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Research Article

Effects of Sunlight Exposure on Flavonol Content and Wine Sensory of the White Winegrape Grechetto Gentile

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Abstract: This aim of this research was to study the effect of sunlight exposure on the composition of white grape and on wine sensory attributes. In 2014 and 2015, vines of the white winegrape Grechetto gentile were subjected to cluster zone leaf removal (LR) after fruit set. Small-scale vinifications of Control and LR grapes were conducted following a standardized protocol designed to verify differences in astringency and bitterness, and the relationship between these mouthfeel attributes and the concentrations of phenolic compounds was investigated. In both years, berry flavonols increased after cluster zone leaf removal, and these compounds were also higher in wine. Berry tannins showed only minor changes in response to the higher solar irradiance, and no difference was detected in wines. In 2014, wine of LR vines was judged more bitter and astringent, while no difference was found between wines of 2015. The higher intensity of phenolic mouthfeel in the first year suggested a large involvement of the

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higher concentration of flavonols also found in that year, while in the second year, the very high pH and alcohol content of all the wines could have masked differences in the perception of astringency and bitterness.

Key words: astringency, bitterness, cluster exposure, flavonol, leaf removal, tannin

Introduction

Cluster sunlight exposure is affected by many factors, such as trellis system, vine vigor, and canopy management. The viticultural technique most used to modify cluster microclimate is leaf removal on the basal part of the shoots, which dramatically alters light interception by the cluster. According to genotype and time and intensity of application, leaf removal may have different impacts on rot infections, grape composition, and yield (Crippen and Morrison 1986, Zoecklin et al. 1992, Jackson and Lombard 1993, Filippetti et al. 2011).

Early studies reported that basal leaf removal was traditionally applied from fruit set to veraison and generally improved the microclimate condition of clusters, increasing the degree of light exposure and decreasing *Botrytis cinerea* infection (Zoecklin et al. 1992). In addition, the increase in light interception enhanced the flavonoid content of red winegrapes (Crippen and Morrison 1986), particularly in cooler regions where very high temperatures are not common (Jackson and Lombard 1993). On the contrary, in warm regions, cluster zone leaf removal could induce excessive fruit temperature (above 35°C) that may have a negative impact on the accumulation of anthocyanins (Spayd et al. 2002, Tarara et al. 2008).

The modification of cluster light exposure can also affect volatile compounds. Monoterpenes and C_{13}-norisoprenoids of white grapes were reported to increase with light

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exposure (Kwasniewski et al. 2010, Skinkis et al. 2010), while a negative correlation between light incidence on clusters and the content of methoxypyrazines was found removing leaves of red winegrapes from 15 days prebloom to 60 days postbloom (Scheiner et al. 2010, Sivilotti et al. 2016).

While the effect of cluster light exposure on the concentration of tannins in grapes and wine is not clear (Joscelyne et al. 2007), there is agreement in the literature that the exposure of clusters to sunlight increases flavonol accumulation in berries (Pastore et al. 2017a), which is also supported by the expression of the gene encoding flavonol synthase in the skins (Downey et al. 2004, Pastore et al. 2013). The effect of light incidence on the flavonols of red winegrapes has been thoroughly investigated (Feng et al. 2015, Pastore et al. 2017a). On the contrary, to our knowledge, the consequences of different sun exposure have not been investigated on white winegrapes, although the evolution of flavonols and their composition at harvest has been well described (Downey et al 2003a). These compounds are present in the berry skin bound to various sugars (glycosides), the most abundant of which are quercetin-3-*O*-glucoside and quercetin-3-*O*-glucoronide (Cheynier and Rigaud 1986). Kampferol and isoramnetin are present at lower levels, while myricetin, laricitin, and syringetin are detected only in red winegrapes (Mattivi et al. 2006).

Flavonols play an important role in the copigmentation of red wines (Boulton 2001), while their impact on sensory properties has not yet been clarified. Even if it is well known that astringency and bitterness are elicited by tannins (Gawel et al. 1998), several studies have described that flavonols also may play an important role in the perception of those mouthfeel sensations. For instance, Preys et al. (2006) observed a relationship between flavonols concentration and bitterness of wines, while Hunfangel and Hoffman (2008) described grape

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flavonols as velvety astringent but not bitter. Moreover, Ferrer-Gallego et al. (2016) reported that the addition of quercetin to red and white wines increased the intensity of astringency and bitterness but, in the case of white wine, decreased the perception of velvety mouthfeel sensation.

Since few studies have been conducted on the role that increased fruit light exposure plays on white grape composition, with particular regard to the phenolic compounds and the mouthfeel they elicit in wines, it was decided to investigate these issues, setting up a trial on the white winegrape Grechetto gentile (*Vitis vinifera* L.). This variety is cultivated in the Bologna area (Italy) for the production of Protected Designation of Origin (PDO) Pignoletto wine with a sensory profile characterized by slight bitterness and astringency that in some case becomes unpleasant. The aim of this trial is to investigate possible relationships between increasing cluster light exposure with the resulting berry flavonoid composition and astringency and bitterness traits in the corresponding wines.

