

Alma Mater Studiorum Università di Bologna
Archivio istituzionale della ricerca

Anthocyanin and flavonol composition response to veraison leaf removal on Cabernet Sauvignon, Nero d'Avola, Raboso Piave and Sangiovese Vitis vinifera L. cultivars

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Pastore, C., Allegro, G., Valentini, G., Muzzi, E., Filippetti, I. (2017). Anthocyanin and flavonol composition response to veraison leaf removal on Cabernet Sauvignon, Nero d'Avola, Raboso Piave and Sangiovese Vitis vinifera L. cultivars. SCIENTIA HORTICULTURAE, 218, 147-155 [10.1016/j.scienta.2017.01.048].

Availability:

This version is available at: <https://hdl.handle.net/11585/624160> since: 2018-02-23

Published:

DOI: <http://doi.org/10.1016/j.scienta.2017.01.048>

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>).
When citing, please refer to the published version.

(Article begins on next page)

This is the final peer-reviewed accepted manuscript of:

Chiara Pastore, Gianluca Allegro, Gabriele Valentini, Enrico Muzzi, *Ilaria Filippetti*, *Anthocyanin and flavonol composition response to veraison leaf removal on Cabernet Sauvignon, Nero d'Avola, Raboso Piave and Sangiovese Vitis vinifera L. cultivars*, Scientia Horticulturae, Volume 218, 2017, Pages 147-155, ISSN 0304-4238,

<https://www.sciencedirect.com/science/article/pii/S0304423817300791>

The final published version is available online at:

<https://doi.org/10.1016/j.scienta.2017.01.048>.

Rights / License:

The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (<https://cris.unibo.it/>)

When citing, please refer to the published version.

1 **Anthocyanin and flavonol composition response to veraison leaf removal on Cabernet Sauvignon,**
2 **Nero d'Avola, Raboso Piave and Sangiovese *Vitis vinifera* L. cultivars.**

3

4 **Chiara Pastore, Gianluca Allegro, Gabriele Valentini, Enrico Muzzi, Ilaria Filippetti***

5 **Dipartimento di Scienze Agrarie, Università di Bologna, viale Fanin 46, 40127 Bologna**

6 **Corresponding author: ilaria.filippetti@unibo.it*

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
11 study was to analyze the effect over two years of leaf removal on anthocyanin and flavonol composition at
12 harvest in four red *Vitis vinifera* L. varieties: Cabernet Sauvignon, Nero d'Avola, Raboso Piave and
13 Sangiovese, characterized by different anthocyanin and flavonol profiles. The concentration of total
14 anthocyanins in berries did not vary among control and defoliated vines in all varieties in both vintages,
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

20 Defoliation is a common crop management practice on grapevine in many viticultural regions. The
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
23 Botrytis bunch rot infection and increases fungicide spray penetration (English et al., 1989; Stapleton and
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

(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 composition. Several authors, mainly reporting the effects of shading on grape color, agreed that low light 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, 1974; Hunter et al., 1991; Iland, 1988; Kliewer and Lider, 1968; Kliewer, 1970; Matus et al., 2009; Zoecklein et al., 1992). Further investigations into the effects of increasing light exposure on grape color gave rise to contradictory results. Some studies reported that high light levels resulted in decreased anthocyanin levels (Bergqvist et al., 2001; Pastore et al., 2013; Spayd et al., 2002), while in other cases no change was observed in total anthocyanin concentration (Downey et al., 2004; Haselgrove et al., 2000; Price et al., 1995; Spayd et al., 2002). When exposure to sunlight is associated with excessive berry temperature, as occurs in warm conditions, this may often lead to berry sunburn that has a negative impact on the color of some red berry grapevine varieties (Kliewer and Torres, 1972; Mori et al., 2005; Mori et al., 2007). It has been pointed out that the lower anthocyanin content in berries under high temperature reflects the combined impact of reduced biosynthesis and increased degradation in which the role of peroxidase enzymes in anthocyanin catabolism is probably involved (Movahed et al., 2016). The modification of bunch light 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 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 researches have shown shifts in anthocyanin composition after bunches microclimatic variation, with an increase in the di-substituted anthocyanin concentration (cyanidin and peonidin) in shaded bunches giving rise to an increased di-substituted to tri-substituted anthocyanins (delphinidin, petunidin and malvidin) ratio (Downey et al., 2004; Ristic et al., 2007; Spayd et al., 2002). Although other authors showed opposite results since bunch light exposure increased the proportion of di- respect to tri-substituted anthocyanins (Chorty et 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 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.

