, F., Guzzon, R., Vrhovsek, U., Stefanini, M., Velasco, R., 2006. Metabolite profiling of
  grape: flavonols and anthocyanins. J. Agric. Food Chem. 54, 7692–7702.
- 456 Matus, J.T., Loyola R., Vega A., Peña-Neira, A., Bordeu E., Arce-Johnson P., Alcalde J.A., 2009.
- 457 Post-veraison sunlight exposure induces MYB-mediated transcriptional regulation of anthocyanin
- and flavonol synthesis in berry skins of *Vitis vinifera*. J. Exp. Bot. 60, 853-867.
- 459 Mazza, G., Francis, F. J., 1995. Anthocyanins in grapes and grape products. Critical Reviews in
- 460 Food Science & Nutrition. 35(4), 341-371.

- Mori, K., Goto-Yamamoto, N., Kitayama, M., Hashizume, K., 2007. Loss of anthocyanins in
  redwine grape under high temperature. J. Exp. Bot. 58(8), 1935-1945.
- Mori, K., Sugaya, S., Gemma, H., 2005. Decreased anthocyanin biosynthesis in grape berries grown
  under elevated night temperature condition. Sci. Hortic. 105, 319–330.
- Movahed, N., Pastore, C., Cellini, A., Allegro, G., Valentini, G., Zenoni, S., Cavallini, E., D'Incà,
  E.,
- Pastore, C., Zenoni, S., Fasoli, M., Pezzotti, M., Tornielli, G.B., Filippetti, I., 2013. Selective
  defoliation affects plant growth, fruit transcriptional ripening program and flavonoid metabolism in
  grapevine. BMC Plant Biology. DOI: 10.1186/1471-2229-13-30
- 470 Percival, D.C., Fisher, K.H., Sullivan, J.A., 1994. Use of fruit zone leaf removal with *Vitis vinifera*471 L. cv Riesling grapevines. II. Effects on fruit composition, yield, and occurrence of bunch rot
- 472 (Botrytis cinerea Pers.). Am. J. Enol. Vitic. 45, 33-139.
- 473 Pereira, G.E., Gaudillere, J.P., Pieri P., Hilbert, G., Maucourt, M., Deborde, C., Moing, A., Roil, D.,
- 474 2006. Microclimate influence on mineral and metabolic profiles of grape berries. J. Agric. Food475 Chem. 54, 6765-6775.
- 476 Pirie, A., Mullins, M.G, 1976. Changes in anthocyanin and phenolics content of grapevine leaf and
  477 fruit tissues treated with sucrose, nitrate, and abscisic acid. Plant Physiol. 58(4), 468-472.
- Poni, S., Intrieri, C., Silvestroni, O., 1994. Interactions of leaf age, fruiting, and exogenous
  cytokinins in Sangiovese grapevines under nonirrigated conditions. I. Gas exchange. Am. J. Enol.
  Vitic. 45, 71-78.
- Price, S.F., Breen, P.J., Valladao, M., Watson, B.T., 1995. Bunch sun exposure and Quercitin in
  grapes and wine. Am. J. Enol. Vitic. 46, 187-194.
- 483 Ristic, R., Downey, M.O., Iland, P.G., Bindon, K., Francis, I.L., Herderich, M., Robinson, S.P.,
- 484 2007. Exclusion of sunlight from Shiraz grapes alters wine colour, tannin and sensory properties.
- 485 Aust. J. Grape Wine Res. 13(2), 53-65.

- 486 Roubelakis-Angelakis K.A., Kliewer, W.M., 1986. Effects of exogenous factors on Phenylalanine
- 487 Ammonia-Lyase activity and accumulation of anthocyanins and total phenolics in grape berries.
  488 Am. J. Enol. Vitic. 37(4), 275-280.
- Sadras, V.O., Petrie, P.R., Moran, M.A., 2013. Effects of elevated temperature in grapevine. II juice
  pH, titratable acidity and wine sensory attributes. Aust. J. Grape Wine Res. 19(1), 107-115.

492 effects on the composition of *Vitis vinifera* cv. Merlot berries. Am. J. Enol. Vitic. 53, 171-182.