Materials and Methods

Plant material and yield components. The study was conducted in the 2014 and 2015 seasons in a 30-year-old, nonirrigated, commercial vineyard of *Vitis vinifera* L. cv. Grechetto gentile grafted onto Kober 5BB rootstock, located in Valsamoggia, Bologna, Italy (latitude 44°28'N; longitude 11°07'E). Vines were spaced 1.5 m within the row and 3.5 m between rows and trained to a vertically shoot positioned (VSP) cane pruning system. Each vine was winter-pruned leaving one cane with 14 nodes. The number of shoots was kept uniform by thinning performed at the BBCH 53 stage – inflorescences visible (Lorenz et al. 1995). Shoots were hedged twice, in June and July from the BBCH 53 stage to the BBCH 81 stage (beginning of ripening), and plants were sprayed to control downy mildew, powdery mildew, and insects (i.e. *Eupoecilia*

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ambiguella, *Lobesia botrana*, and *Scaphoideus titanus*) according to Emilia-Romagna Region standard practices.

A completely randomized design was used and each vine was an experimental unit: on two uniform rows, 20 plants were assigned to the leaf removal treatment (LR) and 20 to the Control (no leaf removal). Leaf removal was applied on 26 June 2014 and 30 June 2015, at BBCH 75 stage (pea-sized berry) after the first shoot hedging and consisted of the removal of all main and lateral leaves from the seven basal nodes of each shoot.

At harvest (23 September 2014 and 15 September 2015), the yield of the tagged plants was weighed and the number of clusters counted. Grapes of the experimental plot were harvested two days before the commercial harvest.

Climate data, berry temperatures and light incidence on cluster. Daily average temperature and rainfall data were kindly provided by the meteorological service of the Emilia-Romagna Region (ARPAE), which has a weather station near the vineyard.

Temperatures of 4 tagged clusters per treatment (8 clusters total) were recorded hourly between BBCH 77 stage (berries beginning to touch) and harvest, using microprobes connected to a datalogger (GMR Strumenti, Florence, Italy). Two probes per tagged cluster were inserted into the subcuticular layers of berry mesocarp, on both sides of the canopy.

Light incidence on cluster was evaluated measuring photosynthetic active radiation (PAR) with a pyranometer (Skye Instruments, Llandrindod Wells, UK) positioned in front of the cluster perpendicular to the sun's rays and was expressed as a percentage of the maximum irradiance measured in an unobstructed ambient. Measurements were taken at 10:00 AM on a day of full sun

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(7 August 2014 and 27 July 2015) when shoot growth had ceased and light interception was recorded on 3 clusters per tagged plant (60 clusters per treatment).

Leaf area measurement. After harvest, 20 fruiting shoots per treatment were randomly selected and removed from extra-vines, within the two rows in which the experiment was set, which were subjected to both treatments. The areas of main and lateral leaves were measured with a LI-3100 A (Li-cor, Lincoln, Nebraska, USA), and the leaf area of each vine was calculated, multiplying the average leaf area of the 20 shoots by the number of shoots per vine.

Berry sampling. At harvest, a sample of 60 berries was taken from each of the 40 tagged plants, representing each experimental unit (2400 berries total). Each 60-berry sample was divided into three subsamples, each consisting of 20 berries, for the following determinations: a) must biochemical parameters, b) skin and seed tannins, and c) skin flavonols. The berries for the determinations of must biochemical parameters were processed immediately, while the remaining samples were frozen and stored at –80°C.

Biochemical analysis of must. Must parameter samples were analyzed to determine the soluble solids concentration using a temperature-compensating Maselli R50 refractometer (Maselli Misure, Parma, Italy). A Crison Titrator (Crison Instruments, Barcelona, Spain) was used to measure must pH and titratable acidity.

Analysis of berry flavan-3-ols and proanthocyanidins. Flavan-3-ols and proanthocyanidins were extracted from the skins and seeds of 20 berries ground separately to a fine powder with liquid nitrogen before extracting 1 mg of the sample in 1mL 70% (v/v) acetone in water, for 24 hours in a dark room (Downey et al. 2003b). Skin and seed extracts were then centrifuged (15 minutes, 13000 rpm), and two 400 µL aliquots of the supernatant were dried under

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vacuum at 20°C. Pellets were stored at -20°C. For the analysis of free monomers, one of these pellets was resuspended in 100 µL methanol acidified with 1% HCl, then neutralized with 100 µL sodium acetate (200 mM, pH 7.5). The other one was used for the analysis of terminal and extension subunits and underwent acid-catalyzed cleavage of the proanthocyanidins in the presence of excess phloroglucinol, following the Kennedy and Jones method (2001). Determinations of the cleaved and uncleaved samples were performed with an HPLC Waters 1525 equipped with a diode array detector (DAD) and a reversed-phase column (RP18 250 x 4 mm, 5 µM) with a pre-column (Phenomenex, Castel Maggiore, Bologna, Italy) following two different procedures proposed by Downey et al. (2003b). For the uncleaved samples, solvent A, 0.2% phosphoric acid, solvent B, 4:1 acetonitrile: 0.2% phosphoric acid (gradient of solvent B: zero min, 0%; 5 min, 10%; 40 min, 10%; 55 min, 17%; 65 min, 19%; 75 min, 19%; 80 min, 100%; 85 min, 100%; 86 min, 0%). For the cleaved samples, solvent A, 0.2% acetic acid, solvent B, methanol (gradient of solvent B: zero min, 1%; 40 min, 1%; 120 min 30%; 120.1 min, 100%; 125 min, 100%; 126 min, 1%). For both methods, 25 µL of sample was injected and run at 25°C with a flow rate of 1 mL/min.