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.

Defoliation treatments and harvest were performed according to the berry ripening trend in each cultivar and 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.

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.

2.2. Temperature monitoring

Berry skin temperature was monitored in 2008 and 2009 in four selected bunches on control and defoliated vines of each tested variety. For each treatment, temperature data were collected from stage 33 (beginning of bunch closure, berries touching, according to Lorenz et al., (1995) until harvest and this fluctuated for each 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 in 2008 and from JD 210 to 261 in 2009. Eight T-type thermocouples (RS components, MI, Italy) were positioned in the sub-cuticular tissues of the berry skin. Four were positioned on two different bunches, two on the east side and two on the west side of the cordon. For each side, one thermocouple was inserted in a berry located in the external part of the bunch and the other in the internal part. Each probe was then 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 bunch exposure was visually estimated in three moments of the day: in the morning (9.00-9.30 a.m.), when the sun position is at its Zenith (1.30- 2.00 p.m.) and in late afternoon (5.30-6.00 p.m.).

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).

2.4 Statistical analyses

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

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.

3. Results

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 both
142 years an increase of around 20 % in the daily average (Table 2).

143

144 **3.2 Vegetative and productive traits**

145 There were only minor differences between the two years in vegetative and productive measurements at
146 harvest following the leaf removal in all tested varieties. Starting from a uniform bunch number per vine, no
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
151 defoliated compared with control vines, whereas only Nero d'Avola had significantly fewer bunches
152 attacked by *Botrytis* on defoliated vines in 2009 (Table 3). It should be noted that the untreated Nero d'Avola
153 was the most sensitive cultivar to *Botrytis*, showing the highest level of attack in 2009. Surprisingly,
154 Sangiovese cv, despite a strong *Botrytis* incidence in 2009, did not respond to leaf removal with significant
155 rot reduction (Table 3). Sugar concentration in must at harvest was not affected by veraison defoliation, but
156 differed in the two vintages, while total acidity and pH in Cabernet Sauvignon, Nero d'Avola and
157 Sangiovese were reduced and increased respectively by defoliation (Table 3).

158 **3.3. Anthocyanins and flavonols**

159 **3.3.1. Univariate analyses**

160 The concentration of total anthocyanins in the berries (mg/g) did not vary among treatments at harvest in
161 both vintages and in all varieties (Table 4). In Sangiovese, where the profile showed only traces of acetate
162 and coumarate anthocyanins, the total concentration corresponded mainly to glycosylate anthocyanins. In
163 Cabernet Sauvignon, Nero d'Avola and Raboso Piave the concentration of glycosylate, acetate and
164 coumarate anthocyanins was not modified following leaf removal treatments compared to the control (Table
165 4).

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

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

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

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

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

180 **3.3.2. Multivariate quantitative data**

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

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

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

195 Sauvignon the two years appear grouped and in Nero d'Avola the two treatments are distinguished mainly
196 according 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.

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

205 **4. Discussion**

206 **4.1. Vegetative and productive traits**

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

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

217 On the other hand, veraison defoliation usually had strong impact on bunches microclimatic conditions. In
218 our study actually we estimated an average daily increase of 20% of bunch exposure in defoliated compared
219 to control vines, in both years, while the berry temperature difference between the treatments within all
220 cultivars and years, expressed as number of hours in which the berries overcome 30°C from veraison to
221 harvest, never exceeded 70 hours. Moreover, during the two seasons the maximum air temperature was
222 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
227 accumulation in grape tissues (Crippen and Morrison, 1986 a; Kliewer and Lider, 1968). On the contrary,
228 temperature has been known for some time to have significant effects on berry acidity, accelerating the
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).

