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Research Article

Effects of Basal Leaf Removal and Cluster Thinning on Monoterpene Accumulation and the Expression of Terpene Biosynthesis Genes in Ripening Moscato Bianco Grape Berries

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Background and Aims: Monoterpenes are key contributors to the aromatic profile of Moscato Bianco grapes and wines. In recent years, producers in the Asti DOCG area (northwestern Italy) have reported a decline in grape aromatic intensity. This study aimed to evaluate whether canopy management practices, basal leaf removal and cluster thinning, can modify bunch-zone microclimate and influence monoterpene biosynthesis and accumulation in Moscato Bianco berries.
Methods and Results: Field trials were conducted over two growing seasons in two vineyards located at different altitudes: Arione (higher altitude) and Vignaioli (lower altitude). Three treatments were applied: (a) basal leaf removal at post-berry set (BBCH 71–73), (b) basal leaf removal at veraison (BBCH 81–85) and (c) cluster thinning at veraison (BBCH 81–85). Grapes were collected at two ripening stages corresponding to commercial harvests for *Asti spumante* (T1) and *Moscato d'Asti* (T2). Pulp and skin tissues were analysed separately for free and bound monoterpenes using GC-MS, and the expression of terpene biosynthesis genes was assessed via RT-qPCR. Microclimatic conditions around clusters were monitored using sensors recording temperature and light intensity data. Leaf removal significantly altered bunch-zone irradiation, with moderate effects on temperature. Terpene synthase (TPS) gene expression showed important treatment-dependent fluctuation. *VvTER* expression was significantly reduced at T2 across vineyards and tissues. In Vignaioli, basal leaf removal and cluster thinning at veraison significantly increased monoterpene accumulation, especially at T2 and most strongly in the glycosylated fraction. In Arione, treatment effects were less pronounced at T1 and showed limited benefits at T2.
Conclusions: Cluster thinning and basal leaf removal at veraison may help preserve the characteristic aromatic profile of Moscato Bianco grapes under variable and changing climatic conditions.
Significance of the Study: This study advances the refinement of canopy management practices for this aromatic variety, clarifies how microclimate and source/sink ratio modulation affect monoterpene biosynthesis and related gene expression, and highlights the importance of site-specific vineyard management.

Keywords: canopy management; free and glycosylated monoterpenes; grape pulp; grape skin; microclimate; volatile aroma compounds (VOCs)

1. Introduction

Moscato Bianco is a highly valued aromatic white-skinned grape variety, primarily grown in the Asti DOCG (Denominazione di Origine Controllata e Garantita) region of Piedmont (northwestern Italy), which encompasses approximately 9900 ha [1]. The vineyards are situated in the rolling hills of Langhe and Monferrato, where ideal mesoclimatic conditions, including cooler temperatures at altitudes of 150–550 m above sea level, help to preserve the characteristic acidity and aromatic freshness of the grapes [2]. Designated as a UNESCO World Heritage Site, in this region two signature wines are produced: *Asti spumante* and *Moscato d'Asti*. The former is a sparkling, fresh wine, while the latter, made from grapes harvested at a more advanced ripening stage, is sweeter and has a lower alcohol content [1, 2]. Both wines are renowned for their fruity and floral aromas, with vibrant notes of orange blossom, jasmine and citrus, complemented by honey and spicy undertones [2]. These distinctive aromas are primarily attributed to terpenoids, particularly monoterpenoids, such as linalool, geraniol and nerol. These compounds are key contributors to the signature flavour of Muscat grape cultivars [3]. Terpenes exist in free forms, which directly contribute to the aroma, and glycosylated forms, which act as precursors, are released during fermentation, and can improve the wine's overall bouquet [4, 5]. Terpene synthesis depends on genetic and environmental factors, including soil composition, altitude, temperature, light exposure and agronomical practices [6].

In the last few years, grape growers and winemakers in the Asti DOCG region have raised concerns regarding a decline in these crucial aroma compounds, which threatens the wine's hallmark aromaticity and, consequently, the reputation among consumers for its distinct quality. Strategies that preserve or enhance aroma compounds are thus urgently needed. Canopy management practices, such as cluster thinning and basal leaf removal, are of particular interest. These techniques, which alter the leaf:fruit ratio and the microclimate of the canopy, are mainly applied to preserve or modify the yield and berry composition, and possibly reduce pathogen infections [7–11]. These practices can also potentially modify the berry aroma profile, with specific effects on monoterpene accumulation [12]. By reducing the crop load, cluster thinning has been widely studied for its effects on berry composition, particularly in terms of sugars, acids and polyphenols [13–15]. The impact of thinning on the aroma compounds has gained increasing attention. For instance, increased monoterpene accumulation was reported in grapes of Muscat Hamburg by cluster thinning performed 2 weeks after flowering [16]. Syrah wines showed increased alcohols, esters and sesquiterpene levels after the application of preveraison cluster thinning [17]. In Cabernet Sauvignon, no effects on terpenes and reduced alcohols, aldehydes, ketones and ester concentrations following pea-sized cluster thinning in a warm, semi-arid climate were observed [18], thus highlighting the influence of cultivar and climate on the effects of this practice.

Basal leaf removal has been shown to alter the microclimate of the grapevine canopy by influencing the temperature, humidity, light and water evaporation [19]. Different results have been reported in the literature, highlighting that cultivar, climate and application timing strongly modulate the effects of these canopy management practices. For example, in Nebbiolo, basal leaf removal applied at BBCH 81 had a greater effect on the accumulation of free and glycosylated volatiles than at BBCH 71. In Aleatico, basal leaf removal at BBCH 71 showed a lower impact on volatile accumulation than that observed in Nebbiolo that underwent the same treatment [20]. An early basal leaf removal (BBCH 71) increased glycosylated monoterpenes and C13-norisoprenoids in Xynisteri [21]. Basal leaf removal applied at about 20% veraison led to greater norisoprenoid accumulation in Chardonnay compared to Pinot Noir berries, with seasonal variations [22]. Li et al. [23] found that, in Cabernet Sauvignon, basal leaf removal applied 10 days before flowering and 35 days after flowering enhanced some C6 compounds over a 3-year experiment, with a higher induction of straight-chain alcohol accumulation in 2017 than in the 2018 and 2019 seasons. Such variability underscores the need for cultivar-specific research. In Sauvignon Blanc, basal leaf removal at veraison was found to increase the accumulation of free monoterpenes, while leaf removal before veraison resulted in a higher concentration of bound monoterpenes. In both defoliation treatments, changes in the expression of terpene biosynthesis genes were also observed [24].

Monoterpene biosynthesis begins with the interconversion of dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP) via two possible pathways: the cytosolic mevalonic acid (MVA) and the plastidial methylerythritol phosphate (MEP) pathways [25]. Within the MEP pathway, *VvDXS* and *VvHDR* are thought to be key rate-limiting enzymes. While *VvDXS1* is located within a QTL associated with Muscat flavour [26], *VvDXS3* expression has been shown to correlate strongly with monoterpene accumulation [27] and has been proposed as a potential marker for monoterpene accumulation [25]. The expression of the *VvDXS3* gene appears to increase with ripening [28] and to be responsive to viticultural practices, such as cluster thinning [29]. *VvHDR* carries out the final reaction, producing IPP and DMAPP. These intermediates are then condensed by geranyl diphosphate synthase (GPPS) to form geranyl pyrophosphate (GPP, 2E-geranyl diphosphate), the direct precursor of monoterpenes [30]. Subsequent modifications are mediated by the terpene synthase (TPS) gene family members, which generate a wide array of monoterpenes. Among these members, *VvTER* is responsible for α-terpineol biosynthesis, a key volatile in Moscato aroma, while *VvLinNer1* contributes to the formation of linalool, a major marker of Moscato typicity [30–32]. Most terpenes undergo glycosylation through the action of glycosyltransferase (GT) genes, which catalyse the attachment of a sugar moiety, enhancing terpene solubility, stability and storage in the grape cells [33–35]. Two GTs,

VvGT7 and *VvGT14*, have been identified for their specific involvement in the glycosylation of monoterpene alcohols, such as linalool, geraniol and nerol, which are major aroma precursors in Moscato grapes [32]. Other GTs, such as *VvGT1, GT3, GT5* and *GT6*, have been associated with the glycosylation of flavonols and other flavonoids, and were therefore not included in our gene expression analysis [36]. Nevertheless, given the complexity and redundancy of the GT gene family in grapevine, additional members might contribute to monoterpene glycosylation. While significant progress has been made in understanding these pathways, the regulation of these genes and their response to environmental and developmental factors remain poorly understood. Such insights are crucial to refine viticultural practices aimed at improving grape aroma.

