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Combined Effects of Early Season Leaf Removal and Climatic Conditions on Aroma Precursors in Sauvignon Blanc Grapes

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Supporting Information

ABSTRACT: Early leaf removal around the cluster zone is a common technique applied in cool climate viticulture, to regulate yield components and improve fruit quality. Despite the increasing amount of information on early leaf removal and its impact on total soluble solids, anthocyanins, and polyphenols, less is known regarding aroma compounds. In order to verify the hypothesis that defoliation, applied before or after flowering, could impact the biosynthesis of thiol precursors, we performed a two year (2013 and 2014) experiment on Sauvignon blanc. We provided evidence that differential accumulation of thiol precursors in berries is affected by the timing of defoliation, and this impact was related to modifications in the biosynthetic pathway. Furthermore, the possible interaction between leaf removal treatment and seasonal weather conditions, and its effect on the biosynthesis of volatile precursors are discussed. Our results suggested that in Sauvignon blanc the relative proportion of 4-*S*-glutathionyl-4-methylpentan-2-one (G-4MSP) and 3-*S*-glutathionylhexan-1-ol (G-3SH) precursors can be affected by defoliation, and this could be related to the induction of two specific genes encoding glutathione-*S*-transferases (*VvGST3* and *VvGST5*), while no significant effects on basic fruit chemical parameters, polyphenols, and methoxypyrazines were ascertained under our experimental conditions.

KEYWORDS: *early leaf removal, cluster exposure, thiol precursors, methoxypyrazines, glutathione S-transferase, Vitis vinifera*

INTRODUCTION

Leaf removal is a common viticultural practice employed to improve cluster microclimate with the aim to influence fruit composition and, ultimately, wine traits. When applied at preflowering stage, leaf removal impacts flower fertility and berry set, leading to smaller and looser clusters,[1] a desired effect in high-yielding cultivars for which this practice can be a powerful tool for controlling excessive crop potential while improving must composition.[2,3]

Despite the increasing amount of available information on the positive effect of early leaf removal on total soluble solids (TSS), anthocyanins, and other polyphenols, mainly by improving the leaf area-to-yield ratio and cluster exposure to sunlight,[4−6] less is known about its effect on aroma compounds biosynthesis. Several studies have pointed out the role of leaf removal on methoxypyrazines concentration in grapes. The accumulation and/or degradation of these compounds, in particular 3-isobutyl-2-methoxypyrazine (IBMP), in the berries and the subsequent wines, is strongly affected by light and therefore by leaf removal.[7,8]

In addition to methoxypyrazines, conferring the classic bell pepper aroma, the most important aroma compounds in Sauvignon blanc wines are varietal thiols, such as 4-methyl-4-sulfanylpentan-2-one (4MSP), reminiscent of box tree and black currant buds, and 3-sulfanylhexan-1-ol (3SH) and 3-sulfanylhexyl acetate (3SHA), responsible for the fruity and citrus notes.[9−11] For instance, the majority of 3SH derives from cysteinylated (Cys-3SH) and glutathionylated (G-3SH) conjugates, while 3SHA is formed during fermentation from 3SH acetylation.[7,8,12,13] Precursor concentrations do not correlate well with free thiol concentrations in wine but no other major source of free thiols has yet been found. Thiol precursors formation pathways are still to be fully elucidated, although some information is available. The first potential precursor, identified in Sauvignon blanc grapes, has been 3-*S*-cysteinylhexan-1-ol (Cys-3SH).[13] Thereafter 3-*S*-glutationylhexan-1-ol (G-

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3SH) was recognized as the tentative pro-precursor of Cys-3SH, suggesting that glutathione detoxification systems would produce G-3SH via glutathione-*S*-transferase (GST) activity, with subsequent catabolism to Cys-3SH.[14] Kobayashi et al.[15] reported that the formation of Cys-3SH and G-3SH in grape berries was increased through various environmental stresses, such as cold or heat shock, UV−C radiation, and biotic stress. Although the genes coding for the enzyme responsible for thiol precursors biosynthesis in ripening berries have not been formally identified, the same authors suggested a novel function of plant GSTs that should indirectly mediate flavor precursor accumulation, by promoting the production of G-3SH from glutathione and (*E*)-2-hexenal in cells under stress conditions.

The lipoxygenase-hydroperoxides lyase (LOX-HPL) pathway has a recognized role in determining volatiles composition in grape by means of C6 compound production, such as hexanal, under stress conditions.[16] Several studies have described that the amounts of glutathionylated and cysteinylated precursors in Sauvignon blanc berries during ripening are related to environmental factors, such as climate and soil composition.[14,17,18] Helwi et al.[19] reported that nitrogen supply enhanced the levels of glutathionylated precursors in grape berries at late berry ripening stages but did not affect the transcript abundance of the glutathione-*S*-transferases genes identified by Kobayashi et al.[15] However, an increase of precursor levels in grapes and musts is not always correlated with an increase of odorous compounds in wine.[20,21]

Little is known about the effect of leaf removal on thiol precursors; therefore, recent works have investigated the impact of cluster microclimate on Sauvignon blanc wines and their chemical composition.[22−24] *S*-Cysteinylated and *S*-glutathionylated thiol precursor levels increase along with TSS accumulation during berry ripening (from 16 to 22°Brix) and were detected in both the skin and the pulp of Sauvignon blanc berries.[8,25−27] Contrasting results have been recently proposed by Martin et al.[22] that investigated the effects of natural variations in cluster microclimate on Sauvignon blanc juice and wine. Authors reported that the variable clusters exposure, naturally occurring in the canopy, does not appear to affect volatile thiol content, leading to the speculation that leaf removal has a more complex effect, probably due to the decrease of photosynthates and other key metabolites that affect the ripening of grape berries.[22]

In order to bridge the knowledge gap between vineyard management and grape aroma composition, we investigated, over a two-year field experiment, the impact of early leaf removal treatments on the biosynthesis of thiol precursors in Sauvignon blanc fruit.

MATERIALS AND METHODS

Chemicals used. 3-Isobutyl-2-methoxypyrazine (IBMP), 3-isopropyl-2-methoxypyrazine (IPMP), gallic acid, L-methionine, L-glutathione (reduced), glutathione oxidized, perchloric acid, acetone and methanol (LC-MS, Chromasolv), were supplied by Sigma-Aldrich (St. Louis, MO, US). 2-Isobutyl-3-methoxy-d_3-pyrazine ([d_3]-IBMP) was supplied by C/D/N/Isotopes (Quebec, Canada). Diethyl ether, pyridine, dichloromethane, 1,4-dioxane, pentane and ethyl acetate were obtained from Merck (Darmstadt, Germany). Formic acid (LC-MS) was obtained from Fluka and ultrapure water of Milli Q gradient purification system from EMD Millipore (Billerica, MA, USA).

Cysteinylated and glutathionylated thiol precursor standards together with deuterium labeled analogues (Cys-4MSP-d_6 and G-4MSP-d_{10}) were synthesized according to the procedures described in details by Vanzo et al.[28] Chemical used for precursor synthesis were analytical or higher grade from Sigma-Aldrich.