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

239 **4.2. Anthocyanins and Flavonols**

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

247 The mechanism that suppresses anthocyanin accumulation in berry skins under high-temperature ripening
248 conditions is not completely clear, but recent evidence suggests that the low anthocyanin content in berries
249 ripened at high temperature reflects the combined impact of reduced biosynthesis and increased degradation
250 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
255 between control and defoliated berries were quite low with a maximum of 70 hours over 30 °C. In fact in a
256 previous research a strong anthocyanins reduction, in Sangiovese berries ripened at more than 140 hours
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

278 delay in sugar accumulation in 2009 clearly linked with the higher yield level, which may be responsible for
 279 the lower anthocyanins level recorded in that year.

280 On the contrary in Raboso Piave, a late ripening variety, since yield level was similar in the two seasons, the
 281 lower anthocyanin concentration at harvest in 2009 may be attributed to a strong sensitivity to temperature as
 282 shown by the high level of sunburned berries registered in defoliated vines in the season with highest air
 283 temperature (Table 3). Although we did not sample sunburned berries, which often exhibit poor color
 284 development (Krasnow et al., 2010), we may argue that the same conditions of higher irradiance and
 285 temperature that induced the sunburn may be responsible for a decrease in anthocyanins as previously
 286 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
310 was almost negligible when they had not been exposed to light and that the subsequent exposure of those
311 tissues to sunlight determined the rise of flavonol accumulation after the increase in the expression of the
312 gene encoding flavonol synthase (Downey et al., 2004; Pastore et al., 2013). Previous research on
313 Sangiovese showed that in similar light conditions, temperature increase caused strong flavonol
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
316 flavonol concentration, revealing that the influence of light is dominant on the synthesis of these compounds
317 compared to the thermal effect, at least under the observed temperature range.

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

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

328 **5. Conclusion**

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

333 which may have reduced anthocyanin concentration counterbalancing the supposed enhancement due to light
334 exposure increase.

335 The strong increase in flavonol concentration in all varieties under defoliation suggests that the influence of
336 light is dominant on the synthesis of these compounds compared to the thermal effect and that they may
337 represent a marker of berries sun exposure. Furthermore, the stimulation of the synthesis of quercetin,
338 derived from the di-substituted branch of the flavonoids pathway, also triggers the production of cyanidin,
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
343 many factors including genotype and the synergistic or antagonistic effect of different levels and extent of
344 both temperature and light intensity experienced by the berries.

345

346 **Figure Captions:**

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

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

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.

364

365

366

367

368

369

370

371

372

373 **Funding.**

374 This work was partially supported by the University of Bologna PhD grant

375

376

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., Kliwer, 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.

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.

Downey, M.O., Dokoozlian, N.K., Krstic, M.P., 2006. Cultural practice and environmental impacts
 on the flavonoid composition of grapes and wine: a review of recent research. *Am. J. Enol. Vitic.*
 57, 257–268.

Downey, M.O., Harvey, J.S., Robinson, S.P., 2003. Synthesis of flavonols and expression of
 flavonol synthase genes in the developing grape berries of Shiraz and Chardonnay (*Vitis Vinifera*
 L.). *Aust. J. Grape Wine Res.* 9, 110-121.

Downey, M.O., Harvey, J.S., Robinson, S.P., 2004. The effect of bunch shading on berry
 development and flavonoid accumulation in Shiraz grapes. *Aust. J. Grape Wine Res.* 10, 55-73.

English, J.T., Thomas, C.S., Marois, J.J., Gubler, W.D., 1989. Microclimates of grapevine canopies
 associated with leaf removal and control of Botrytis bunch rot. *Phytopathology.* 79, 395- 401.

411 Flamini, R., Mattivi, F., Rosso, M.D., Arapitsas, P., Bavaresco, L., 2013. Advanced knowledge of
 412 three important classes of grape phenolics: anthocyanins, stilbenes and flavonols. *Int. J. Mol. Sci.*
 413 14(10), 19651-19669.