This study aimed to address some of these critical gaps by investigating the effects of cluster thinning and basal leaf removal, performed at different phenological stages, on monoterpene accumulation. The expression of terpene biosynthesis–related genes was examined in the skin and pulp tissues of Moscato Bianco grapes harvested at two sequential ripening stages, the first (T1) for the production of *Asti spumante* and the second (T2) for the production of *Moscato d'Asti* wine.

2. Materials and Methods

2.1. Vineyards and Experimental Design. The trials were conducted during the 2020 and 2021 growing seasons in two vineyards located within the Asti DOCG production area. The first site, Vignaioli Santo Stefano (belonging to the Ceretto group), is located in Calosso (Asti, Italy) at an altitude of 285 m above sea level, with a 24% slope, west-facing exposure and vine spacing of 250 × 80 cm (44.7407° N, 8.2612° E). The second site, Arione, is situated in Mango (Cuneo, Italy) at 412 m above sea level, with a 16% slope, north-facing exposure and a vine spacing of 250 × 90 cm (44.7015° N, 8.1698° E). Both vineyards adhere to the production guidelines recognized by the European and national system of organization and regulation of DOP (Protected Designation of Origin). Vignaioli vineyard was selected because it represents the average altitude where Moscato Bianco grapes are grown within the Asti DOCG region, while Arione vineyard represents higher altitude conditions. A fully randomized block design was used, consisting of three blocks per vineyard. Within each block, all treatments (described below) were applied to entire rows of vines that were randomly assigned to each treatment. No buffer rows were included between treatments. At each sampling time, the biological replicates were obtained by randomly collecting clusters from six treated vines within each block, resulting in a total of 18 vines sampled per treatment across the three blocks.

Three different vineyard management protocols were tested: basal leaf removal at postberry set (POST) (BBCH 71–73), basal leaf removal at veraison (VER) (BBCH 81–85) and cluster thinning at veraison (THIN) (BBCH 81–85), according to the extended BBCH scale for grapevine phenological development [37]. The basal leaf removal consisted of removing the leaves from the first 5 nodes, from the shoot base to the second cluster. The lateral shoots were not removed. Cluster thinning was carried out reducing the crop load by about 30%. Control vines (CK) did not receive any thinning or basal leaf removal treatment. Climatic conditions were monitored using the values recorded by two weather stations of the agrometeorological network of the Piedmont region, located close to the experimental sites (Piedmont Agrometeorological Network, Piedmont Region). In detail, climatic data for Vignaioli and Arione farms have been obtained from the weather stations located in Calosso (Asti, Italy; GPS coordinates: 44.7485° N, 8.2306° E) and Mango (Cuneo, Italy; GPS coordinates: 44.7074° N, 8.1471° E), respectively. The data provided by the weather stations were average, minimum and maximum temperature, rainfall, vapour pressure deficit (VPD) and solar radiation. From these data, the daily temperature delta between max and min values (ΔT) and the water stress (WS) ratio as the ratio $(ET_0 - ET_c)/ET_0$ were calculated.

2.2. Sampling. Grapes were harvested when the average TSS (°Brix) reached the value of 21 ± 1 (T1, commercial harvest for *Asti spumante* wine) and the value of 24 ± 1 (T2, commercial harvest for *Moscato d'Asti* wine). As reported in Table S1 (Supporting Information), the harvest dates were the same for the different canopy manipulations, with the exception of THIN treatment: in fact and as expected, the THIN grapes reached the fixed TSS values about 1 week before the CK, POST and VER grapes in Arione (T1, 2020) and Vignaioli (T1 and T2, 2021).

To collect berry samples homogenous in terms of ripening stage, a flotation method was employed based on the relationship between sugar content and density in NaCl solutions. This method relies on the principle that grape berries with higher sugar content have a higher density than the salt solution and will sink in less concentrated salt solutions, while those with lower sugar content will float. Ten solutions (110–200 g/L, increments of 10 g/L) were prepared in 2-L trays. A representative sample (400–500 berries) was collected from different clusters to ensure they reflected the vineyard's maturity level, and a portable refractometer was used to determine, on a few berries, the initial sugar concentration (Cx) for flotation. Berries were immersed in the corresponding salt solution. Floating berries (lower density and lower sugar) were washed, dried and transferred to the next lower concentration (Cx − 1), while sinking berries (higher density and higher sugar) were collected and stored. This iterative process continued until all berries were classified by density. A frequency histogram identified the most representative maturity class, from which samples were selected to standardize ripeness across treatments. Must

density was correlated with salt solution concentration. Homogeneous berries in terms of TSS (Table S1, Supporting Information) have been collected, and, at each sampling time, berries were manually dissected: the pulp was gently and carefully removed from the skin using a sharp razor, the seeds were discarded, and the isolated skin and pulp tissues were immediately frozen in liquid nitrogen and stored at −80°C until further analysis.

2.3. Microclimatic Parameters. Given the extreme importance of temperature and light in the biosynthesis of volatile terpene compounds, during the two seasons, 2020 and 2021, specific sensors (Rossi Strumenti SRLS, Italy) were installed to monitor microclimatic conditions. Sensors were placed directly within the cluster zone. In each vineyard, two canopy sides were monitored (north and south). On each side, two thermocouples for temperature measurement were installed on two different bunches, for a total of four temperature sensors per treatment in each of the two vineyards. The thermocouples were positioned inside single berries, considering the berries in the intermediate section of the bunch, one on the outside (sun exposed) and one on the inside of the bunches. Light intensity was monitored with silicon-based global solar radiation sensors (400–1100 nm) placing one sensor per canopy side (north and south), for a total of two light sensors per treatment in each of the two farms. Light sensors were oriented perpendicular to the row orientation. Sensors were installed on control rows and on rows subjected to defoliation. No sensors were installed on rows subjected to cluster thinning, as this treatment was not expected to substantially alter light exposure or berry temperature, assuming that the cluster thinning treatment was comparable to the control in terms of microclimatic conditions. Sensors recorded the temperature and irradiation level every 10 min starting, each year, from 9 July (after fruit set) until the harvest date. Supporting Figure S1 provides a visual example of the sensor installation and positioning within the canopy.

2.4. Volatile Organic Compound (VOC) Analysis

2.4.1. Sample Preparation. The skin and pulp tissues were extracted separately. For the skin, 5 mL of methanol was added to 85 g of sample for each replicate. For the pulp, 0.5 g of sodium metabisulfite was added to 15 g sample for each replicate. After 30 min, samples were homogenized using an immersion blender (ULTRA-TURRAX; IKA, Staufen, Germany). A pectolytic enzyme (Ferrari Biotecnologie—Cytolase M102) was added to the extract following the procedure of Di Stefano [38], and, when two phases were formed, the solution was centrifuged at 6000 rpm for 5 min. The supernatant was collected in a flask, and the pellet was washed with an additional 10 mL of ultrapure water, before a further centrifugation. The supernatant was added to the first one, and the volume was adjusted to 100 mL by adding ultrapure water.