Location, plant material, and experimental design. The experiment was conducted in a vineyard of Sauvignon blanc (clone R3, rootstock Kober 5BB) located in Oslavia (latitude: 45° 58′ 16″ N, longitude: 13° 36′ 16″), within the D.O.C. Collio viticultural area of the Friuli Venezia Giulia region (North-Eastern Italy), during two consecutive seasons (2013 and 2014). The vineyard was planted in 1996 with a plant density of 3787 plants/ha (1.20 m between vines and 2.20 m between rows). Rows were planted East−west and vines were winter pruned to a double Guyot (locally called "Cappuccina") with a vertical shoot positioned (VSP) trellis system retaining 20 buds/vine. The canopy height was 1.20 m and the last couple of catch wires was positioned 1.00 m above basal main supporting wire. During both seasons shoot trimming was manually performed (on 30 June 2013 and 25 June 2014) when the shoot tips were more than 20 cm above the catch wires. The experiment was a fully randomized design consisting of three treatments and three replicates with 20 vines selected per each plot. Treatments comparison was: (i) untreated control (CONT), where all basal leaves were retained in each shoot; (ii) before-flowering leaf removal (BFLR), where five to six leaves were removed 15 days (21 May) and 10 days (21 May) before flowering in the years 2013 and 2014, respectively; and (iii) after-flowering leaf removal (AFLR), where two to three leaves were removed 15 days (20 June) and 11 days (11 June) after flowering in 2013 and 2014, respectively. If present, laterals were retained at both timings of leaf removal. The defoliation intensity was different between BFLR and AFLR since in case of the former treatment the strong decrease of leaf area is required to limit berry-set, while as for the latter leaf removal is performed only to improve cluster sun exposure. The dates of the major phenological stages were also recorded. Flowering (50% cap fall) occurred on 5 June 2013 and 31 May 2014. Veraison (50% soft berries) occurred on 4 August 2013 (60 days after flowering, DAF) and on 29 July 2014 (59 DAF). The grapes were harvested when the TSS reached 19°Brix in the CONT, that was on 4 September (91 DAF) and on 3 September (95 DAF) in 2013 and 2014, respectively.

Leaf-Area measurements. Leaf area was assessed on the main and lateral shoots at five different times during the growing season: before and after the application of each leaf removal treatment and at harvest. For each plot, 6 shoots randomly selected were tagged in different vines and the length of the main vein of each leaf was measured, keeping separated data of main and lateral leaves. The correlation between main vein length (v) and leaf area (LA) was calculated on a sample of 50 leaves of different sizes ($LA = 0.753v^2 + 3.958v$; $R^2 = 0.9152$). Leaves were scanned and images processed with ImageJ for the determination of leaf area.[29] Total leaf area × shoot was then determined keeping separated main and lateral leaves. The total leaf area per vine (TLA) was then calculated by multiplying the number of shoots and the shoot leaf area. The leaf area-to-yield ratio (LA/Y) was calculated by rating total leaf area per vine (m^2) measured at harvest and the yield per vine (kg).

Yield parameters, berry sampling and juice analysis. Yield and cluster number per vine were determined at harvest on 10 vines per plot; average cluster weight was calculated by rating the yield and the number of clusters. Berry samples were collected during maturation on each plot. One set of samples of 50 berries each was collected every 7−10 d from approximately 60 DAF until harvest. The samples were stored in a portable cooler and transported to the laboratory within 2 h; the samples were hand-squeezed and analyzed for soluble solids (°Brix), titratable acidity (g/L) and pH with a WineScanTM FT120 Basic (FOSS, Hillerød, Denmark).

At harvest 50 intact berries were snipped with the pedicels from different clusters, selecting them randomly from different positions. In order to prevent metabolic changes after sampling, grapes were immediately frozen in liquid nitrogen, transferred to the laboratory in dry ice and stored at −80 °C. In order to analyze total polyphenols, methoxypyrazines, thiol precursors and gene expression, pedicels were removed and the berries were ground to a fine powder using an

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analytic mill (A11B IKA, Königswinter, Germany) under liquid nitrogen and stored back at −80 °C.

Both sets of samples were collected around midday from each plot in order to have 3 comparable biological replicates.

Determination of total polyphenols in berries. For each sample, an aliquot of 2 g of frozen berry powder was weighted and extracted with 5 mL of MeOH/H_2O (70:30 v:v) solution for 10 min in a ultrasound bath. The samples were then centrifuged for 10 min at 2000 rpm, the supernatant poured, and another aliquot of 3 mL of MeOH/H_2O was added to the pellet and the process was repeated. The two supernatant fractions were combined and adjusted to 10 mL with the MeOH/H_2O solution. The extracts were then filtered using 0.45 μm syringe filters (Chromafil Xtra, Macherey-Nagel, Düren, Germany), and the analysis of total polyphenols was performed using the Folin-Ciocalteau method described in Singleton and Rossi.[30] A 7-point calibration curve was created using different concentrations of gallic acid and for each concentration three replications were prepared. Grape samples were diluted to fit the range of concentration of the calibration curve. Total polyphenols were expressed as mg gallic acid equivalent (mg GAE/g of berry).

Determination of methoxypyrazines in berries. The content of 3-isobutyl-2-methoxypyrazine (IBMP) and 3-isopropyl-2-methoxypyrazine (IPMP) were determined as described in Šuklje et al.[31] and modified by Sivilotti et al.[32] Briefly, the samples were prepared by placing 3 g of NaCl into a 20 mL SPME vial along with a stir bar, followed by 2 g of grape powder, 6 mL of Milli-Q-purified water, 2 mL of 4 M NaOH and 100 μL of solution of [d_3]-IBMP (0.5 μg/L). The vial was closed and placed onto a magnetic stir plate to dissolve the NaCl. Calibration standards were prepared in same way with addition of standard solution of IBMP and IPMP (50 and 56 ng/L respectively). The samples were analyzed using a gas chromatograph (Agilent Technologies 7890A) equipped with a Gerstel MPS2 multipurpose sampler and connected to a mass spectrometer (Agilent Technologies 5975C, upgraded with triple axis detector).

Determination of thiol precursors in grapes. The analysis of thiol precursors was carried out using the method described by Vanzo et al.[28] A 10 g aliquot of pulverized frozen grapes was rapidly transferred into cold, deoxygenated methanol (1:4 w/v), spiked with deuterium labeled internal standards (Cys-4MSP-d_6 and G-4MSP-d_{10}), vortexed, extracted and centrifuged. The extract (200 μL) was filtered through a 0.22 μm PVDF Millipore filter (Billerica, MA, USA) and directly injected onto UHPLC-MS/MS (Agilent Technologies, Palo Alto, USA). Direct injection enabled quantification of glutathione, oxidized glutathione and thiol precursors present in grape extract above analytical quantification limits (ten times signal-to-noise). When the thiol precursors (mostly G-4MSP) were below analytical limits for evaluation by direct injection, grape extracts were concentrated and purified by the use of a Dowex 50WX4−100 ion-exchange resin and subsequently a hydrophobic cartridge (6 mL, 500 mg, Strata SDB-L). The eluate was evaporated to dryness under reduced pressure on a rotavapor (Bűchi, Germany) at 38 °C, redissolved in 200 μL mixture of methanol:water 1:1, filtered through a 0.22 μm PVDF filter and analyzed using UHPLC-MS/MS. Thiol precursors after purification procedure were quantified considering recoveries of deuterated internal standards. Recovery of G-4MSP-d_{10} was used for quantification of G-4MSP, whereas recovery of Cys-4MSP-d_6 was used for Cys-4MSP. 3SH precursors were usually above the LOQ with direct injection of grape extracts. If this was not the case, recovery of Cys-4MSP-d_6 was used to determine Cys-3SH, whereas G-4MSP-d_{10} was used for G-3SH.