The concentrations of free monomers and hydrolyzed terminal subunits were determined from standard curves prepared with commercial standards of catechin, epicatechin, epicatechin-gallate, and epigallocatechin (Extrasynthese, Genay, France) by measuring absorbance at 280 nm (Downey et al. 2003b). The concentration of extension subunit-phloroglucinol adducts was calculated from published molar extinction coefficients (Kennedy and Jones 2001). The mean degree of polymerization (mDP) was calculated by summing terminal and extension subunits and dividing by terminal subunits (Downey et al. 2003b).

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159 **Analysis of berry flavonols.** Flavonols were extracted from the skins of 20 berries by
160 soaking the peeled skins in 100 mL methanol for 24 hours in a dark room at 20°C, and 5 mL of
161 supernatant underwent acid hydrolytic cleavage of the flavonol glycosides (Mattivi et al. 2006).
162 The HPLC instrument was equipped as described above and the concentrations of quercetin,
163 kaempferol, and myricetin aglycons were determined from standard curves prepared with
164 commercial standards of these compounds (Extrasynthese, Genay, France) by measuring
165 absorbance at 370 nm.

166 The contents of flavonols, flavan-3-ols, and proanthocyanidins were expressed as mg per
167 kg of berries (mg kg^{-1}), in order to compare the concentrations in grape with those in the resulting
168 wines.

169 **Small-scale vinifications.** At harvest, grapes of each treatment were divided into two lots,
170 and all wines were produced for both vintages as small-scale batch fermentations of about 40 kg
171 each (four vinifications per year) at the ASTRA experimental winery (Tebano, Ravenna, Italy).
172 Each fermentation was conducted with about 25 L of must.

173 Given that alcohol level and acid concentration can affect the mouthfeel of phenolics
174 (Gawel et al., 2013), the vinification protocol was designed to allow uniform fermentation and
175 similar levels of alcohol and acids between treatments. In these conditions, possible variations of
176 astringency and bitterness may be related to differences in phenolic compounds.

177 After grapes were destemmed and crushed by a destemmer-crusher Cingano POS01 DND
178 (Della Toffola, Treviso, Italy), a cold prefermentative maceration was performed in stainless steel
179 containers at 10°C in the absence of oxygen for 24 hours to enhance the aromatic profile of the
180 wines. Recently, some winery has started applying this technique since Grechetto gentile wines

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are quite poor in varietal aromas. Sugar content of the musts was then adjusted before alcoholic fermentation with glucose, and the acidic profile of the wines was uniformed before bottling by adding tartaric and malic acid.

The remaining part of the operations followed a standard protocol used for the vinification of white grapes. After the cold prefermentative maceration, must was separated with a Speidel hydraulic press (Inderst, Bolzano, Italy), performing one cycle at 0.3 MPa for 12 min and was sulphited by adding 50 mg/L of SO_2 as potassium metabisulfite. Must was kept at 8°C for 24 hours for clarification in 30 L stainless steel containers and then racked in similar containers. Juice yields ranged between 65 and 68% (equal to 650 to 680 mL of must per kg of grape). Must was inoculated with 20 mg/L of a commercial yeast strain (Zymaflore® VL2, Laffort, Bordeaux, France) for the alcoholic fermentation that took place at 18°C for 15 days in 2014 and 19 days in 2015. After alcoholic fermentation, wines were sulphited by adding 30 mg/L of SO_2, cooled to 8°C for 24 hours, and racked in stainless steel containers. Wines were stored in these containers with N_2, to prevent oxidation until bottling. At bottling, performed after five months of storage, wines were not filtered and 20 mg/L of SO_2 were added.

Chemical analysis of wines. The determinations of wine tannins were performed using the methyl cellulose precipitable assay (MCP) proposed by Sarneckis et al. (2006). The analyses of alcohol content, residual sugars, pH, volatile acidity, and organic acids were conducted in the ASTRA laboratory (Tebano, Ravenna, Italy), following the International Organization of Vine and Wine official method (OIV, 2017), while the analyses of flavonols and hydroxycinnamic acids were performed in the laboratory of the "Fondazione E. Mach – IASMA" (San Michele all'Adige,

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Trento, Italy), following the protocol proposed by Mattivi et al. (2006) and an internal protocol respectively.