2.4.2. Extraction of Free and Bound Aromatic Compounds. A total of 200 µL of 1-heptanol (40 µg mL^{-1}) as internal standard was added to 10 mL of extract obtained during the sample preparation. The solution was passed through a cartridge C18 360 mg (Waters—Sep-Pak C18 Plus Short Cartridge, 55–105 µm) previously activated by 4 mL methanol and 5 mL of ultrapure water. After the sample had been loaded, salts, sugars and additional polar compounds were removed by washing the cartridge with 5 mL of ultrapure water. The fraction containing free compounds was recovered by elution with 10 mL of dichloromethane, dehydrated with sodium sulphate anhydrous and concentrated in a small volume (500 µL) using a Vigreux column in a temperature-controlled water bath at 70°C before analysis. A second fraction containing glycoside compounds was recovered with 10 mL of methanol. The methanol solution was evaporated to dryness under vacuum rotavapor at 40°C. The residue was dissolved in 4 mL of a citrate–phosphate buffer at pH 5, which was then added to 200 µL of a glycosidic enzyme (Ferrari Biotecnologie—Cytolase M102) and kept at 40°C overnight. The solution was then added to 200 µL of 1-heptanol and centrifuged, and the resulting solution was passed through a cartridge C18 360 mg (Waters—Sep-Pak C18 Plus Short Cartridge, 55–105 µm), previously activated by 4 mL of methanol and 5 mL of ultrapure water. The fraction containing the aglycones was then eluted with 10 mL of dichloromethane, dehydrated with sodium sulphate anhydrous and concentrated to 500 µL again using a Vigreux column in a temperature-controlled water bath at 70°C.

2.4.3. Analysis on Gas Chromatography–Mass Spectrometry (GC-MS). Free and glycosylated terpenoid content was quantified using GC-MS. A GC Agilent 7890 B and Agilent 7010, with split/splitless injector set at 230°C and a PAL RSI 85 autosampler, were used for the analyses. The GC oven heating program was 40°C × 1 min, 60°C/min up to 60°C and 4°C/min up to 230°C. Source temperature was set at 230°C. Volatiles were separated on a J&W DB WAX polyethylene glycol column (30 m, 0.25 mm ID, 0.25 µm film; DBWAXetr from Agilent Technologies, USA). Helium was used as carrier gas with a constant flow rate of 1 mL min^{-1}. Compounds were identified based on their retention indices and by comparing their mass spectra with entries in the National Institute of Standards and Technology 2020 MS library using the total ion chromatogram (TIC). Quantification was performed using 1-heptanol as internal standard, as previously reported by published literature [39]. Due to the limited availability of commercial standards for all target compounds, a semiquantitative approach was applied. Results were calculated as the ratio of peak area of each compound to that of the internal standard, assuming a response factor of 1 and expressing the values as µg of 1-heptanol equivalents/kg FW.

Monoterpene quantification was carried out on three biological replicates (one per experimental block) for the season 2020. However, for the season 2021 only two

replicates have been analysed due to logistical constraints. Despite this limitation, the 2021 data were included in the final multivariate analysis and used to reinforce and validate the trends observed analysing data from season 2020.

2.5. Total RNA Extraction and qRT-PCR Analysis. RNA was extracted from 100 mg of ground tissue using the Spectrum Plant Total RNA Kit (Sigma-Aldrich, Italy), following the manufacturer's protocol, which included DNA digestion with the On-Column DNase I Digestion Set (Sigma-Aldrich, Italy). RNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Italy), ensuring an absorbance ratio of 260/280 nm around 1.82 and 260/230 nm around 1.32.

Reverse transcription of RNA into cDNA was performed using the ReadyScript cDNA Synthesis Mix (Sigma-Aldrich, Italy). The relative expression of key genes involved in terpenoid biosynthesis and metabolism (*VvDXS3, VvHDR, VvTER, VvLinNer1, VvGT7 and VvGT14*) was analysed through real-time quantitative PCR (RT-qPCR). Although *VvDXS1* is known to be associated with monoterpene accumulation in Muscat varieties, this gene was not included in the present analysis because the different primer pairs designed and tested during the experiment did not meet the required amplification efficiency and specificity criteria during the assay validation. *VvActin* was used as the housekeeping gene. Gene expression analyses were conducted on samples collected in both the 2020 and 2021 growing seasons; however, only results from 2020 are presented and discussed in detail, as for this season a complete set of data for both gene expression and VOC analyses has been produced. Data from 2021 season are included only in the overall multivariate analysis performed including results from both seasons.

All primers were synthesized by Sigma-Aldrich (Italy), and their sequence is listed in Table S2 (Supporting Information). RT-qPCR was conducted on three biological replicates (one per block), with nontemplate controls (NTCs) included as negative controls in each reaction.

The relative expression levels of individual genes were calculated using the 2^−ΔΔCt method [40]. Expression levels from the three biological replicates were normalized to those of the control (CK) at time point T1.

2.6. Statistical Analyses. The analyses of gene expression, VOCs and microclimatic parameters were performed using R Version 4.0.1. Data normality was assessed using the Shapiro–Wilk test. To verify the assumption of homogeneity of variance, Levene's test was applied. For the analysis of gene expression and VOCs, normally distributed data were evaluated using one-way analysis of variance (ANOVA) ($p \leq 0.05$), followed by a post hoc Dunnett test to compare each treatment against the control. For non-normally distributed data, a Kruskal–Wallis one-way ANOVA was performed, followed by Dunn's test with significance levels set at $p < 0.05$. The Bonferroni–Holm procedure was applied to correct for multiple testing. For microclimatic parameters, normally distributed data were analysed using one-way ANOVA followed by a post hoc HSD test to explore differences among all treatments. Principal component analysis (PCA) was performed to investigate climatic data measured on site (2 years and two vineyards) using JMP Pro 17.0 (JMP SAS, Drive Cary, NC, USA). Partial least squares-discriminant analysis (PLS-DA) was performed using the mixOmics package of R software [41] to classify treatments and study their separation across vintages. A three-component model was built for the season 2020, while for the PLS-DA model built including data from both seasons (2020 and 2021), two components were used. Considering this latter PLS-DA model, each data point represents the average value of three biological replicates ($n = 2$ for 2021 VOC results only). This approach avoids potential bias introduced by the limited availability of replicates regarding VOC data recorded in 2021 season. For both models, the number of variables contributing to each component was optimized through repeated fivefold cross-validation, using the Mahalanobis distance as the primary performance metric. Treatments were used as response variables, while VOCs, gene expression data and microclimatic parameters were used as predictor variables. Plots were generated using the R software package 'ggplot2'. To determine the influence of microclimatic data on skin and pulp glycosylated and free terpene compounds, forward-stepwise multiple regressions were calculated including data from both harvest times (T1 and T2). The independent variables used for the regressions were minimum and maximum daily temperature (Tmin_G-V, Tmin_V-H, Tmax_G-V and Tmax_G-H), the daily temperature delta between max and min values (ΔT_G-V – ΔT_V-H), the number of days with maximum temperature above 35°C (n°D_over_35) and the mean daily radiation (MeanRAD_G-V-MeanRAD_V-H). All these parameters were separately calculated and included in the analysis as before and after veraison (G-V and G-H). In these abbreviations, G refers to the green growing stage of the berries (BBCH 72–75), V to veraison (BBCH 81–85) and H to harvest, indicating the time intervals over which the variables were calculated. The selection of variables was carried out based on F-to enter of 3, to avoid a nonsignificant contribution of some variables in the regression.

3. Results

3.1. Climatic Parameters

3.1.1. Climatic Conditions Characterizing the Different Vineyards and Seasons. To investigate the influence of seasonal and vineyard-specific climatic conditions, we conducted a PCA of the key climatic parameters recorded over the two years (2020 and 2021) by the weather stations installed close to the two vineyards (Arione and Vignaioli) (Figure 1). Principal components (PC) 1 and PC2 described 82.3% of the total variation, discriminating the effects of years and vineyards which were separated into the four quadrants built on the first two PCs. The year 2020 was mainly associated with a higher total rainfall and WS ratio after veraison (TOTrain_V-H and WSratio_V-H,

respectively) and a higher maximum daily temperature from veraison to harvest (Tmax_V-H). The 2021 season was associated with higher total rainfall, daily mean radiation and minimum daily temperature values before veraison (TOTrain_G-V, RADmean_G-V and Tmin_G-V, respectively). The vineyards also clustered separately in the PCA. Arione was related to higher VPD values and daily mean radiation, whereas Vignaioli was associated with a higher maximum daily temperature and daily thermal delta both before and after veraison (Figure 1).