RNA extraction, cDNA synthesis and Real-Time PCR. The three pools of berries collected at harvest for each treatment, powdered and frozen, were considered for transcriptional analyses. Total RNA was extracted with the "Spectrum Plant total RNA" kit (Sigma-Aldrich) from 0.2 g of cryogenically ground frozen berries. RNA was quantified by NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific), electrophoretically separated on a 1% agarose gel to check integrity and stored at −80 °C. The reverse transcription of RNA samples was performed with the QuantiTect Reverse Transcription Kit (Qiagen), according to the manufacturer's instructions. cDNA was stored at −20 °C. All cDNA samples were then diluted 30-fold and amplified in three technical replicates by quantitative real-time PCR, using CFX96 Real-Time PCR Detection system (Bio-Rad) and SsoFast EvaGreen Supermix (Bio-Rad). PCR conditions and specific oligonucleotide primer pairs for *VvGSTs* genes were the same as described in Kobayashi et al.[15] All quantifications were normalized to *VviUbiquitin* housekeeping gene, as described in Falginella et al.[33] Relative expression was calculated using the $\Delta\Delta Ct$ method.[34]

Statistical analysis. Line-scatters, histograms, and radar charts were constructed using SigmaPlot 13 (Systat Software GmbH, Erkrath, Germany). Software from SAS Institute Inc. (JMP 7.0) was used for statistical analyses. The data were analyzed through two -way ANOVA with treatments and years as fixed factors and when the differences were significant, means were separated with Tukey's HSD test ($p < 0.05$).

RESULTS

Weather conditions. The two seasons (2013 and 2014) were characterized by very different meteorological conditions, in terms of both temperatures and rain (Figure 1). Although in

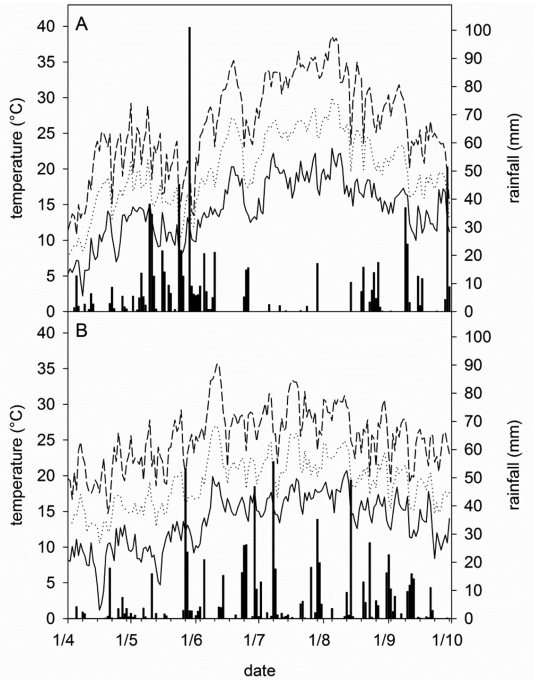

Figure 1. Daily values (April through September) of rainfall (histograms) and min, mean, and max temperatures (lines) recorded at the weather station of Capriva del Friuli in the years 2013 (A) and 2014 (B) (ARPA FVG − OSMER, http://www.meteo.fvg.it/).

2013 abundant rainfalls occurred in May, the rest of the season was characterized by moderate rainfalls, with only 106 mm from the beginning of July until the end of August. In contrast, abundant rainfall occurred in 2014, with more than 100 mm/month in the period from May to September, and with almost 200 mm/month in June and July. Higher heat accumulation, calculated as Growing Degree Days (GDD, base 10 °C), was recorded in 2013 than in 2014 (+181 GDD for the period from first of April to 30th of September), with the highest differences in July (+112 GDD in 2013) and August (+86 GDD in 2013). Solar radiation followed a similar trend to GDD in the two seasons, and it was higher in 2013 than in 2014, mainly in July (+24%), August (+14%), and September (+13%).

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Table 1. Yield Components, Grape Composition, and Thiol Precursors in "Sauvignon Blanc" Vines Subjected to Leaf Removal Treatments in 2013 and 2014

	treatment (T)				season (S)			interaction (T × S)
	CONT	BFLR	AFLR	*significance*[z]	2013	2014	*significance*	*significance*
Clusters per vine	25.8	25.0	26.1	*ns*	24.4	26.9	*	*ns*
Yield (kg/vine)	3.1 a[y]	2.8 b	3.2 a	*	2.5	3.5	***	*ns*
Cluster weight (g)	118 a	109 b	120 a	*	106	125	***	*ns*
Berry weight (g)	1.73	1.68	1.69	*ns*	1.36	2.04	***	*ns*
Berry per cluster	70.0	66.1	73.8	*ns*	78.5	61.4	***	*ns*
LA/Y ratio (m^2/kg)	1.30 b	1.86 a	1.31 b	***	1.46	1.52	*ns*	*ns*
TSS (°Brix)	19.3	19.1	19.6	*ns*	20.2	18.4	***	*ns*
Titratable acidity (g/L)	9.13	9.32	9.00	*ns*	7.29	11.01	***	*
pH	3.09	3.06	3.07	*ns*	3.16	2.99	***	*
Total polyphenols (mg/kg)	2210	2208	2386	*ns*	2568	2008	***	*ns*
IBMP (ng/kg)	10.8	12.2	12.8	*ns*	10.7	13.2	**	*ns*
Cys-4MSP (μg/kg)	1.98	2.14	1.51	*ns*	2.99	0.76	***	*ns*
Cys-3SH (μg/kg)	3.49	3.05	3.35	*ns*	4.40	2.19	**	*ns*
G-4MSP (μg/kg)	0.22	0.27	0.18	*ns*	0.32	0.13	***	*
G-3SH (μg/kg)	14.5	20.9	22.0	*ns*	24.8	13.5	***	*
GSH (mg/kg)	62.20	67.45	74.60	*ns*	68.53	67.64	*ns*	*ns*
GSSG (mg/kg)	2.59	2.35	2.26	*ns*	2.23	2.14	*ns*	*ns*

[z]Data were analyzed through two-way ANOVA (*ns*, not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$) with treatment and season as fixed factors, and when differences were significant, the means were separated using Tukey's HSD test ($p < 0.05$). [y]Different letters identify significantly different means. CONT, untreated control; BFLR, before-flowering leaf removal; AFLR, after-flowering leaf removal.

Leaf area and yield components. BFLR dropped the total leaf area by 47.0% at the time of application which was statistically lower when compared with the other two treatments. In contrast, at the time of AFLR the shoots were more developed and a lower number of leaves was removed; thus, the decrease of total leaf area was milder and accounted for 6.9% of the actual leaf area. At harvest no differences in leaf area were ascertained among treatments. Meteorological differences probably accounted for significantly lower main and higher lateral leaf area development in the warmer season 2013, as compared to 2014 which was cooler.

The timing of leaf removal significantly impacted yield parameters. As expected, the number of clusters was not significantly different between treatments (Table 1). On the other hand, the yield was lower in BFLR (−10%), as a result of a lower mean cluster weight (−8%). Berry weight and number of berries per cluster were not significantly different between treatments. Comparing the two seasons, the yield was significantly higher in 2014, because of both the higher average cluster weight and higher cluster number per vine.