Sensory analysis of wines. Descriptive analyses of wines were conducted three months after bottling at ASTRA laboratory by a group of twenty panelists: twelve females and eight males for 2014 wines, eleven females and nine males for 2015 wines. Their ages ranged from 26 to 59 years. ASTRA laboratory continuously trains its panelists, and the group that evaluated our Grechetto gentile wines had lengthy experience in white wine sensory analysis. Three training sessions were conducted to recognize and rate in a similar and reproducible manner the perceived intensity of the following parameters: color, floral aroma, fruity aroma, vegetal aroma, acidity, sapid taste, body, astringency, and bitterness. Information about the reference standard of the sensory parameters is included as supplemental data (Supplemental Table 1).

Wine sensory analyses were performed in one tasting session per vintage, conducted in separate booths, at 21°C ambient temperature. Two samples (40 mL) for each replicate were served in standard ISO 3591 glasses, labeled with different letters. Panelists evaluated each sample for about 5 min and scored the perceived intensity of nine previously selected attributes on a 9 cm unstructured linear scale. The left-side end of the scale was “low intensity” and the right-side “high intensity.” Panelists rested 3 min between samples, and in the meantime, they ate an unsalted cracker and rinsed their mouths with deionized water.

Statistical analysis. All data were subjected to a combined analysis of variance over years performed using the mixed procedure available in SAS v9.0 (SAS Institute, Inc., Cary, NC, USA). Treatment comparisons were analyzed using the Tukey’s honestly significant difference (HSD) test for pairwise comparison with mean separation by alpha = 0.05.

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Results and Discussion

Environmental condition, light incidence, and berry temperature. Seasons of 2014 and 2015 were characterized by different climatic conditions: during the period from July to September, rainfall sums were 262.9 mm in 2014 while only 71.2 mm in 2015, and the average air temperature was 21.2°C in the first year and 23.7°C in the second (Figure 1).

As expected, clusters of LR vines were highly exposed to sunlight, while the light incidence on Control was significantly lower (Table 1). Regardless of treatment, higher cluster exposure was found in 2015 than in 2014. In both seasons, berry temperatures of LR vines were higher, as were the number of hours in which berry temperature exceeded 30°C and the number of hours in which it rose above 35°C. These thresholds were adopted for the study because several authors reported detrimental effects on the biosynthesis of anthocyanins and flavonols above these temperatures (Spayd et al. 2002, Tarara et al. 2008, Pastore et al. 2017b).

Leaf area, yield components, and grape composition. All leaves on the basal nodes of the shoots were removed in the LR treatment, considerably reducing main and lateral leaf area (Table 2). In 2014, lateral leaf area was higher than in 2015, and this may be linked with the abundance of rainfall in the former. Similarly, an overall increase in pruning wood was observed in the first year, without differences between treatments.

Cluster zone leaf removal did not influence crop load, cluster, or berry weights (Table 2), as previously reported when basal leaf removal was applied postbloom (Feng et al. 2015).

Sugar concentration at harvest was not affected by leaf removal as, despite the leaf removal, all values of the fruit-to-leaf ratio (Table 3) were very high. In 2014, basal leaf removal raised must pH and lowered titratable acidity. These changes may be linked to the increase of berry

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246 temperature (Table 1), which is involved in the reduction of malic acid (Lakso and Kliewer 1975).
247 In 2015, pH and acidity were higher and lower respectively than in the previous year, but no
248 difference appeared between Control and LR: potentially the hotter climate of 2015 led to the high
249 temperature threshold values also being reached in Control berries (286 hours above 30°C), which
250 may have determined a malic acid reduction as happened in LR berries.

251 **Skin and seed phenolic compounds.** The HPLC analysis did not detect free monomers
252 from the skin tissues, and so data of terminal and extension subunits were reported. Cluster zone
253 leaf removal did not affect total skin tannins (Table 4) as reported on Merlot by Sivilotti et al.
254 (2016), but just the concentration of terminal subunits, as again found on Merlot by Yu et al.
255 (2016). The concentration of extension subunits, which represents the largest part of skin tannins
256 (Downey et al. 2003b), was not affected by leaf removal. Similar results were found on Shiraz by
257 Ristic et al. (2010) in a comparison between berries naturally shaded by foliage and berries highly
258 exposed to sunlight via leaf removal.

259 Previous studies investigated the role of light on the fate of skin tannins by comparing
260 intense artificial shading treatments with a control whose vines were not subjected to leaf removal,
261 and an overall decrease of these compounds was observed in the shaded berries of Shiraz (Ristic
262 et al. 2007). It has been reported in the literature that the absence of light, caused by artificial
263 cluster shading, can have a detrimental effect on skin tannins, but our results showed that the
264 increment of light interception induced by cluster zone leaf removal does not stimulate any
265 additional skin tannins accumulation compared to natural shading. In our study, the temperature
266 also increased due to the higher solar irradiance (following leaf removal), but it did not modify the
267 accumulation of skin tannins, as previously evidenced altering berry temperature by forced

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convection on clusters of Merlot grapes without modification of cluster light exposure (Cohen et al. 2012).