3.1.2. Treatment Effects on the Microclimatic Parameters of the Grape Clusters. To evaluate the impact of the applied treatments on the microclimatic parameters of the grape clusters, a set of sensors was installed in the two vineyards as described in the Materials and Methods section. As previously specified, for the control (CK) and thinned (THIN) samples, only one set of sensors per vineyard, installed on the control vines, was employed, as cluster thinning does not affect the microclimatic conditions of the clusters. Table 1 reports the mean values of irradiation, temperature and ΔT-thermal (Tmax–Tmin) values recorded by the sensors for the different samples in the considered vineyards and seasons. The average temperature showed no significant differences among treatments across vineyards (Arione and Vignaioli), harvest dates (T1 and T2) and years (2020 and 2021). Irradiation levels were notably influenced by the treatments. In Vignaioli, POST and VER consistently exhibited the highest average irradiation levels compared to THIN and CK, regardless of the season or sampling time. In contrast, Arione presented a more variable pattern. In 2020, the highest irradiation values at both T1 and T2 were recorded for POST, while VER values were comparable to those of CK and THIN. However, in 2021 highest irradiation values were recorded for VER, whereas the lowest values among all treatments were recorded for POST. This discrepancy may be attributed to the rapid lateral bud development induced by POST treatment, which may have increased the canopy shading over the grape clusters. Thermal delta also demonstrated notable differences among treatments, except for Vignaioli in 2020, where no significant variations were observed. POST samples consistently showed the highest thermal delta values across vineyards, seasons and sampling times, with significant differences compared to CK and THIN at both T1 and T2. These data indicate a strong and consistent effect of POST treatment in modifying temperature within the canopy microclimate. Conversely, VER only resulted in a higher thermal delta than CK and THIN in Arione in 2021. Regarding Tmax, POST generally showed the highest maximum temperatures in Vignaioli in both years and in Arione during 2020, with Tmax trends largely mirroring those of the thermal delta. However, in Arione in 2020, CK and THIN consistently had higher Tmax values at both T1 and T2. Notably, the increase in irradiation observed following VER and, to a lesser extent, POST treatments was more pronounced in Vignaioli than in Arione, which suggests a stronger treatment effect on light exposure in the Vignaioli vineyard.

3.2. Effects of Basal Leaf Removal and Cluster Thinning on Monoterpene Biosynthesis and Accumulation. Monoterpene biosynthesis and accumulation were investigated by analysing the expression level of six key genes coding for enzymes regulating this specific pathway [30–32], as well as quantifying a set of 30 monoterpenes. Two aromatic alcohols, which are biosynthesized via the shikimate pathway, namely benzyl alcohol and 2-phenylethanol, were also quantified. More specifically, linalool, α-terpineol, citronellol, nerol, geraniol, 3,7-dimethylocta-l,5-diene3,7-(diol_I), 3,7-dimethylocta-l,7-diene-3,6- (diol_II) and 2, 6,7-dihydro-7-hydroxylinalool (endiol) were assessed in both their free and glycosylated forms, while hotrienol was assessed only in its free form. The two aromatic alcohols and the remaining terpene derivatives, specifically those with hydroxyl functional groups, were assessed exclusively in their glycosylated forms (Table S3, Supporting Information). For both molecular and biochemical analyses, skin and pulp tissues were analysed separately to gain a deeper understanding of the common and tissue-specific responses to the treatments. Only two biological replicates were analysed in terms of VOC profiles in 2021 due to technical problems. Considering the overall set of collected samples and the data analysis, and for the sake of clarity, the following section focuses on the molecular and biochemical results obtained in the 2020 season, where three biological replicates were analysed; however, a comprehensive multivariate analysis, along with a stepwise regression analysis incorporating data from both seasons, is presented in the final section.

3.2.1. Expression of Key Genes Regulating Volatile Monoterpene Biosynthesis. The expression of the six key genes (*VvHDR, VvDXS3, VvLinNer1, VvTER, VvGT7* and *VvGT14*) previously identified as central regulators of the monoterpene biosynthetic pathway was monitored in pulp and skin samples collected from the Arione and Vignaioli vineyards in 2020 (Figures 2 and 3). To focus on biologically relevant changes, only genes showing significant differences in the respective tissues are presented. *VvLinNer1* and *VvTER* showed significant alterations in the skin samples from both vineyards, while *VvDXS3* and *VvGT14* showed significant changes in Vignaioli and Arione, respectively (Figure 2).

Considering skin tissue samples collected in Vignaioli vineyard at T1, none of the analysed genes showed significant differences across treatments compared to control grapes (Figure 2). In Arione at T1, significant increases in gene expression were observed. *VvTER* was upregulated in the POST skin sample, while *VvLinNer1* showed higher expression levels in the THIN berry skin. These data suggest that in Arione, which is located at a higher altitude, terpene biosynthesis–related genes started to react to the applied treatments earlier in the season.

At T2, more pronounced differences were induced by the treatments in skin tissue samples in both vineyards (Figure 2). In Vignaioli, *VvDXS3* was significantly downregulated by the THIN treatment, while a significant increase in *VvLinNer1* expression was observed in the skin

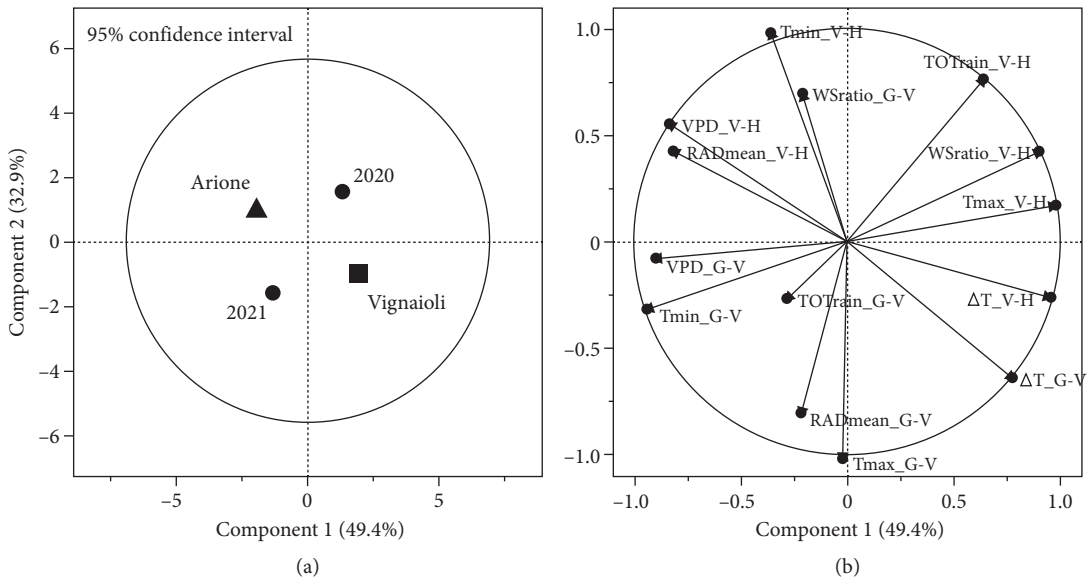

Figure 1: Principal component analysis (PCA) of key climatic parameters across two vineyards (Arione and Vignaioli) and 2 years. (a) (scores plot) shows the separation of the vineyards and years based on the first two principal components (PC1 and PC2). (b) (loadings plot) displays the climatic variables associated with each principal component. G refers to the first growing stage of the berries (BBCH 72–75), V to veraison (BBCH 81–85) and H to harvest. Legend: Tmin, Tmax, minimum and maximum daily temperature; ΔT, daily temperature delta between max and min values; VPD, vapour pressure deficit; WSratio, water stress; MeanRAD, the mean daily radiation. These parameters were separately calculated and included in the analysis as before and after veraison (G-V and G-H, where G refers to the green growing stage of the berries, V to veraison and H to harvest, indicating the time intervals over which the variables were calculated).