The leaf area-to-yield ratio at harvest (Table 1) was significantly modified (+30% compared to control vines) by the BFLR as a clear consequence of the yield drop, while in the case of AFLR the values were much lower and similar to CONT (Table 1).

Berry composition. The application of BFLR and AFLR did not promote any significant modification in basic fruit chemistry parameters at harvest (TSS, TA, and pH), total polyphenols, and IBMP (Table 1). On the other hand, the dynamic of berry maturation was affected by the meteorological conditions of the seasons. The increase of sugars and pH was faster in 2013 than in 2014 (Figure 2A, 2C), and, by the time of harvest, it reached significantly higher values in 2013 (Table 1). In contrast, the rate of degradation of titratable acidity was higher in 2013 (Figure 2B), and significantly lower values were registered at harvest when compared to 2014 (Table 1). In addition, the concentration of total polyphenols and IBMP at harvest was not significantly different among treatments, even if slightly higher values of IBMP could be observed in the case of both BFLR and AFLR (Table 1). The concentration of IPMP was below the analytical limit of detection (LOD) at the time of harvest (data not reported).

The composition of thiol precursors was slightly modified by both leaf removal treatments, although statistical differences were observed only for G-3SH and G-4MSP in 2013 (Table 1, Figure 3B,3D). On the other hand, significantly lower concentrations of all precursors were observed in the second season. As shown in Table 1 and Figure 3A and 3C, the concentrations of Cys-4MSP and Cys-3SH were not significantly modified by leaf removal treatments. As far as the G-4MSP trend is concerned (Figure 3B), significant differences were recorded in 2013, between BFLR and AFLR, with higher values in the case of the former, even if both the treatments did not show significant variation from CONT. Similarly, the effect of the treatments on the G-3SH concentration was significant only in 2013, with G-3SH levels the highest in AFLR, followed by BFLR and CONT (Figure 3D). The concentrations of GSH and GSSG were not significantly affected by both season and leaf removal treatment (Table 1).

Gene expression analysis. At harvest, *VvGST1* and *VvGST4* showed very low expression levels in berries, in both experimental years, even if *VvGST4* appeared significantly responsive to treatment in 2014. Conversely, *VvGST2*, *VvGST3*, and *VvGST5* were more highly expressed, with *VvGST5* transcripts being the most abundant (Figure 4). Interesting results were obtained in the 2013 season, when *VvGST3* and *VvGST5* genes, mirroring the G-3SH precursor accumulation pattern, appeared significantly up-regulated by leaf removal, with the lowest level in CONT, increased level in BFLR, and highest level in AFLR berries (Figure 4A). On the other hand, in 2014, no significant differences were found in the highly expressed genes which, similarly to the G-3SH precursor, appeared mostly unaffected by leaf removal treatments (Figure 4B).

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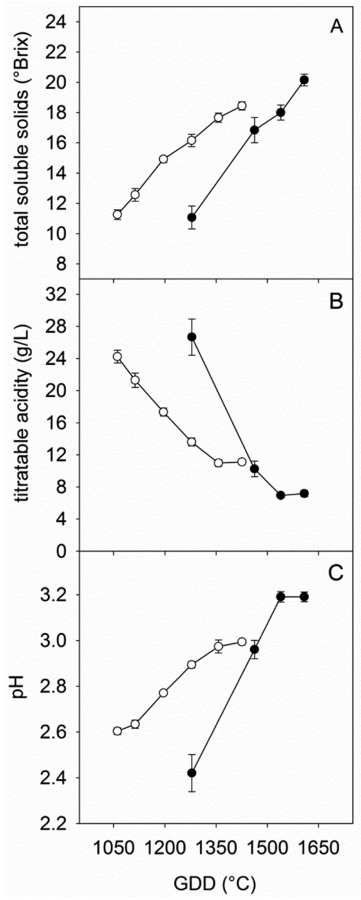

Figure 2. Evolution of soluble solids (A), titratable acidity (B), and pH (C) in *Vitis vinifera* "Sauvignon blanc" grapes in 2013 (●) and 2014 (○) as related to the cumulated growing day degrees (GDD, °C) calculated from the 1st of April. Each point represents the average of all treatments and replicates in comparison, and bars indicate standard error.

DISCUSSION

Our experiment carried out in Oslavia, by comparing leaf removal, applied before and after flowering, confirmed previous research and provided new insights on thiol precursors in Sauvignon blanc grapes. Although the implications of leaf removal applied in the preflowering stage are well-known and widely explained in the literature,[1,2,32,35−38] our research aimed to describe the effects of this treatment on the accumulation of thiol precursors in grape berries and to investigate the possible role of glutathione-*S*-transferase encoding genes in this process.

In our study, berry maturation parameters (i.e., TSS, TA, pH) were not significantly affected by both leaf removal treatments (Table 1), as already reported by several authors.[32,36,38−41] Similarly, the concentration of polyphenols was not significantly influenced by the leaf removal treatments, while a significant change was noticed between the two seasons (Table 1). This represents an interesting issue when considering that most of the studies on leaf removal have been carried out on red grape varieties, demonstrating the positive effect of this viticultural technique on the accumulation and concentration of anthocyanins,[5,9] flavonols,[5,6] and flavan-3-ols[5,32,42] at harvest, whereas little is known on the biosynthesis of phenolic compounds, accumulated at lower concentration, in white varieties.

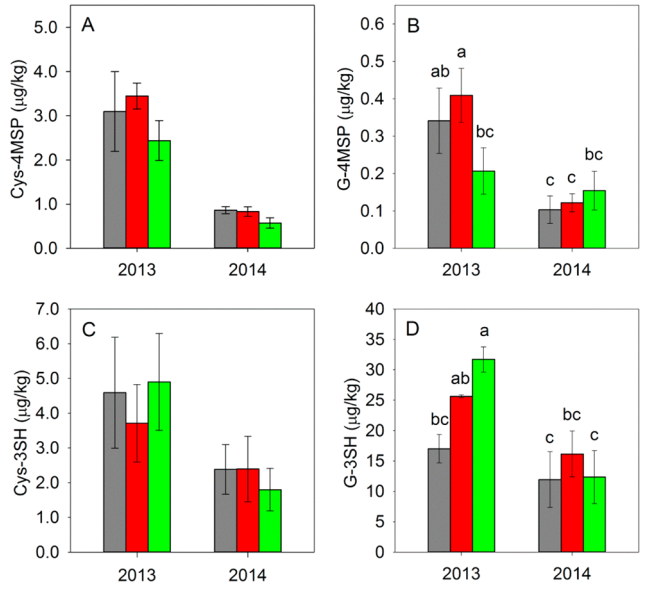

Figure 3. Concentrations of Cys-4MSP (A), G-4MSP (B), Cys-3SH (C), and G-3SH (D) in *Vitis vinifera* "Sauvignon blanc" berries at harvest time in 2013 and 2014 as affected by leaf removal treatments. Treatment × season interaction was significant with two-way ANOVA full factorial analysis (Table 1), and means were separated with Tukey's HSD test ($p < 0.05$). Different letters identify significantly different means, and bars indicate standard error. (gray) CONT, untreated control; (red) BFLR, leaf removal before flowering; (green) AFLR, leaf removal after flowering.