Spayd, S.E., Tarara, J.M., Mee, D.L., Ferguson, J.C., 2002. Separation of sunlight and temperature

- 493 Stapleton, J.J., R. Stanley Grant, 1992. Leaf removal for nonchemical control of the summer bunch
  494 rot complex of wine grapes in the San Joaquin Valley. Plant disease. 76.2, 205-208.
- Tarara, J.M., Lee, J., Spayd, S.E., Scagel, C.F., 2008. Berry temperature and solar radiation alter
  acylation, proportion, and concentration of anthocyanin in merlot grapes. Am. J. Enol. Vitic. 59(3),
  é235–247.
- 498 Tornielli, G.B., Filippetti, I., 2016. The grapevine VviPrx 31 peroxidase as a candidate gene
  499 involved in anthocyanin degradation in ripening berries under high temperature. J. Plant Res.
  500 129(3), 513-526.
- Yamane, T., Jeong S.K., Goto-Yamamoto, N., Koshita ,Y., Kobayashi, S., 2006. Effects of
  temperature on anthocyanin biosynthesis in grape berry skins. Am. J. Enol. Vitic. 57, 54-59.
- Zoecklein, B. W., Wolf, T. K., Duncan, N. W., Judge, J. M., Cook, M. K., 1992. Effects of fruit
  zone leaf removal on yield, fruit composition, and fruit rot incidence of Chardonnay and white
- 505 Riesling (*Vitis vinifera* L.) grapes. Am. J. Enol. Vitic. 43.2, 139-148.

- 1 Table 1. Julian Day on which veraison defoliation treatment and harvest took place in 2008 and 2009 for
- 2 Cabernet Sauvignon, Nero d'Avola, Raboso Piave and Sangiovese.

	20	08	2009			
	Defoliation	Harvest	Defoliation	Harvest		
Cabernet Sauvignon	226	276	217	271		
Nero d'Avola	226	276	217	271		
Raboso Piave	226	287	225	281		
Sangiovese	211	266	210	261		

Table 2. Number of hours during which berry temperature was higher than 30 °C on control (C) and 1 defoliated (D) vines during the experimental period. For each variety and year the period of measurements 2 ranges from leaf removal to harvest and are as follows: Cabernet Sauvignon and Nero d'Avola from JD 226 3 to 276 in 2008 and from JD 217 to 271 in 2009; Raboso Piave from JD 226 to 287 in 2008 and from JD 225 4 5 to 281 in 2009; Sangiovese from JD 211 to 265 in 2008 and from JD 210 to 261 in 2009. Values represent 6 means of eight replicates. Average of percentage of bunch exposure estimated in 2008 and 2009. For each 7 variety and year the measurements were performed in three days during August at 9.00 am, 1.30 pm and 5.30 8 pm.

Parameter	20	008	20	)09	Signifi		cance		
	С	D	С	D	Treat.	Year	Treat. x Year		
Cabernet Sauvignon									
h>30 °C	147	202	214	270	**	*	ns		
Average bunch exposure (%)	5.2	24.8	6.2	26.4	**	ns	ns		
		Ner	o d'Avola						
h>30 °C	145	205	212	263	**	*	ns		
Average bunch exposure (%)	3.3	23.4	4.2	25.3	**	ns	ns		
		Rat	oso Piave						
h>30 °C	147	202	164	206	**	*	ns		
Average bunch exposure (%)	2.1	23.8	3.2	24.6	**	ns	ns		
Sangiovese									
h>30 °C	269	300	256	324	**	*	ns		
Average bunch exposure (%)	5.4	26.4	6.7	26.3	**	ns	ns		

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Table 3. Yield components and main grape composition parameters recorded at harvest in Cabernet
 Sauvignon vines subjected to defoliation at veraison (D) in comparison to control vines (C) in 2008 and
 Botrytis and sunburn were expressed as average percentage of surface area with symptoms for each

- 4 bunch at harvest.