Table 1: Average values of temperature (˚C), irradiation (w/m^2), ΔT-thermal excursion (Tmax–Tmin; ˚C) and Tmax (˚C) of the grape clusters collected at Arione and Vignaioli vineyards during 2020 and 2021 seasons.

			T1				T2			
	Season	**Treatment**	**Irradiation (w/m^2)**	**Temperature (˚C)**	**ΔT-thermal delta**	**Tmax (˚C)**	**Irradiation (w/m^2)**	**Temperature (˚C)**	**ΔT-thermal delta**	**Tmax (˚C)**
Arione	2020	CK	78.9 b	23.2	13.5 b	36.8	77.7 b	23.0	13.7 b	37.00 a
		THIN	81.5 b	23.4	13.6 b	36.5	88.8 b	23.0	13.5 b	37.00 a
		POST	106.4 a	23.7	14.9 a	35.7	105.7 a	23.5	15.0 a	35.4 ab
		VER	81.7 b	24.4	14.9 a	34.9	82.1 b	23.4	15.0 a	34.8 b
	2021	CK/THIN	80.3 b	22.9	13.3 b	30.5 b	82.7 b	22.9	13.5 b	30.7 b
		POST	79.5 b	23.3	16.0 a	33.2 a	78.5 b	23.3	16.4 a	33.3 a
		VER	94.1 a	23.0	13.3 b	31.1 b	94.9 a	22.9	13.9 b	31.1 b
Vignaioli	2020	CK/THIN	68.5 c	24.5	17.0	36.8 b	68.9 c	24.6	17.3	37.2 b
		POST	106.4 b	24.9	17.4	37.6 b	103.2 b	25.0	17.4	37.8 b
		VER	130.1 a	24.9	18.5	39.5 a	129.3 a	25.1	18.7	39.78 a
	2021	CK	69.0 b	24.5	18.0 b	36.0 ab	67.8 b	24.4	18.4 b	35.8 ab
		THIN	70.4 b	24.8	17.6 b	35.9 ab	69.0 b	24.5	18.0 b	36.0 ab
		POST	121.7 a	24.9	19.3 a	37.4 a	122.0 a	24.8	19.7 a	37.2 a
		VER	125.5 a	24.4	17.1 b	35.0 b	124.8 a	24.3	17.3 b	34.7 b

Note: Temperature and irradiation were recorded every 10 min using sensors installed on individual plants in the field. Data collection occurred from 09 July 2020 to 2021 until the harvest date (Table S1 Supporting Information). Treatments: CK, control; POST, basal leaf removal at post-berry set; VER, basal leaf removal at veraison; THIN, cluster thinning at veraison. Different letters within columns indicate significant differences among treatments, calculated separately for the two seasons (ANOVA, HSD post hoc test, $p \leq 0.05$).

samples from both basal leaf removal treatments (POST and VER). *VvLinNer1* upregulation was also recorded in the skin tissue of VER and THIN samples collected in Arione. The gene *VvTER* showed a significant reduction in expression across all treatments at T2 in both vineyards ($p < 0.01$ for VER; $p < 0.001$ for THIN and POST), suggesting

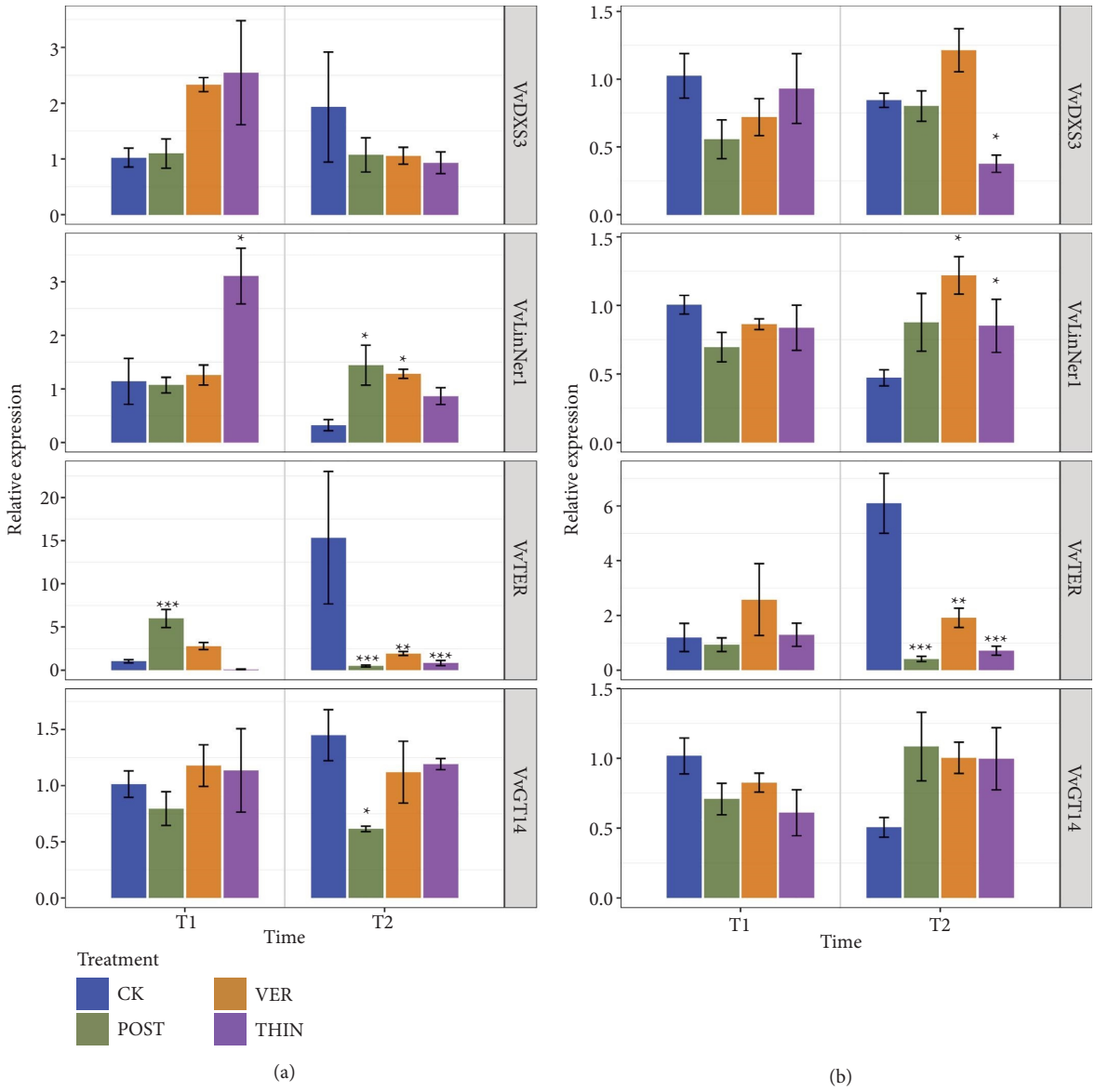

FIGURE 2: Relative expression of *VvDXS3, VvLinNer1, VvTER* and *VvGT14* in the skin of Moscato Bianco grapes collected in Arione (a) and Vignaioli (b) vineyards at T1 and T2 in 2020. Blue colour represents control (CK), green colour represents basal leaf removal at post-berry set (POST), orange colour represents basal leaf removal at veraison (VER), and purple colour represents samples collected on vines subjected to cluster thinning (THIN). Each bar represents the average of the expression level analysed in the three biological replicates, normalized to the expression level of the control CK at T1. Bars indicate the standard error. Asterisks denote statistically significant differences between control and canopy management treatment ($p < 0.05^{*}$, $p < 0.01^{**}$, $p < 0.001^{***}$), assessed using Dunnett's test or Kruskal–Wallis and Dunn's post hoc test, depending on whether the assumptions for parametric or nonparametric tests were met.

a repression of this gene at T2 following canopy management practices. In Arione samples only, *VvGT14* showed a significant decrease in expression in POST skin samples at T2.