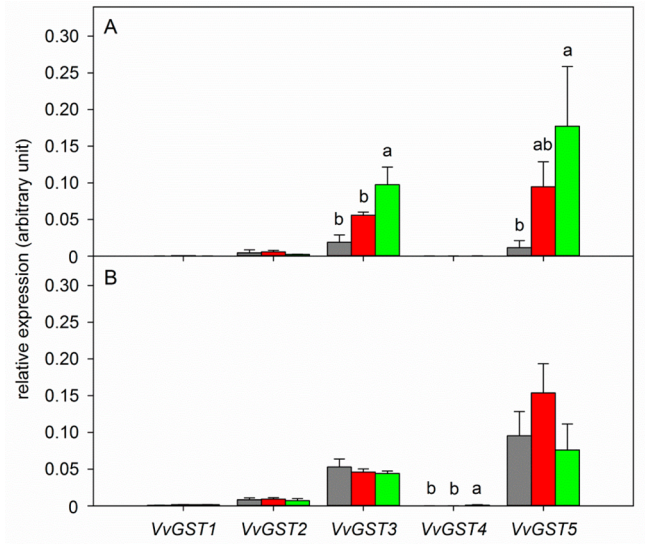

Figure 4. Relative expression of genes coding for glutathione-*S*-transferase (*VvGST*) in *Vitis vinifera* "Sauvignon blanc" berries at harvest time in the seasons 2013 (A) and 2014 (B), as affected by leaf removal treatments. Data were analyzed by one-way ANOVA, and when the differences were significant, means were separated with Tukey's HSD test ($p < 0.05$). Different letters identify significantly different means, and bars indicate standard error. (gray) CONT, untreated control; (red) BFLR, leaf removal before flowering; (green) AFLR, leaf removal after flowering.

The concentration of IBMP at harvest was nearly unaffected by the early leaf removal, when performed both at preflowering and at postflowering time (Table 1). Several scientific contributions highlighted the impact of light and temperature

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on IBMP concentration in the berries[43−46] as well as the importance of a balanced leaf area-to-yield ratio.[44,47,48] Methoxypyrazines are synthesized at very early stages of berry development, reaching a peak at veraison, and they decrease during berry maturation.[43−45,49] The only difference recorded, in our study concerning IBMP concentration, seems related to the seasonal climatic variation in the two experimental years. It is likely that, as suggested by Hunter et al.,[50] this reflects a balance between the biochemical formation of these compounds preveraison and their degradation affected by light and temperature postveraison. Moreover, previous research carried out in a similar growing region (North-Eastern Italy) highlighted that the leaf removal effect on IBMP concentration at harvest in Sauvignon blanc is overwhelmed by warm meteorological conditions (mainly light and temperature).[51]

Thiol precursors did not show significant differences across the treatments, although interseason variability greatly affected their accumulation. In the case of glutathionylated compounds, a significant interaction between year and treatment was observed (Table 1). In detail, an important season-dependent effect of the treatments was observed for these precursors that displayed a stronger response in the warmer 2013 as compared to cooler 2014, where the differences between treatments were negligible. Capone et al.[26] reported that the concentration may fluctuate during ripening and even decline with increasing TSS, but several studies have shown that the berry ripening stage mainly accounts for the biosynthesis of thiol precursors in the berries, and they demonstrated that their concentration is 4- to 5-fold higher in berries with higher sugar concentration.[8,25,27] These findings are consistent with our results, which also showed a lower thiol precursors accumulation along with a lower sugar concentration, as occurred with grapes harvested in 2014, regardless of defoliation treatments (Table 1). The 2014 season was characterized by several rainfall events and correlated with lower temperature and solar radiation that limited the impact of leaf removal in improving the cluster microclimate condition and thus thiol precursors accumulation. In fact, the primary objective of leaf removal is to improve the cluster microclimate by increasing sunlight penetration in the canopy and subsequently achieving higher berry temperature.[52]

As is known, cluster exposure is reported to differentially impact thiols in wine, either improving their concentration[23,24] or not affecting their amount in the finished wines.[22] For this reason, canopy management practices impact cluster microclimate and grape, juice and wine composition,[53] but the controversial results probably arise from the differences related to the climatic conditions in which the experiments were carried out. However, thiol precursors in berries are not reported in the above-mentioned trials, and our study provided the first evidence for an interactive effect between climatic conditions and leaf removal on these compounds in the berries, at harvest.

As a result, the strength of our research lies in the integration of data from the same treatments in striking dissimilar climatic conditions. We pointed out that the efficacy of early leaf removal in improving thiol precursors content is influenced not only by the timing, defined with respect to the grape phenological stages, but even more by the climatic conditions. Most likely, the season 2014, characterized by abundant rainfalls and lower solar radiation and temperature, did not provide environmental conditions favorable for thiol precursors accumulation, and canopy management practices, employed in this experiment, did not positively affect the biosynthesis of these compounds.

Accordingly, the impact of defoliation on G-3SH, in the 2013 experiment, might be the result of a modification of the microclimate at the level of cluster zone promoting precursors biosynthesis. Indeed, as suggested by previous studies, the accumulation of thiol precursors depends on light, radiation, and temperature through their biosynthesis or degradation, even if more research is needed on this relevant subject.[15] Interestingly, in the favorable season 2013, a different effect can be ascribed to the timing of defoliation in the diverse glutathionylated precursors. While G-4MSP precursor content appears not significantly modified by treatments, when compared to control, G-3SH is found to be barely increased in BFLR and significantly in AFLR berries. Our findings substantiate the hypothesis that the two compounds are derived from different biosynthetic pathways subjected to specific regulation and that the accumulation of G-3SH precursor is highly affected by environmental conditions.[18,54−56]

In our work, defoliation practice did not affect, in any way, cysteinylated precursors levels; on the other hand, the information available in the literature regarding the impact of stresses on these compounds is lacking and quite controversial,[15,19] and further analysis would be needed to better understand their biosynthesis dynamics. In contrast, leaf removal impacted the content of G-3SH, further confirming the existence of the committed pathway, as hypothesized by Kobayashi et al.[15] and Thibon et al.[56] for the biosynthesis of this compound.

For these reasons, we carried out a transcriptional analysis that allowed us to hypothesize that the precursor biosynthesis, influenced by canopy management practices, is possibly regulated by the glutathione-*S*-transferase enzymes (VvGST). Kobayashi et al.[15] reported the induction of *VvGST* encoding genes in grape leaves by UV-C irradiation and in grape berry skins by downy mildew infection. The authors hypothesized that GSTs enzymes could act downstream of the LOX/HPL pathway, allowing glutathione to conjugate with (*E*)-2-hexenal. Our analysis achieved compatible, but only partially overlapping results, most probably given the differences in the tissues examined and experimental conditions. In Sauvignon blanc grapes, G-3SH is more or less equally distributed in skin and pulp,[8] and for this reason we carried out our analysis on the whole berry, including both tissues. Consistently with the previous study, *VvGST1* and *VvGST2* genes lack any apparent relationship with G-3SH accumulation in fruit, and *VvGST3* confirms a behavior compatible with a role in precursor biosynthesis. Remarkably, in our condition, *VvGST5* transcript is the most abundant, with an accumulation pattern mirroring G-3SH, when exposed to leaf removal. This result can be ascribed to the tissue-specificity of the gene that is expressed preferentially in the pulp, largely represented in our samples. Although the role of *VvGST5* seems unusual in light of other works, its expression in the whole berry tissues, under stress conditions, has been recently shown.[57] Additionally, although *VvGST4* shows an expression pattern consistent with precursor accumulation, its transcripts display almost unnoticeable levels, probably because skin tissues are under-represented in our samples.