Skin tannin mean degree of polymerization (mDP) was lower in LR treatment, since in the calculation of mDP the denominator (terminal subunits) was higher and the numerator (total proanthocyanidins) unchanged. The composition of skin tannins was not affected by basal leaf removal, but in the hotter season (2015), higher percentages of epicatechin were found counterbalanced by a lower level of epigallocatechin. The effect of high temperature on epigallocatechin and epicatechin is still not well understood, but in recent researches, it appears that it might be related to variety because with higher temperature Merlot and Shiraz showed an increase of epigallocatechin and decrease of epicatechin, while on Cabernet Sauvignon no change of epigallocatechin was detected (Hochberg et al. 2015, Yu et al. 2016).

Cluster zone leaf removal decreased the concentration of seed flavan-3-ol free monomers in both years, while no difference was found in the concentration of terminal and extension subunits (Table 5). The concentration of total seed flavanols was lower in LR berries only in 2015, when the values of LR and Control berries reached higher values than those in 2014. Basal leaf removal did not affect the composition of seed flavanols nor their mDP. The effect of sun exposure on seed flavanols is not clear: Ristic et al. (2010) found no statistical difference between exposed and naturally shaded cv. Shiraz berries, whereas Yu et al. (2016) reported a slight increase following leaf removal in only one of two studied years. No effect of artificial shading was noted in concentration and composition of cv. Shiraz berry seed flavanols (Downey et al. 2004).

The free forms of myricetin, quercetin, and kaempferol were detected after acid hydrolysis (Table 6). In 2014, myricetin was found only in traces in Control berries; it was reported to be

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absent in white winegrapes (Mattivi et al. 2006), but cluster zone leaf removal strongly stimulated the accumulation of this flavonol. Quercetin and kaempferol also increased drastically after leaf removal, and a 30-fold increase of total flavonols was observed. A similar effect of leaf removal was also found in 2015, with a 3-fold increase of their concentration.

The results of basal leaf removal on the white winegrape Grechetto gentile are coherent with the overall increase of flavonols found in previous studies conducted on different red winegrapes (Feng et al. 2015, Pastore et al. 2017a), confirming the high sensitivity of flavonols to changes in environmental conditions. In particular, flavonol biosynthesis has been extensively studied in response to its induction by UV-containing light, reflecting its role as UV protectant (Spayd et al. 2002, Pastore et al. 2013).

In Control berries, the quercetin percentage was above 70%, which is a common value for many white winegrapes (Mattivi et al. 2006), but leaf removal modified the flavonol profile by an increase in the kaempferol percentage counterbalanced by a decrease in quercetin. The myricetin percentage was higher in 2014, while no difference was noted in 2015.

Considering the results on LR clusters in the two years, the lower level of flavonols found in 2015 compared to that of 2014 is probably due to the very high temperatures (above 35°C) that occurred mainly in July and to a lesser extent in August to which berries were subjected. Indeed, recent papers (Degu et al. 2016, Pastore et al. 2017b) reported that temperatures higher than 35°C had a detrimental effect on the concentration of flavonols. However, considering only the Control clusters, the more intense light incidence reported in 2015 than in 2014 (Table 1) could have induced the flavonol rise.

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311 **Chemical composition and sensory attributes of wines.** As expected, due to sugar
312 concentration and tartaric and malic acid standardization done within each vintage, no difference
313 was detected in the alcohol, in pH, and in each acid content of wines of the same years (Table 7).
314 Changes observed in grape composition due to different climatic conditions of the two
315 years determined differences in the wine: in 2015, the alcohol content was higher than that of 2014
316 and reached 15% v/v, while acidity, in particular malic acid, was much lower.
317 The phenolic compounds detected in wines were tannins, hydroxycinnamic acids, and
318 flavonols. The analysis of tannins did not show any difference between Control and LR wines, and
319 the absence of changes is coherent with the similar contents detected in the skin. Seed flavanols
320 should not be present in our wines because the condition at which the cold prefermentative
321 maceration was conducted (low temperature, absence of alcohol, and limited duration) should
322 avoid their extraction. No differences were shown in hydroxycinnamic acids. The only changes
323 regarding wine phenolic compounds were found in flavonols: LR wines showed higher
324 concentrations than Control in both years, resembling the differences found in grapes.
325 The results of the organoleptic analysis are reported in Table 8. In the cooler year (2014),
326 color was more intense in LR than Control, while the vegetal aroma was higher in Control wines.
327 The reduced vegetal aroma in LR wines found in the cooler year may be due to the decrease of
328 methoxypyrazines in exposed clusters (Ryona et al. 2008). In 2015, no change in vegetal aroma
329 was noticed, and it seems that in the warmer year, sunlight exposure had less effect on temperature-
330 dependent aromas (e.g., methoxypyrazines), probably because high temperatures also had a strong
331 detrimental effect on the shaded grapes.