A stronger effect of the treatments was generally recorded in pulp tissue samples collected in the Arione vineyard (Figure 3), confirming the findings reported for skin tissue. In the Arione vineyard, all the genes analysed exhibited significant alterations in the expression patterns in response to the applied treatments. In the pulp samples of Vignaioli, only *VvLinNer1*, *VvGT7* and *VvGT14* were significantly affected by the treatments. At T1 only, *VvLinNer1* showed significant upregulation in the VER samples. At T2, *VvLinNer1* expression increased significantly in the THIN samples. Considering genes involved in the glycosylation of terpene compounds, namely *VvGT7* and *VvGT14*, their expression level decreased at T2 in the POST and VER samples for *VvGT7*, and in the POST samples for *VvGT14*. At T1 in the Arione pulp, *VvGT7* showed significantly lower expression levels in the THIN sample, while *VvTER* showed

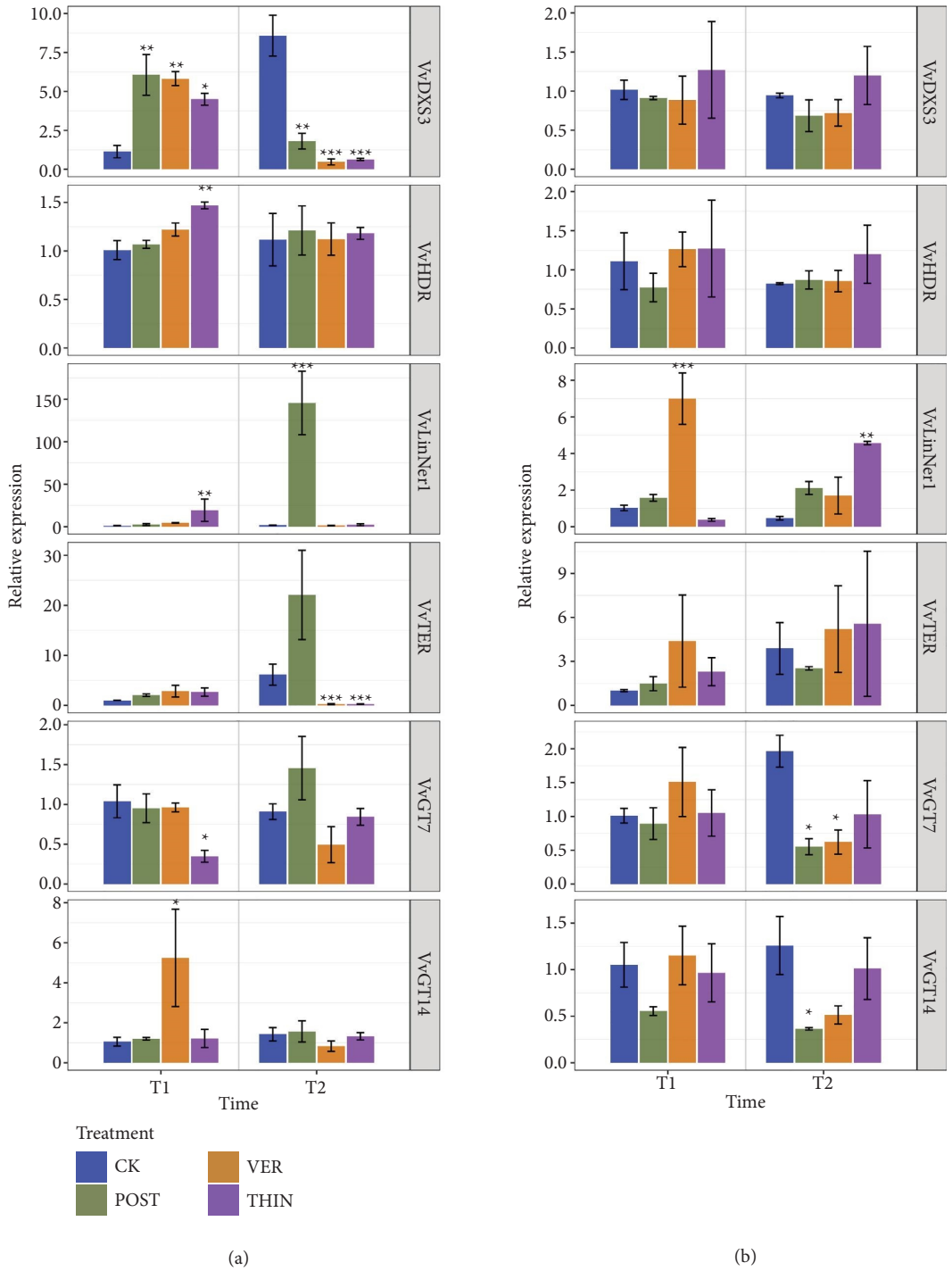

FIGURE 3: Relative expression of *VvDXS3, VvHDR, VvLinNer1, VvTER, VvGT7* and *VvGT14* in the pulp of the Moscato Bianco grapes collected in Arione (a) and Vignaioli (b) vineyards at T1 and T2 in 2020. The treatments CK (control), POST (basal leaf removal at post-berry set), VER (basal leaf removal at veraison) and THIN (cluster thinning) are represented by the blue, green, orange and purple colours, respectively. Each bar represents the average of the expression level analysed in the three biological replicates, normalized to the expression level of the control CK at T1. Bars indicate the standard error. Asterisks denote statistically significant differences between control and canopy management treatment ($p < 0.05^{*}$, $p < 0.01^{**}$, $p < 0.001^{***}$) assessed using Dunnett's test or Kruskal–Wallis and Dunn's post hoc test, depending on whether the assumptions for parametric or nonparametric tests were met.

no significant variations. Notably, *VvDXS3* exhibited increased expression across all treatments, while *VvHDR* and *VvLinNer1* genes were significantly upregulated in THIN samples and *VvGT14* in VER berries. At T2, in pulp tissue of Arione samples, *VvDXS3* and *VvTER* showed decreased expression levels across all treatments. The gene *VvLinNer1*

also showed a marked increase in POST samples collected in the Arione vineyard, and all the other genes were not affected by the different viticultural practices.

3.2.2. Accumulation Pattern of Terpene Compounds. To better understand the physiological effects of the treatments on monoterpene accumulation, monoterpene profiling (including free and glycosylated compounds) was performed on all samples, analysing skin and pulp tissues separately. These molecules included oxide monoterpenes (oxide_A, oxide_B, oxide_C and oxide_D), diol monoterpenes with two hydroxyl groups (diol_1 and diol_2), alcohol monoterpenes with hydroxyl groups (-OH), such as linalool, nerol, geraniol, α-terpineol, citronellol and hotrienol, as well as hydroxy derivatives, which were only found in their glycosylated form (hydroxycitronellol, 8-hydroxydihydrolinalool, hydroxynerol, trans-8-hydroxylinalool and hydroxygeraniol). Two benzenic compounds, benzyl alcohol and 2-phenylethanol, were also included in the analysis due to their high abundance and sensory relevance, although they are not monoterpenes. Figures 4 and 5 illustrate the impact of each treatment on total monoterpene concentrations, along with specific monoterpenes that showed statistically significant differences. A more in-depth analysis of the effects of treatments on the accumulation of the analysed monoterpenes in grape tissues at T1 and T2 is provided in Figures S2 and S3 (Supporting Information).