414 Gollop, R., Even, S., Colova-Tsolova, V., Perl, A., 2002. Expression of the grape dihydroflavonol
 415 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
 418 and flesh of white-fleshed and teinturier grape berries. *Planta.* 243(1), 23-41.

419 Guidoni, S., Ferrandino, A., Novello, V., 2008. Effects of seasonal and agronomical practices on
 420 skin anthocyanin profile of Nebbiolo grapes. *Am. J. Enol. Vitic.* 59(1), 22-29.

421 Guidoni, S., Ferrandino, A., Novello, V., 2008. Effects of seasonal and agronomical practices on
 422 skin anthocyanin profile of Nebbiolo grapes. *Am. J. Enol. Vitic.* 59 (1), 22-29.

423 Hale, C.R., Buttrose, M.R., 1974. Effect of temperature on ontogeny of berries of *Vitis Vinifera* L.,
 424 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.

428 Hunter, J.J., De Villiers, O.T., Watts, J.E., 1991. The effect of partial defoliation on quality
 429 characteristics of *Vitis vinifera* L. cv. Cabernet Sauvignon grapes. II. Skin color, skin sugar, and
 430 wine quality. *Am. J. Enol. Vitic.* 42 (1), 13-18.

431 Iland, P.O., 1988. Leaf removal effects on fruit composition. In *Proceedings of the Second*
 432 *International Symposium for Cool Climate Viticulture and Oenology.* RE Smart et al.(eds.). 2, 137-
 433 138.

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.

436 Kliewer, W.M., Lider, L.A., 1968. Influence of bunch exposure to the sun on the composition of
 437 Thompson Seedless fruit. *Am. J. Enol. Vitic.* 19, 175-184.

438 Kliewer, W.M., 1970. Effect of day temperature and light intensity on colouration of *Vitis vinifera*
 439 L. grapes. *J. Amer. Soc. Hort. Sci.* 95, 693-697.

440 Kliewer, W.M., Torres, R.E., 1972. Effect of controlled day and night temperatures on grape
 441 coloration. *Am. J. Enol. Vitic.* 23 (2), 71-77.

442 Krasnow, M., Matthews, M., Smith, R., Benz, J., Weber, E., Shackel, K., 2010. Distinctive
 443 symptoms differentiate four common types of berry shrivel disorder in grape. *California*
 444 *Agriculture.* 64(3), 155-159.

445 Lakso, A.N., Kliewer, W.M., 1975. The influence of temperature on Malic acid metabolism in
 446 Grape berries. *Plant Physiol.* 56, 370-372.

447 Lemut S.M., Trost, K., Sivilotti, P., Arapitsas, P., Vrhovsek, U., 2013. Early versus late leaf
 448 removal strategies for Pinot Noir (*Vitis vinifera* L.): effect on colour- related phenolics in young
 449 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.
 452 *vinifera*)—codes and descriptions according to the extended BBCH scale. *Aust. J. Grape Wine Res.*
 453 1(2), 100-103.

454 Mattivi, F., Guzzon, R., Vrhovsek, U., Stefanini, M., Velasco, R., 2006. Metabolite profiling of
 455 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
 458 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.

461 Mori, K., Goto-Yamamoto, N., Kitayama, M., Hashizume, K., 2007. Loss of anthocyanins in
 462 redwine grape under high temperature. J. Exp. Bot. 58(8), 1935-1945.

463 Mori, K., Sugaya, S., Gemma, H., 2005. Decreased anthocyanin biosynthesis in grape berries grown
 464 under elevated night temperature condition. Sci. Hortic. 105, 319–330.

465 Movahed, N., Pastore, C., Cellini, A., Allegro, G., Valentini, G., Zenoni, S., Cavallini, E., D’Inca,
 466 E.,

467 Pastore, C., Zenoni, S., Fasoli, M., Pezzotti, M., Tornielli, G.B., Filippetti, I., 2013. Selective
 468 defoliation affects plant growth, fruit transcriptional ripening program and flavonoid metabolism in
 469 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. Food
 475 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.