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In 2014, LR wines had a higher level of astringency and bitterness while no difference in sensorial properties was found between wines of the following vintage. In concordance with the findings reported by Ferrer-Gallego et al. (2016), our results of the 2014 vintage showed that wines with higher concentration of flavonol were more astringent and bitter. The lack of difference in the mouthfeel of 2015 can be explained by the findings of Gawel et al. (2013), who reported that the mouthfeel of phenolics is more evident at low pH and moderate alcohol level. In our study, changes in mouthfeel were found only when wine pH was around 3.20 and alcohol content about 13% and not when higher values of pH and alcohol were reached, as happened in 2015. We can speculate that the astringency and bitterness differences found in 2014 wines might be favored by the direct contribution of low pH to these mouthfeel sensations (Gawel et al. 2013).

Although it is well known that phenolics elicit astringency and bitterness, it is still very difficult to explain the mouthfeel of each compound, also because it was demonstrated that tannins and hydroxycinnamic acids have a synergistic effect on the perception of their flavor (Ferrer-Gallego et al. 2014). In our study, higher astringency and bitterness were perceived in wines with higher concentration of flavonols and similar concentration of tannins and hydoxycinnamic acids, but only when pH and alcohol content were moderate. Given the role that flavonols play in enhancing the mouthfeel of other phenolic compounds (Scharbert and Hofmann 2005), we can speculate that the difference in the concentration of flavonols, although of small magnitude, may have increased the perception of astringency and bitterness of tannins and hydoxycinnamic acids.

Finally, it is to be considered that the cold prefermentative maceration, performed to improve wine aroma, may have increased the extraction of phenolic compounds and consequently the perception of astringency and bitterness.

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Conclusions

Our findings for Grechetto gentile grown in northern Italy revealed that the increase in cluster light exposure after leaf removal induced a grape acidity decrease in the cooler season and a rise of flavonols in both years, while no effect was found on skin tannins. Small-scale vinifications were conducted following a protocol designed to verify differences in the mouthfeel of phenolics, including a cold prefermentative maceration. The latter technique, despite not frequently performed in white wine vinification, sometimes is adopted to enhance the aromatic profile of Grechetto gentile wines. Wine obtained from LR grapes was more astringent and bitter in the first season, when pH and alcohol were moderate. Our study showed that the increase in light incidence on white winegrape clusters may intensify undesired sensations in wine. Further research is needed to confirm the role that flavonols and other phenolic compounds play on the sensation of astringency and bitterness, and it will be important to verify these findings on other white winegrapes, in particular those cultivated worldwide.

In conclusion, given that in the Bologna area Grechetto gentile grapes are, in most cases, harvested at relatively low levels of sugar concentration and pH for the production of sparkling wines, the canopy should be managed to prevent high cluster exposure during ripening to avoid unpleasant astringency and bitterness.

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Table 1 Light incidence on cluster and berry temperatures recorded in Grechetto gentile vines subjected to cluster zone leaf removal on stage BBCH 53 (LR) or Control, in 2014 and 2015.

Parameter	2014		2015		Year effect[a]	Treatment x year interaction[a]
	Control	LR	Control	LR		
Light incidence on cluster (%)[b]	4.4 b[c]	61.4 a	12.4 b	67.3 a	*	ns
Average berry temperature (°C)[d]	20.0 b	21.2 a	23.3 b	24.3 a	*	ns
Maximum berry temperature (°C)	32.4 b	42.4 a	39.0 b	43.2 a	*	ns
Berry temperature above 30 °C (h)	40 b	160 a	286 b	395 a	*	ns
Berry temperature above 35 °C (h)	0 b	8 a	4 b	108 a	*	ns

[a]Asterisks indicate significance at: *, $\alpha < 0.05$; ns, not significant.
[b]Light incidence was measured at 10:00 AM on a day of full sun (7 August 2014 and 27 July 2015) with a pyranometer.
[c]Different letters within a row for a given year indicate significant differences after Tukey test.
[d]Temperature measurements taken hourly from 12 July to 19 September 2014 and from 3 July to 10 September 2015, using microprobes connected to a datalogger.

Table 2 Vegetative parameters and yield components recorded in Grechetto gentile vines subjected to cluster zone leaf removal on stage BBCH 53 (LR) or Control, in 2014 and 2015.

Parameter	2014		2015		Year effect[a]	Treatment x year interaction[a]
	Control	LR	Control	LR		
Main leaf area (m^2 / vine)	4.38 a[b]	2.21 b	4.10 a	2.15 b	ns	ns
Lateral leaf area (m^2 / vine)	9.11 a	6.29 b	7.24 a	5.71 b	**	ns
Cluster (n° / vine)	23.4	23.6	24.5	25.1	ns	ns
Yield/vine (kg)	4.18	4.65	4.49	4.54	ns	ns
Cluster weight (g)	177.9	185.8	183.4	181.9	ns	ns
Berry weight (g)	2.15	2.08	1.98	2.00	ns	ns
Pruning wood (kg / vine)	3.63	3.19	2.31	2.21	*	ns

[a]Asterisks indicate significance at: *, $\alpha < 0.05$; **, $\alpha < 0.01$; ns, not significant.
[b]Different letters within a row for a given year indicate significant differences after Tukey's HSD test.

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Table 3 Grape composition at harvest and leaf-to-fruit ratio recorded in Grechetto gentile vines subjected to cluster zone leaf removal on stage BBCH 53 (LR) or Control, in 2014 and 2015.