In a recent study, several candidate *VvGSTs* were identified by RNA-seq as affected by nitrogen status, in the berries.[19] Those authors point out the presence of several genes encoding GST in the *Vitis vinifera* genome, suggesting that data from

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other *loci* should help in elucidating this issue. However, the results achieved by our targeted-approach provide interesting clues, indicating *VvGST3* and *VvGST5* as the genes most likely involved in the mediation of thiol precursors increase in berries from grapevines subjected to early leaf removal.

Recently, the metabolic and physiological impact of leaf removal on quality-associated metabolites has been determined in Sauvignon blanc.[58] The study confirmed that leaf removal treatment, at early stages of berry development, affects quality-associated metabolites (mainly monoterpenes and C_{13}-norisoprenoids), whereas differences in the concentrations of sugars and organic acids were marginally influenced. Finally, the same authors emphasized the need of more insights into the transcriptional regulatory networks controlling the observed metabolic plasticity. For this reason we carried out a study in order to elucidate the influence of a common viticultural practice on the aroma potential of Sauvignon blanc. Our data are not conclusive, and more work is needed, especially to relate fruit chemical composition to wine sensory attributes. However, it is likely that the different pattern of thiol precursor accumulation in berries, affected by the timing of defoliation and the season, reflects a response of the related biosynthetic pathway to dissimilar physiological conditions. The multi-approach study presented here pointed out that early leaf removal, both before and after flowering, could be an ineffective technique to increase precursors levels in grapes, when applied in a cold and rainy season, but it has a positive effect in the warmer and sunny season. The lack of differences among leaf removal treatments in the colder season could be related with the incomplete maturation of the grapes (easily understandable from Figure 2). Thiol precursors concentration increases exponentially when maturation is reached,[27] and we can speculate that the effects of leaf removal could have appeared by postponing the harvest time by 1 or 2 weeks, but this was not possible because of the occurrence of rots.

We acknowledge that leaf removal has a large impact on the modification of microclimate and triggers a general transcriptome and metabolome reprogramming;[58] therefore, we are far from a comprehensive understanding of the mechanisms underlying the regulation of thiol precursors production. However, we argue that two genes, encoding GSTs, might be involved in the biosynthesis of aroma compound precursors in berries, and their transcription can be influenced by canopy management strategies.

We also indicate that, in favorable meteorological conditions, early leaf removal can provide a tool to alter the biosynthesis of thiol precursors in Sauvignon blanc grapes, by modifying the relative proportion of 4MSP and 3SH precursors, in relation to the timing of application, without any effect on basic fruit chemical parameters, polyphenols, and IBMP.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.7b03508.

Monthly values of growing degree days (GDD), rainfall, and solar radiation recorded at the weather station of Capriva del Friuli; and evolution of main, lateral, and total leaf area of Sauvignon blanc vines exposed to leaf removal treatments in 2013 and 2014 (PDF)

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Notes

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■ REFERENCES

(1) Caspari, H. W.; Lang, A.; Alspach, P. Effects of girdling and leaf removal on fruit set and vegetative growth in grape. *Am. J. Enol. Vitic.* **1998**, *49*, 359−366.

(2) Poni, S.; Casalini, L.; Bernizzoni, F.; Civardi, S.; Intrieri, C. Effects of early defoliation on shoot photosynthesis, yield components, and grape composition. *Am. J. Enol. Vitic.* **2006**, *57*, 397−407.

(3) Poni, S.; Bernizzoni, F.; Civardi, S. The effect of early leaf removal on whole-canopy gas exchange and vine performance of *Vitis vinifera* L. "Sangiovese. *Vitis - J. Grapevine Res.* **2008**, *47*, 1−6.

(4) Palliotti, A.; Gardi, T.; Berrios, J. G.; Civardi, S.; Poni, S. Early source limitation as a tool for yield control and wine quality improvement in a high-yielding red *Vitis vinifera* L. cultivar. *Sci. Hortic. (Amsterdam, Neth.)* **2012**, *145*, 10−16.

(5) Sternad Lemut, M.; Sivilotti, P.; Franceschi, P.; Wehrens, R.; Vrhovsek, U. Use of metabolic profiling to study grape skin polyphenol behavior as a result of canopy microclimate manipulation in a "Pinot noir" vineyard. *J. Agric. Food Chem.* **2013**, *61*, 8976−8986.

(6) Diago, M. P.; Ayestarán, B.; Guadalupe, Z.; Poni, S.; Tardáguila, J. Impact of prebloom and fruit set basal leaf removal on the flavonol and anthocyanin composition of Tempranillo grapes. *Am. J. Enol. Vitic.* **2012**, *63*, 367−376.

(7) Allen, T.; Herbst-Johnstone, M.; Girault, M.; Butler, P.; Logan, G.; Jouanneau, S.; Nicolau, L.; Kilmartin, P. A. Influence of grape-harvesting steps on varietal thiol aromas in Sauvignon Blanc wines. *J. Agric. Food Chem.* **2011**, *59*, 10641−10650.

(8) Roland, A.; Schneider, R.; Charrier, F.; Cavelier, F.; Rossignol, M.; Razungles, A. Distribution of varietal thiol precursors in the skin and the pulp of Melon B. and Sauvignon Blanc grapes. *Food Chem.* **2011**, *125*, 139−144.

(9) Tominaga, T.; Dubourdieu, D. Identification of 4-Mercapto-4-methylpentan-2-one from the Box Tree (*Buxus sempervirens* L.) and Broom (*Sarothamnus scoparius* (L.) Koch.). *Flavour Fragrance J.* **1997**, *12*, 373−376.

(10) Tominaga, T.; Darriet, P.; Dubourdieu, D. Identification de l'acétate de 3-mercaptohexanol, composé à forte odeur de buis, intervenant dans l'arôme des vins de Sauvignon. *Vitis - J. Grapevine Res.* **1996**, *35*, 207−210.

(11) Darriet, P.; Tominaga, T.; Lavigne, V.; Boidron, J. N.; Dubourdieu, D. Identification of a powerful aromatic component of *Vitis vinifera* L. var. Sauvignon wines: 4-Mercapto- 4-methylpentan-2-one. *Flavour Fragrance J.* **1995**, *10*, 385−392.