Parameter	2	008	20	09	Average 2008-2009 Sig		gnificanc	gnificance			
	С	D	С	D	C	D	Т	Y	ΤxΥ		
Cabernet Sauvignon											
Bunches /vine	24	24	24	25	24	25	ns	ns	ns		
Yield /vine (kg)	2.71	3.17	3.48	3.91	3.09	3.54	ns	*	ns		
Berry mass (g)	1.27	1.39	1.69	1.62	1.48	1.50	ns	***	**		
Botrytis (%)	0.00	0.00	5.00	0.00	2.50	0.00	ns	ns	ns		
Sunburn (%)	0.00	0.00	0.00	0.00	0.00	0.00	ns	ns	ns		
Total Soluble Solids (°Brix)	22.83	22.54	21.49	20.99	22.16	21.77	ns	***	ns		
Titratable acidity (g/L)	7.35	6.25	6.80	5.57	7.07	5.91	***	**	ns		
pH	3.61	3.69	3.60	3.71	3.61	3.70	*	ns	ns		
			Nero	d'Avola							
Bunches /vine	23	21	20	18	22	20	ns	ns	ns		
Yield /vine (kg)	4.3	4.42	4.71	3.92	4.51	4.17	ns	ns	ns		
Berry mass (g)	2.01	2.01	2.80	2.60	2.40	2.31	ns	***	ns		
Botrytis (%)	0.00	0.40	19.00	3.00	9.50	1.70	*	*	**		
Sunburn (%)	0.00	0.00	0.00	2.00	0.00	1.00	ns	ns	ns		
Total Soluble Solids (° Brix)	22.92	22.49	21.22	21.29	22.07	21.89	ns	***	ns		
Titratable acidity (g/L)	8.24	7.73	7.33	6.39	7.79	7.06	*	***	ns		
pH	3.33	3.36	3.38	3.48	3.36	3.42	*	***	ns		
			Rabo	so Piave							
Bunches /vine	11	11	12	11	12	11	ns	ns	ns		
Yield /vine (kg)	3.81	3.82	4.59	2.67	4.20	3.24	ns	ns	**		
Berry mass (g)	1.88	1.69	2.10	2.06	1.99	1.87	*	***	ns		
Botrytis (%)	0.00	0.00	1.00	0.00	0.50	0.00	ns	ns	ns		
Sunburn (%)	0.00	0.00	2.20	37.20	1.10	18.50	**	**	**		
Total Soluble Solids (° Brix)	22.19	21.50	22.50	22.26	22.34	21.88	ns	*	ns		
Titratable acidity (g/L)	12.02	12.69	10.39	10.62	11.21	11.65	ns	***	ns		
pH	3.15	3.16	3.27	3.30	3.21	3.23	ns	***	ns		
			San	giovese							
Bunches /vine	17	16	16	16	16	16	ns	ns	ns		
Yield /vine (kg)	6.33	5.55	7.08	5.88	6.71	5.72	ns	ns	ns		
Berry mass (g)	2.37	2.34	2.65	2.50	2.51	2.42	ns	*	ns		
Botrytis (%)	4.90	2.20	14.50	11.7	9.70	6.95	ns	**	ns		
Sunburn (%)	0.30	6.00	1.20	13.10	0.75	9.55	**	**	ns		
Total Soluble Solids (° Brix)	20.77	20.67	21.01	22.17	20.89	21.42	ns	*	ns		
Titratable acidity (g/L)	7.62	6.65	6.94	6.20	7.28	6.42	***	**	ns		
pH	3.38	3.45	3.43	3.52	3.41	3.49	***	**	ns		
*, **, ***, ns indicate significance at P<0.05. P<0.01 and P<0.001 or not significant. respectively.											

- 1 Table. 4. Concentration of total anthocyanins, sum of glycosylate, acetate and coumarate anthocyanins (mg/g
- 2 skin) and ratio between di-substituted and tri-substituted anthocyanins at harvest in Cabernet Sauvignon,
- 3 Nero d'Avola, Raboso Piave and Sangiovese vines subjected to defoliation at veraison (D) in comparison to
- 4 control vines (C) in 2008 and 2009.