Parameter	2014		2015		Year effect[a]	Treatment x year interaction[a]
	Control	LR	Control	LR		
Soluble solids (°Brix)	21.3	22.1	24.6	23.8	***	**
pH	3.21 b[b]	3.32 a	3.60	3.58	***	*
Titratable acidity (g/L)	10.70 a	8.31 b	5.36	5.58	***	*
Leaf-to-fruit ratio (m^2 / kg)	3.49 a	1.98 b	2.68 a	1.77 b	ns	ns

[a]Asterisks indicate significance at: *, $\alpha < 0.05$; **, $\alpha < 0.01$; ***, $\alpha < 0.001$; ns, not significant.
[b]Different letters within a row for a given year indicate significant differences after Tukey's HSD test.

Table 4 Berry-skin tannin concentration, mDP and composition recorded in Grechetto gentile vines subjected to cluster zone leaf removal on stage BBCH 53 (LR) or Control, in 2014 and 2015.

Parameter	2014		2015		Year effect[a]	Treatment x year interaction[a]
	Control	LR	Control	LR		
Terminal subunits (mg / kg berries)	77.6 b[b]	85.6 a	68.4 b	85.0 a	ns	ns
Extension subunits (mg / kg berries)	1510	1517	1050	1188	ns	ns
Total tannins (mg / kg berries)	1587	1603	1118	1273	ns	ns
mDP[c]	20.5 a	18.7 b	16.4 a	15.0 b	**	ns
Catechin (%)	6.4	7.2	7.6	8.4	ns	ns
Epicatechin (%)	43.3	43.8	53.4	53.8	**	ns
Epigallocatechin (%)	47.6	46.3	36.9	35.7	**	ns
Epicatechin-gallate (%)	2.7	2.7	2.1	2.1	ns	ns

[a]Asterisks indicate significance at: **, $\alpha < 0.01$; ns, not significant.
[b]Different letters within a row for a given indicate significant differences after Tukey's HSD test.
[c]mDP: mean degree of polymerization.

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Table 5 Seed flavanol concentration, mDP and composition recorded in 'Grechetto gentile' vines subjected to cluster zone leaf removal on stage BBCH 53 (LR) or Control, in 2014 and 2015.

Parameter	2014		2015		Year effect[a]	Treatment x year interaction[a]
	Control	LR	Control	LR		
Free Monomers (mg / kg berries)	230.8 a[b]	147.2 b	320.3 a	175.3 b	**	ns
Terminal subunits (mg / kg berries)	160.5 b	195.1 a	229.8 a	200.5 b	**	**
Extension subunits (mg / kg berries)	872.3	940.1	1065.6	1024.5	**	ns
Total flavanol (mg / kg berries)	1264	1282	1616 a	1400 b	**	**
mDP[c]	6.45	5.92	5.71	6.15	ns	ns
Catechin (%)	23.1	23.3	26.3	24.1	ns	ns
Epicatechin (%)	54.9	52.5	52.0	52.1	ns	ns
Epicatechin-gallate (%)	22.1	24.3	21.7	23.8	ns	ns

[a]Asterisks indicate significance at: ** $\alpha < 0.01$; ns not significant.
[b]Different letters within a row for a given indicate significant differences after Tukey's HSD test.
[c]mDP: mean degree of polymerization.

Table 6 Berry flavonol concentration and composition recorded in 'Grechetto gentile' vines subjected to cluster zone leaf removal on stage BBCH 53 (LR) or Control, in 2014 and 2015.

Parameter	2014		2015		Year effect[a]	Treatment x year interaction[a]
	Control	LR	Control	LR		
Quercetin (mg / kg berries)	2.70 b[b]	71.82 a	15.38 b	42.14 a	**	**
Kampferol (mg / kg berries)	0.26 b	30.37 a	4.16 b	17.99 a	**	**
Myricetin (mg / kg berries)	0.03 b	2.73 a	2.11 b	5.66 a	**	**
Total flavonols (mg / kg berries)	2.99 b	104.9 a	21.65 b	65.78 a	**	**
Myricetin (%)	0.6 b	2.6 a	10.1	8.7	**	ns
Quercetin (%)	92.5 a	68.8 b	71.8 a	64.2 b	**	ns
Kampferol (%)	6.9 b	28.6 a	18.1 b	27.0 a	**	ns

[a]Asterisks indicate significance at: ** $\alpha < 0.01$; ns not significant.
[b]Different letters within a row for a given year indicate significant differences after Tukey's HSD test.

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Table 7 Chemical composition of 'Grechetto gentile' wines obtained from leaf removal treatment (LR[a]) or 'Control' vines, in 2014 and 2015. Wines were analyzed three months after bottling.