DOI: 10.1021/acs.jafc.7b03508
J. Agric. Food Chem. 2017, 65, 8426−8434

(12) Benkwitz, F.; Nicolau, L.; Lund, C.; Beresford, M.; Wohlers, M.; Kilmartin, P. A. Evaluation of key odorants in Sauvignon Blanc wines Using Three different Methodologies. *J. Agric. Food Chem.* **2012**, *60*, 6293−6302.
(13) Tominaga, T.; Peyrot des Gachons, C.; Dubourdieu, D. A new type of flavor precursors in *Vitis vinifera* L. cv. Sauvignon Blanc: S-cysteine conjugates. *J. Agric. Food Chem.* **1998**, *46*, 5215−5219.
(14) Peyrot des Gachons, C.; Tominaga, T.; Dubourdieu, D. Sulfur Aroma Precursor Present in S-glutathione Conjugate Form: Identification of S-3-(Hexan-1-ol)-glutathione in Must from *Vitis vinifera* L. cv. Sauvignon Blanc. *J. Agric. Food Chem.* **2002**, *50*, 4076−4079.
(15) Kobayashi, H.; Takase, H.; Suzuki, Y.; Tanzawa, F.; Takata, R.; Fujita, K.; Kohno, M.; Mochizuki, M.; Suzuki, S.; Konno, T. Environmental stress enhances biosynthesis of flavor precursors, S-3-(hexan-1-ol)-glutathione and S-3-(hexan-1-ol)-L-cysteine, in grapevine through glutathione S-transferase activation. *J. Exp. Bot.* **2011**, *62*, 1325−1336.
(16) Podolyan, A.; White, J.; Jordan, B.; Winefield, C. Identification of the lipoxygenase gene family from *Vitis vinifera* and biochemical characterisation of two 13-lipoxygenases expressed in grape berries of Sauvignon Blanc. *Funct. Plant Biol.* **2010**, *37*, 767−784.
(17) Lacroux, F.; Tregoat, O.; van Leeuwen, C.; Pons, A.; Tominaga, T.; Lavigne-Cruège, V.; Dubourdieu, D. Effect of Foliar Nitrogen and Sulphur Application on Aromatic Expression of *Vitis vinifera* L. cv Sauvignon Blanc. *J. Int. Sci. Vigne Vin* **2008**, *42*, 1−8.
(18) Choné, X.; Lavigne-Cruège, V.; Tominaga, T.; Van Leeuwen, C.; Castagnède, C.; Saucier, C.; Dubourdieu, D. Effect of vine nitrogen status on grape aromatic potential: Flavor precursors (S-cysteine conjugates), glutathione and phenolic content in *Vitis vinifera* L. cv. Sauvignon Blanc grape juice. *J. Int. des Sci. la Vigne du Vin* **2016**, *40*, 1−6.
(19) Helwi, P.; Guillaumie, S.; Thibon, C.; Keime, C.; Habran, A.; Hilbert, G.; Gomes, E.; Darriet, P.; Delrot, S.; Leeuwen, C. Van Vine nitrogen status and volatile thiols and their precursors from plot to transcriptome level. *BMC Plant Biol.* **2016**, *16*, 173.
(20) Subileau, M.; Schneider, R.; Salmon, J.-M.; Degryse, E. Nitrogen catabolite repression modulates the production of aromatic thiols characteristic of Sauvignon Blanc at the level of precursor transport. *FEMS Yeast Res.* **2008**, *8*, 771−780.
(21) Pinu, F. R.; Jouanneau, S.; Nicolau, L.; Gardner, R. C.; Villas-boas, S. G. Concentrations of the Volatile Thiol 3-Mercaptohexanol in Sauvignon Blanc Wines: No Correlation with Juice Precursors. *Am. J. Enol. Vitic.* **2012**, *63*, 407−412.
(22) Martin, D.; Grose, C.; Fedrizzi, B.; Stuart, L.; Albright, A.; McLachlan, A. Grape cluster microclimate influences the aroma composition of Sauvignon Blanc wine. *Food Chem.* **2016**, *210*, 640−647.
(23) Šuklje, K.; Antalick, G.; Buica, A.; Langlois, J.; Coetzee, Z. A.; Gouot, J.; Schmidtke, L. M.; Deloire, A. Clonal differences and impact of defoliation on Sauvignon Blanc (*Vitis vinifera* L.) wines: a chemical and sensory investigation. *J. Sci. Food Agric.* **2016**, *96*, 915−926.
(24) Šuklje, K.; Antalick, G.; Coetzee, Z.; Schmidtke, L. M.; Baša Česnik, H.; Brandt, J.; du Toit, W. J.; Lisjak, K.; Deloire, A. Effect of leaf removal and ultraviolet radiation on the composition and sensory perception of *Vitis vinifera* L. cv. Sauvignon Blanc wine. *Aust. J. Grape Wine Res.* **2014**, *20*, 223−233.
(25) Cerreti, M.; Esti, M.; Benucci, I.; Liburdi, K.; de Simone, C.; Ferranti, P. Evolution of S-cysteinylated and S-glutathionylated thiol precursors during grape ripening of *Vitis vinifera* L. cvs Grechetto, Malvasia del Lazio and Sauvignon Blanc. *Aust. J. Grape Wine Res.* **2015**, *21*, 411−416.
(26) Capone, D. L.; Sefton, M. A.; Jeffery, D. W. Analytical investigations of wine odorant 3-mercaptohexan-1-ol and its precursors. In *Flavor Chemistry of Wine and Other Alcoholic Beverages*; Qian, M. C., Shellhammer, T., Eds.; American Chemical Society: Washington, DC, 2012; pp 15−35.
(27) Capone, D. L.; Sefton, M. A.; Jeffery, D. W. Application of a modified method for 3-mercaptohexan-1-ol determination to investigate the relationship between free thiol and related conjugates in grape juice and wine. *J. Agric. Food Chem.* **2011**, *59*, 4649−4658.
(28) Vanzo, A.; Janeš, L.; Požgan, F.; Velikonja Bolta, S.; Sivilotti, P.; Lisjak, K. Liquid chromatography mass spectrometry determination of thiol precursors, methionine, glutathione and oxidized glutathione in Sauvignon Blanc grapes considering sample preparation steps which prevent postharvest metabolic changes. Accepted for publication in *Sci. Rep.*
(29) Schneider, C. A.; Rasband, W. S.; Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **2012**, *9*, 671−675.
(30) Singleton, V. L.; Rossi, J. A. J. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144−158.
(31) Šuklje, K.; Lisjak, K.; Baša Česnik, H.; Janeš, L.; Du Toit, W.; Coetzee, Z.; Vanzo, A.; Deloire, A. Classification of grape berries according to diameter and total soluble solids to study the effect of light and temperature on methoxypyrazine, glutathione, and hydroxycinnamate evolution during ripening of Sauvignon Blanc (*Vitis vinifera* L.). *J. Agric. Food Chem.* **2012**, *60*, 9454−9461.
(32) Sivilotti, P.; Herrera, J. C.; Lisjak, K.; Baša Česnik, H.; Sabbatini, P.; Peterlunger, E.; Castellarin, S. D. Impact of Leaf Removal, Applied Before and After Flowering, on Anthocyanin, Tannin, and Methoxypyrazine Concentrations in "Merlot" (*Vitis vinifera* L.) Grapes and Wines. *J. Agric. Food Chem.* **2016**, *64*, 4487−4496.
(33) Falginella, L.; Di Gaspero, G.; Castellarin, S. D. Expression of flavonoid genes in the red grape berry of "Alicante Bouschet" varies with the histological distribution of anthocyanins and their chemical composition. *Planta* **2012**, *236*, 1037−1051.
(34) Livak, K. J.; Schmittgen, T. D. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. *Methods* **2001**, *25*, 402−408.
(35) Palliotti, A.; Gatti, M.; Poni, S. Early Leaf Removal to Improve Vineyard Efficiency: Gas Exchange, Source-to-Sink Balance, and Reserve Storage Responses. *Am. J. Enol. Vitic.* **2011**, *62*, 219−228.
(36) Risco, D.; Pèrez, D.; Yeves, A.; Castel, J. R.; Intrigliolo, D. S. Early defoliation in a temperate warm and semi-arid Tempranillo vineyard: vine performance and grape composition. *Aust. J. Grape Wine Res.* **2014**, *20*, 111−122.
(37) Tardaguila, J.; de Toda, F. M.; Poni, S.; Diago, M. P. Impact of early leaf removal on yield and fruit and wine composition of *Vitis vinifera* L. Graciano and Carignan. *Am. J. Enol. Vitic.* **2010**, *61*, 372−381.
(38) Sternad Lemut, M.; Sivilotti, P.; Butinar, L.; Laganis, J.; Vrhovsek, U. Pre-flowering leaf removal alters grape microbial population and offers good potential for a more sustainable and cost-effective management of a Pinot Noir vineyard. *Aust. J. Grape Wine Res.* **2015**, *21*, 439−450.
(39) Lee, J.; Skinkis, P. Oregon "Pinot noir" grape anthocyanin enhancement by early leaf removal. *Food Chem.* **2013**, *139*, 893−901.
(40) Downey, O. M.; Dokoozlian, K. N.; Krstic, P. M. Cultural Practice and Environmental Impacts on the Flavonoid Composition of Grapes and Wine: A Review of Recent Research. *Am. J. Enol. Vitic.* **2006**, *57*, 257−268.
(41) Gatti, M.; Bernizzoni, F.; Civardi, S.; Poni, S. Effects of cluster thinning and preflowering leaf removal on growth and grape composition in cv. Sangiovese. *Am. J. Enol. Vitic.* **2012**, *63*, 325−332.
(42) Kemp, B. S.; Harrison, R.; Creasy, G. L. Effect of mechanical leaf removal and its timing on flavan-3-ol composition and concentrations in *Vitis vinifera* L. cv. Pinot Noir wine. *Aust. J. Grape Wine Res.* **2011**, *17*, 270−279.
(43) Scheiner, J. J.; Sacks, G. L.; Pan, B.; Ennahli, S.; Tarlton, L.; Wise, A.; Lerch, S. D.; Vanden Heuvel, J. E. Impact of severity and timing of basal leaf removal on 3-isobutyl-2-methoxypyrazine concentrations in red winegrapes. *Am. J. Enol. Vitic.* **2010**, *61*, 358−364.
(44) Ryona, I.; Pan, B. S.; Intrigliolo, D. S.; Lakso, A. N.; Sacks, G. L. Effects of cluster light exposure on 3-isobutyl-2-methoxypyrazine accumulation and degradation patterns in red wine grapes (*Vitis*