478 Poni, S., Intrieri, C., Silvestroni, O., 1994. Interactions of leaf age, fruiting, and exogenous
 479 cytokinins in Sangiovese grapevines under nonirrigated conditions. I. Gas exchange. Am. J. Enol.
 480 Vitic. 45, 71-78.

481 Price, S.F., Breen, P.J., Valladao, M., Watson, B.T., 1995. Bunch sun exposure and Quercetin in
 482 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.

489 Sadras, V.O., Petrie, P.R., Moran, M.A., 2013. Effects of elevated temperature in grapevine. II juice
 490 pH, titratable acidity and wine sensory attributes. Aust. J. Grape Wine Res. 19(1), 107-115.

491 Spayd, S.E., Tarara, J.M., Mee, D.L., Ferguson, J.C., 2002. Separation of sunlight and temperature
 492 effects on the composition of *Vitis vinifera* cv. Merlot berries. Am. J. Enol. Vitic. 53, 171-182.

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.

495 Tarara, J.M., Lee, J., Spayd, S.E., Scagel, C.F., 2008. Berry temperature and solar radiation alter
 496 acylation, proportion, and concentration of anthocyanin in merlot grapes. Am. J. Enol. Vitic. 59(3),
 497 235–247.

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.

501 Yamane, T., Jeong S.K., Goto-Yamamoto, N., Koshita ,Y., Kobayashi, S., 2006. Effects of
 502 temperature on anthocyanin biosynthesis in grape berry skins. Am. J. Enol. Vitic. 57, 54-59.

503 Zoecklein, B. W., Wolf, T. K., Duncan, N. W., Judge, J. M., Cook, M. K., 1992. Effects of fruit
 504 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.

	2008		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

1 Table 2. Number of hours during which berry temperature was higher than 30 °C on control (C) and
2 defoliated (D) vines during the experimental period. For each variety and year the period of measurements
3 ranges from leaf removal to harvest and are as follows: Cabernet Sauvignon and Nero d’Avola from JD 226
4 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
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	2008		2009		Significance		
	C	D	C	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
Nero d’Avola							
h>30 °C	145	205	212	263	**	*	ns
Average bunch exposure (%)	3.3	23.4	4.2	25.3	**	ns	ns
Raboso 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

9

10

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 2009. Botrytis and sunburn were expressed as average percentage of surface area with symptoms for each bunch at harvest.

Parameter	2008		2009		Average 2008-2009		Significance		
	C	D	C	D	C	D	T	Y	T x Y
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
Titrateable 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
Titrateable 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
Raboso 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
Titrateable 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
Sangiovese									
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
Titrateable 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	2008		2009		Average 2008-2009		Significance		
	C	D	C	D	C	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 ratio	0.076	0.101	0.059	0.099	0.068	0.101	***	*	ns
Raboso Piave									
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 ratio	1.031	1.212	1.387	1.482	1.209	1.347	ns	**	ns
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

5 *, **, ***, ns indicate significance at P< 0.05. P< 0.01 and P < 0.001 or not significant, respectively. (1) Sangiovese has
6 only traces of acetate anthocyanins, so the total anthocyanins are mostly glycosylate anthocyanins.
7

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	2008		2009		Average 2008-2009		Significance		
	C	D	C	D	C	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	*	***	***
Raboso 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

4 *, **, ***, ns indicate significance at P< 0.05. P< 0.01 and P < 0.001 or not significant, respectively.
5