Parameter	2014		2015		Year effect[b]	Treatment x year interaction[b]
	Control	LR	Control	LR		
Alcohol (% v/v)	13.3	13.3	14.7	14.7	**	ns
Residual sugars (g / L)	< 1	< 1	< 1	< 1	ns	ns
Total dry extract (g / L)	22.2	21.4	19.9	19.5	ns	ns
pH	3.21	3.24	3.68	3.60	**	ns
Volatile acidity (g / L)	0.25	0.20	0.41	0.50	**	ns
Tartaric acid (g / L)	1.60	1.45	0.80	0.80	**	ns
Malic acid (g / L)	5.10	4.85	2.45	2.50	**	ns
Lactic acid (g / L)	0.32	0.32	0.30	0.30	ns	ns
Citric acid (g / L)	0.41	0.36	0.30	0.30	*	ns
Tannins (mg / L)	38.6	44.3	47.6	54.7	ns	ns
Hydroxycinnamic acids (mg / L)	46.6	52.9	36.7	43.7	ns	ns
Flavonols (mg / L)	0.65 b[c]	1.15 a	0.38 b	1.75 a	ns	ns

[a]LR vines were subjected to cluster zone leaf removal at stage BBCH 53.
[b]Asterisks indicate significance at: * $\alpha < 0.05$; ** $\alpha < 0.01$; ns not significant.
[c]Different letters within a row for a given year indicate significant differences after Tukey's HSD test.

Table 8 Perceived intensity of sensorial traits of 'Grechetto gentile' wines obtained from leaf removal treatment (LR[a]) or 'Control' vines, in 2014 and 2015. Wines were analyzed three months after bottling. Lowest intensity is scored 0, highest intensity is scored 9.

Parameter	2014		2015		Year effect[b]	Treatment x year interaction[b]
	Control	LR	Control	LR		
Color intensity	4.05 b[c]	5.47 a	5.01	5.06	ns	**
Floral aroma	3.90	4.15	4.09	3.95	ns	ns
Fruity aroma	3.99	3.97	4.09	3.87	ns	ns
Vegetal aroma	3.77 a	3.40 b	3.04	3.02	*	**
Acidity	4.44	4.63	3.89	3.81	*	ns
Sapid taste	4.43	3.99	3.86	4.27	ns	**
Body	4.28	4.35	4.75	4.76	*	ns
Astringency	3.52 b	3.90 a	3.12	3.10	**	ns
Bitterness	3.15 b	3.55 a	3.31	3.32	ns	ns

[a]LR vines were subjected to cluster zone leaf removal at stage BBCH 53.
[b]Asterisks indicate significance at: * $\alpha < 0.01$; ** $\alpha < 0.01$; ns not significant.
[c]Different letters within a row for a given year indicate significant differences after Tukey's HSD test.

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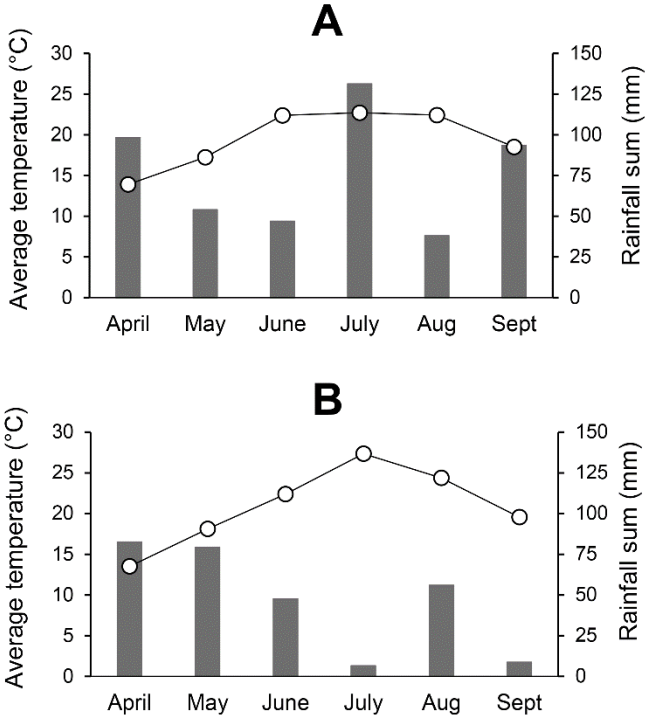

Figure 1 Average air temperature and rainfall sum from April to September in 2014 (**A**) and 2015 (**B**). Data were registered close to the experimental site (Valsamoggia, Italy) and provided by the meteorological service of the Emilia-Romagna Region (ARPAE). Bars indicate mm of rainfall; line with dots indicates temperatures.

Supplemental Table 1 Reference standards used for training the panelists.

Parameter	**Reference standard**	**Range of variation**
Floral aroma	Benzyl acetate (mg/L)	0.05 - 1
Fruity aroma	Isoamyl acetate (ppm)	5 - 100
Vegetal aroma	cis-3-Hexen-1-ol (mL/L)	0.05 - 1
Acidity	Citric acid (g/L)	0.1 - 2
Sapid taste	Sodium chloride (g/L)	0.2 - 5
Astringency	Aluminium potassium sulfate (g/L)	0.25 - 1
Bitterness	Quinine monohydrochloride dihydrate (g/L)	0.025 - 0.1
Color intensity	Low versus high-colored white wine	
Body	Light versus full-bodied white wine	