In both vineyards and tissues, the total glycosylated fraction of monoterpenes was more abundant than the free fraction. Generally, the most abundant glycosylated compounds identified in both vineyards and tissues were the monoterpenes linalool, nerol, geraniol and oxide_A, along with the aromatic alcohol benzyl alcohol, together accounting for over 50% of the total volatile profile (Figures S4 and S5, Supporting Information). In the free fraction, linalool was the dominant monoterpene, contributing approximately 35% of the total free monoterpenes. In both vineyards, nerol and geraniol were almost absent in the pulp, contributing less than 2% to the total aroma compounds (Figures S4 and S5, Supporting Information).

Focusing on the effects of the applied treatments on the canopy, in both vineyards and tissues, at T1 no significant changes were observed in the accumulation of total monoterpenes, either in their free or glycosylated forms. The only exception was in the Vignaioli vineyard, where cluster thinning (THIN) induced a significantly higher accumulation of total glycosylated aroma compounds in the pulp (Figure 4). At T2, no differences were observed in the skin of Arione berries. In the pulp, there was a marked decrease in the concentration of total both free and glycosylated aroma compounds, across all treatments. The only exception was for total glycosylated aroma compounds in THIN grapes, where no significant differences were detected. In contrast, in Vignaioli at T2 an increase in total aroma compound accumulation was observed in both pulp and skin following the treatments. This increase was significant in the pulp for total glycosylated compounds in THIN samples, and in the skin for total glycosylated compounds in POST, VER and THIN grapes. Additionally, a significant increase in total free aroma compounds was recorded in the skin of THIN samples (Figure 4).

Considering the individual monoterpene compounds, in the skin of grapes collected in the Arione vineyard, at both T1 and T2 (Figure 5(a), A), limited changes in monoterpene accumulation, regardless of their form (free or glycosylated), were observed in the comparison of different treatments. At T1, a significantly higher accumulation of glycosylated geraniol and free diol-2 was recorded in VER samples. Significant decreases in glycosylated nerol and free α-terpineol were recorded in THIN samples. A similar decrease was observed for glycosylated α-terpineol in VER grapes. However, at T2 only POST samples showed a significant reduction in free citronellol.

In the skin tissue of the Vignaioli samples (Figure 5(a), B), at T1 a significant increase in glycosylated citronellol was registered for both POST and THIN grapes. Significant decreases were also detected, as in the case of free geraniol in both leaf removal treatments (POST and VER) and in free nerol and free α-terpineol in samples from all treatments. At T2, the applied treatments led to a significant increase in glycosylated forms of several compounds, including linalool, nerol, geraniol, oxides and diols. Glycosylated linalool increased in THIN grapes, glycosylated geraniol increased in POST and VER samples, glycosylated nerol and diol-2 increased in all treatments, and glycosylated oxides increased in VER and THIN grapes. Free monoterpenes showed less consistent trends, though THIN and VER occasionally showed higher levels of monoterpenes. Free citronellol decreased in POST and VER samples, and α-terpineol also showed lower levels after POST and THIN treatments.

In pulp tissue of berries collected in the Arione vineyard (Figure 5(b), A), a significant increase was observed at T1 in VER samples for glycosylated geraniol and nerol, and for the free form of diol-2 in both VER and POST grapes. Glycosylated linalool also showed higher concentrations in POST samples. At T2, a marked decrease in the concentration of both free and glycosylated monoterpenes was evident across most of the treatments. Free linalool levels and glycosylated oxides decreased in samples from all treatments. The only observed increase at T2 in the Arione vineyard was that of free diol-2 after VER and POST treatments.

In the pulp tissue of the Vignaioli samples (Figure 5(b), B), at T1 no or a slight difference in the monoterpene profile was observed in response to the applied treatments, with only a significantly higher concentration of glycosylated hydroxylated monoterpenes (gly_OH_VOCs) detected in POST and THIN samples. At T2, THIN and VER grapes showed a significant increase in glycosylated geraniol, while glycosylated diol-2 increased only in VER samples. Free geraniol and nerol concentrations also increased in the pulp of THIN berries, in addition to free diol-2 in VER samples. POST grapes showed no significant modifications at T2, except for a slight, but significant, lower accumulation of free α-terpineol.

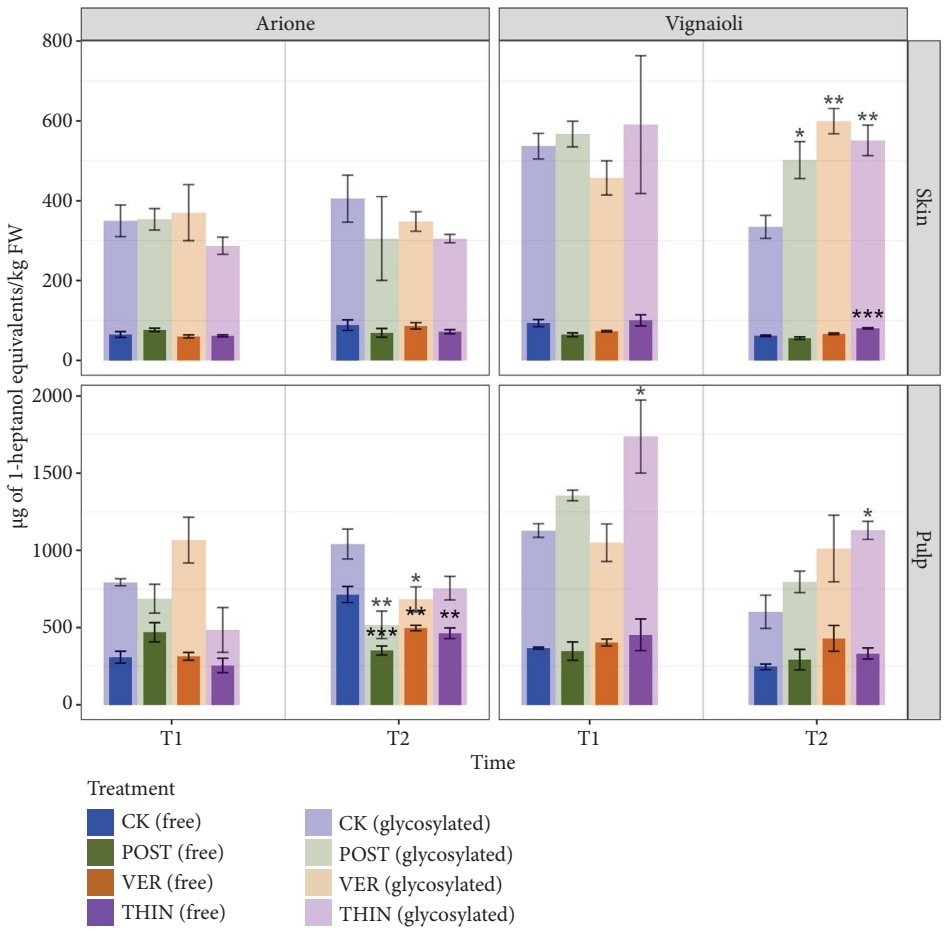

FIGURE 4: Bar plots representing the accumulation of total free and glycosylated monoterpenes in Moscato Bianco in skin and pulp of berries collected in Arione and Vignaioli vineyards at the considered sampling times (T1, commercial harvest for *Asti Spumante* wine production, and T2, commercial harvest for *Moscato d'Asti* wine production) in 2020. The treatments CK (control), POST (basal leaf removal at post-berry set), VER (basal leaf removal at veraison) and THIN (cluster thinning) are represented by the blue, green, orange and purple colours, respectively. Lighter colours were used to depict glycosylated aroma compounds, while darker and shinier colours were used for free aroma compounds. Each value is the average of the three biological replicates, and bars represent the standard error. Asterisks denote statistically significant differences in comparison with the control ($p < 0.05^{*}$, $p < 0.01^{**}$, $p < 0.001^{***}$), assessed using Dunnett's test or Kruskal–Wallis and Dunn's post hoc test, depending on whether the assumptions for parametric or nonparametric tests were met.