DOI: 10.1021/acs.jafc.7b03508
J. Agric. Food Chem. 2017, 65, 8426−8434

vinifera L. Cv. Cabernet Franc). *J. Agric. Food Chem.* **2008**, *56*, 10838−10846.
(45) Koch, A.; Ebeler, S. E.; Williams, L. E.; Matthews, M. A. Fruit ripening in *Vitis vinifera*: light intensity before and not during ripening determines the concentration of 2-methoxy-3-isobutylpyrazine in Cabernet Sauvignon berries. *Physiol. Plant.* **2012**, *145*, 275−285.
(46) Dunlevy, J. D.; Soole, K. L.; Perkins, M. V.; Nicholson, E. L.; Maffei, S. M.; Boss, P. K. Determining the Methoxypyrazine Biosynthesis Variables Affected by Light Exposure and Crop Level in Cabernet Sauvignon. *Am. J. Enol. Vitic.* **2013**, *64*, 450−458.
(47) Šuklje, K.; Baša Česnik, H.; Janeš, L.; Kmecl, V.; Vanzo, A.; Deloire, A.; Sivilotti, P.; Lisjak, K. The effect of leaf area to yield ratio on secondary metabolites in grapes and wines of *Vitis vinifera* L. cv. Sauvignon Blanc. *J. Int. Sci. Vigne Vin* **2016**, *47*, 83−97.
(48) Chapman, D. M.; Matthews, M. A.; Guinard, J.-X. Sensory Attributes of Cabernet Sauvignon Wines Made from Vines with Different Crop Yields. *Am. J. Enol. Vitic.* **2004**, *55*, 325−334.
(49) Roujou de Boubée, D.; Cumsille, A. M.; Pons, M.; Dubordieu, D. Location of 2-methoxy-3-isobutylpirazine in Cabernet Sauvignon bunches and its extractability during vinification. *Am. J. Enol. Vitic.* **2002**, *53*, 1−5.
(50) Hunter, J. J.; Volschenk, C. G.; Marais, J.; Fouchè, G. W. Composition of Sauvignon Blanc grapes as affected by pre-vèraison canopy manipulation and ripeness level. *South African J. Enol. Vitic.* **2004**, *25*, 13−18.
(51) Mosetti, D.; Herrera, J. C.; Sabbatini, P.; Green, A.; Alberti, G.; Peterlunger, E.; Lisjak, K.; Castellarin, S. D. Impact of leaf removal after berry set on fruit composition and bunch rot in "Sauvignon Blanc. *Vitis - J. Grapevine Res.* **2016**, *55*, 57−64.
(52) Sabbatini, P.; Howell, G. S. Effects of Early Defoliation on Yield, Fruit Composition, and Harvest Season Cluster Rot Complex of Grapevines. *HortScience* **2010**, *45*, 1804−1808.
(53) Jackson, D. I.; Lombard, P. B. Environmental and Management Practices Affecting Grape Composition and Wine Quality - A Review. *Am. J. Enol. Vitic.* **1993**, *44*, 409−430.
(54) Helwi, P.; Habran, A.; Guillaumie, S.; Thibon, C.; Hilbert, G.; Gomes, E.; Delrot, S.; Darriet, P.; Van Leeuwen, C. Vine Nitrogen Status Does Not Have a Direct Impact on 2-Methoxy-3-isobutylpyrazine in Grape Berries and Wines. *J. Agric. Food Chem.* **2015**, *63*, 9789−9802.
(55) Kobayashi, H.; Matsuyama, S.; Takase, H.; Sasaki, K.; Suzuki, S.; Takata, R.; Saito, H. Impact of Harvest Timing on the Concentration of 3-Mercaptohexan-1-ol Precursors in *Vitis vinifera* Berries. *Am. J. Enol. Vitic.* **2012**, *63*, 544−548.
(56) Thibon, C.; Cluzet, S.; Mérillon, J. M.; Darriet, P.; Dubourdieu, D. 3-sulfanylhexanol precursor biogenesis in grapevine cells: The stimulating effect of *Botrytis cinerea*. *J. Agric. Food Chem.* **2011**, *59*, 1344−1351.
(57) Savoi, S.; Wong, D. C. J.; Arapitsas, P.; Miculan, M.; Bucchetti, B.; Peterlunger, E.; Fait, A.; Mattivi, F.; Castellarin, S. D. Transcriptome and metabolite profiling reveals that prolonged drought modulates the phenylpropanoid and terpenoid pathway in white grapes (*Vitis vinifera* L.). *BMC Plant Biol.* **2016**, *16*, 67.
(58) Young, P. R.; Eyeghe-bickong, H. A.; Plessis, K.; Alexandersson, E.; Jacobson, D. A.; Coetzee, Z.; Deloire, A.; Vivier, M. A. Grapevine Plasticity in Response to an Altered Microclimate: Sauvignon Blanc Modulates Specific Metabolites in Response to Increased Berry Exposure. *Plant Physiol.* **2016**, *170*, 1235−1254.

DOI: 10.1021/acs.jafc.7b03508
J. Agric. Food Chem. 2017, 65, 8426−8434