Parameter	20	08	20	)09	Average 2	2008-2009		Significance			
	С	D	С	D	С	D	Treat.	Year	Treat. X Year		
Cabernet Sauvignon											
Total anthocyanins	7.19	6.58	4.74	4.08	5.96	5.33	ns	***	ns		
Sum of glycosylate	4.71	4.14	2.99	2.54	3.84	3.34	ns	***	ns		
Sum of acetate	1.88	1.73	1.32	1.08	1.60	1.40	ns	***	ns		
Sum of coumarate	0.60	0.71	0.43	0.46	0.52	0.59	ns	**	ns		
Di-Tri substituted	0.098	0.113	0.312	0.120	0.205	0.117	***	***	**		
·	Nero d'Avola										
Total anthocyanins	8.30	8.14	8.53	8.88	8.42	8.51	ns	ns	ns		
Sum of glycosylate	5.92	5.98	5.65	6.21	5.80	6.09	ns	ns	ns		
Sum of acetate	1.12	1.06	1.13	1.05	1.12	1.06	ns	ns	ns		
Sum of coumarate	1.26	1.10	1.75	1.62	1.50	1.36	ns	***	ns		
Di-Tri substituted	0.076	0.101	0.059	0.099	0.068	0.101	***	*	ns		
1410				Rahoso Pia	ave						
Total anthocyanins	13.39	10.83	5.45	8.65	9.42	9.68	ns	***	***		
Sum of glycosylate	11.39	9.17	4.69	7.54	8.03	8.32	ns	***	***		
Sum of acetate	1.21	1.09	0.34	0.49	0.78	0.77	ns	**	ns		
Sum of coumarate	0.79	0.57	0.42	0.62	0.61	0.59	ns	**	***		
Di-Tri substituted	1.031	1.212	1.387	1.482	1.209	1.347	ns	**	ns		
ratio											
	Sangiovese (1)										
Total anthocyanins	4.87	4.33	4.30	4.71	4.58	4.52	ns	ns	ns		
Di-Tri substituted ratio	0.709	1.273	0.951	1.639	0.830	1.456	***	**	ns		

\*, \*\*, \*\*\*, ns indicate significance at P< 0.05. P< 0.01 and P < 0.001 or not significant, respectively. (1) Sangiovese has

only traces of acetate anthocyanins, so the total anthocyanins are mostly glycosylate anthocyanins.

- 1 Table 5. Concentration of total and single flavonol compounds (mg/g skin) at harvest in Cabernet Sauvignon,
- 2 Nero d'Avola, Raboso Piave and Sangiovese vines subjected to defoliation at veraison (D) and in control
- 3 vines (C) in 2008 and 2009.

Parameter	20	2008		2009		Average 2008-2009		Significance			
	С	D	С	D	С	D	Treat.	Year	Treat. X Year		
Cabernet Sauvignon											
Total flavonols	0.16	0.35	0.24	0.62	0.20	0.48	*	**	ns		
Myricetin	0.08	0.16	0.13	0.26	0.10	0.21	*	**	ns		
Quercetin	0.08	0.17	0.10	0.30	0.09	0.23	*	*	ns		
Kaempferol	0.00	0.02	0.01	0.06	0.01	0.04	*	***	**		
Nero d'Avola											
Total flavonols	0.33	0.60	0.32	0.95	0.32	0.77	*	***	***		
Myricetin	0.18	0.30	0.22	0.46	0.20	0.38	*	***	**		
Quercetin	0.13	0.26	0.09	0.40	0.11	0.33	*	*	***		
Kaempferol	0.01	0.03	0.01	0.09	0.01	0.06	*	***	***		
				Rat	ooso Piave						
Total flavonols	0.17	0.44	0.11	0.54	0.14	0.49	**	ns	ns		
Myricetin	0.10	0.10	0.04	0.12	0.07	0.11	*	*	**		
Quercetin	0.07	0.32	0.07	0.38	0.07	0.36	**	ns	ns		
Kaempferol	0.00	0.02	0.00	0.04	0.00	0.03	*	*	ns		
Sangiovese											
Total flavonols	0.32	0.67	0.40	0.69	0.36	0.68	***	ns	ns		
Myricetin	0.06	0.07	0.06	0.08	0.06	0.07	*	ns	ns		
Quercetin	0.25	0.57	0.32	0.55	0.28	0.56	***	ns	ns		
Kaempferol	0.01	0.03	0.02	0.06	0.02	0.05	***	**	ns		

\*, \*\*, \*\*\*, ns indicate significance at P< 0.05. P< 0.01 and P < 0.001 or not significant, respectively.

# Figure 1