Figure 1

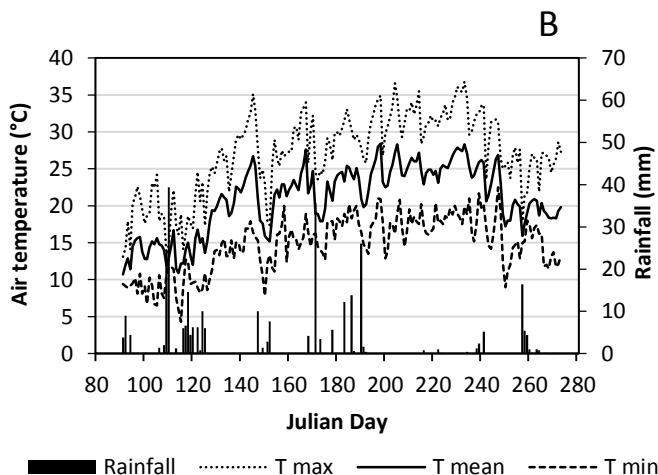
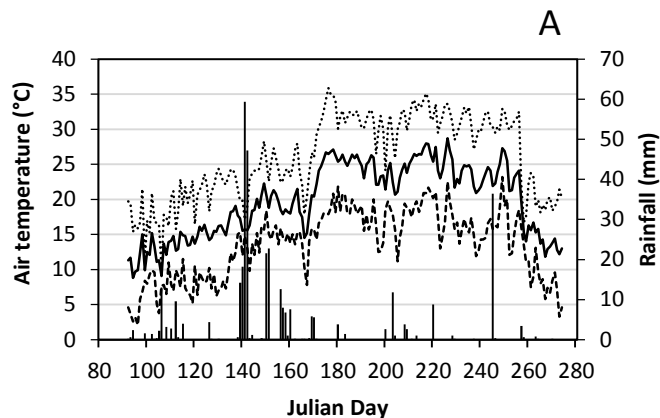
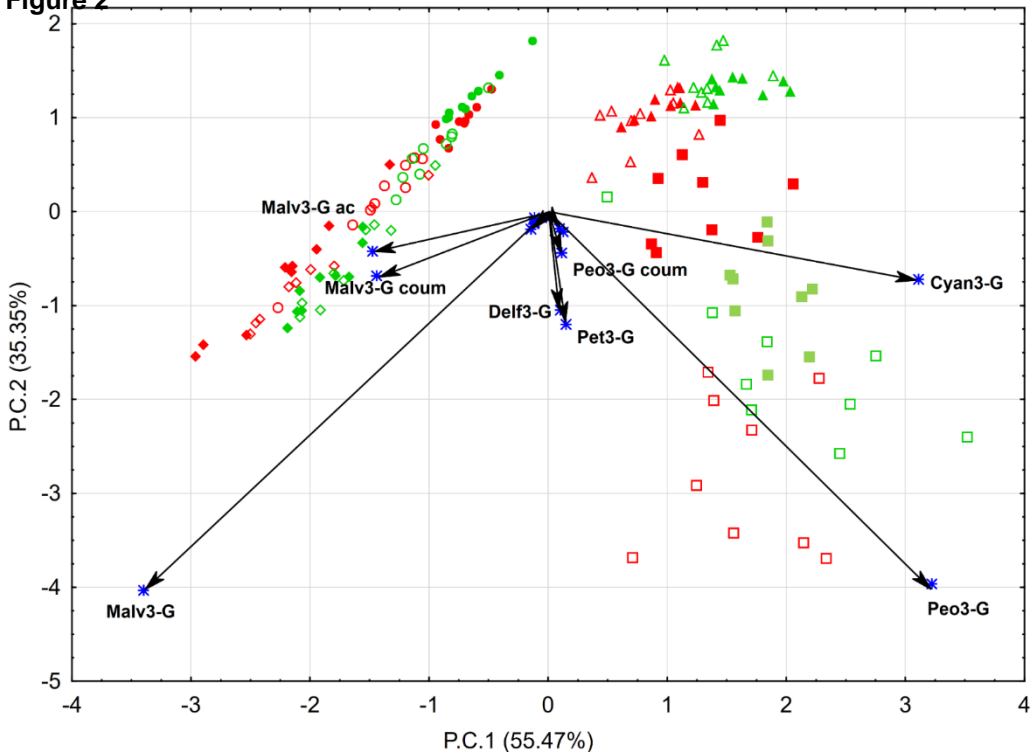


Figure 2



△ Sangiovese □ Raboso Piave ○ Cabernet Sauvignon ◇ Nero d'Avola

Figure 3