3.3. Overall Analysis of the Two Vineyards Throughout the Two-Season Experiment. The detailed analysis of individual monoterpenes highlighted that the treatments applied had specific and, sometimes, contrasting effects on the accumulation of key aroma compounds in both vineyards and at different sampling times. To gain a broader understanding of how the accumulation of these compounds, microclimatic factors and gene expression levels interact in shaping the aromatic profile, multivariate statistical approaches were applied by combining all the data from the two vineyards produced in 2020 season only (Figure 6), as well as combining the two harvesting seasons (Figure 7). A stepwise regression analysis was also performed to better link microclimatic parameters to the observed accumulation patterns of the analysed monoterpenes (Table 2).

3.3.1. Multivariate Analysis. Figure 6 presents the first PLS-DA model produced including all the data (microclimatic parameters, gene expression and volatile profiling) collected during the 2020 season from both Arione and Vignaioli vineyards. This model was created to explore the overall differences and similarities among the applied treatments across the two vineyards. The volatiles identified, along with the expression levels of the selected key genes and measured microclimatic parameters, were used as predictor variables, while the treatments served as response variables. Among the model components, we chose to report the biplot built using components 1 and 3, which together explained 66% of the total variability. This combination was selected because component 3, despite explaining slightly lower variance than component 2, provides clearer discrimination between CK and THIN groups and enhances the separation between POST and VER treatments (see Figure S6 for the biplot of components 1 and 2 in the Supporting Informations).

The model revealed clear separation patterns along the first and third components. Along the horizontal axis

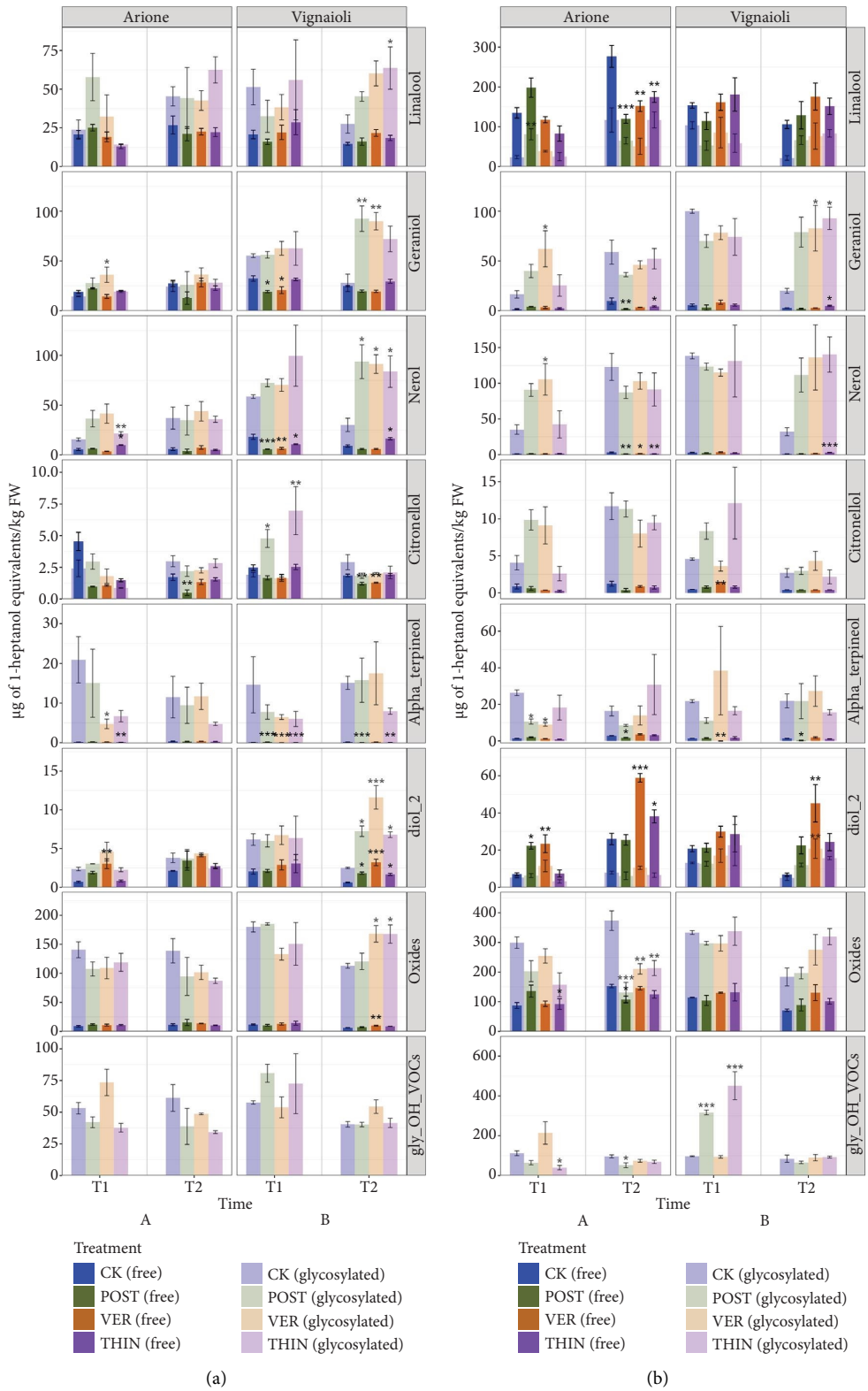

FIGURE 5: Bar plots representing the accumulation of specific free and glycosylated monoterpenes in Moscato Bianco berry skin (a) and pulp (b) of Arione and Vignaioli vineyards at T1 (harvest for *Asti Spumante*) and T2 (harvest for *Moscato d'Asti*) in 2020. The treatments CK (control), POST (basal leaf removal at post-berry set), VER (basal leaf removal at veraison) and THIN (cluster thinning) are represented by blue, green, orange and purple colours, respectively. Lighter colours were used to depict glycosylated aroma compounds, while darker and shinier colours were used for free aroma compounds. Each value is the average of the three biological replicates, and bars represent the standard error. Asterisks denote statistically significant difference in comparison with the control ($p < 0.05^{*}$, $p < 0.01^{**}$, $p < 0.001^{***}$), assessed using Dunnett's test or Kruskal–Wallis and Dunn's post hoc test, depending on whether the assumptions for parametric or nonparametric tests were met.

FIGURE 6: Biplot of the PLS-DA model built for the 2020 season. Vineyards (Arione and Vignaioli), grape berry tissues (skin and pulp) and time points (T1 and T2) were considered together. The model was created using the identified monoterpenes, the gene expression levels and the microclimatic parameters as predictor variables, while treatments (CK, POST, VER and THIN) were employed as response variables. The treatments CK (control), POST (basal leaf removal at post-berry set), VER (basal leaf removal at veraison) and THIN (cluster thinning) are represented by the blue, green, orange and purple colours, respectively. The vineyards Arione and Vignaioli are represented by triangles and squares, respectively. Empty shapes indicate skin tissue, while filled shapes represent pulp tissue.

(component 1), CK and THIN clustered on the right, while VER and POST samples were grouped on the left. This suggests that grape samples subjected to basal leaf removal treatments (POST and VER) shared a greater similarity within each other than the CK and THIN samples. Further differentiation emerged along the vertical axis (component 3), where POST and VER samples appeared to be separated. POST and VER samples clustered at the bottom and top of the quadrants, respectively. This highlights the clear effects of the two basal leaf removal protocols. The volatile accumulation pattern of the grape samples from the VER treatment appeared to be predominantly characterized by higher concentrations of free oxides, diols and linalool. This indicates that the basal leaf removal at veraison strongly influenced the accumulation of these compounds. In contrast, glycosylated monoterpenes were more closely associated with the CK treatment. Microclimatic parameters also showed strong associations, as they were altered

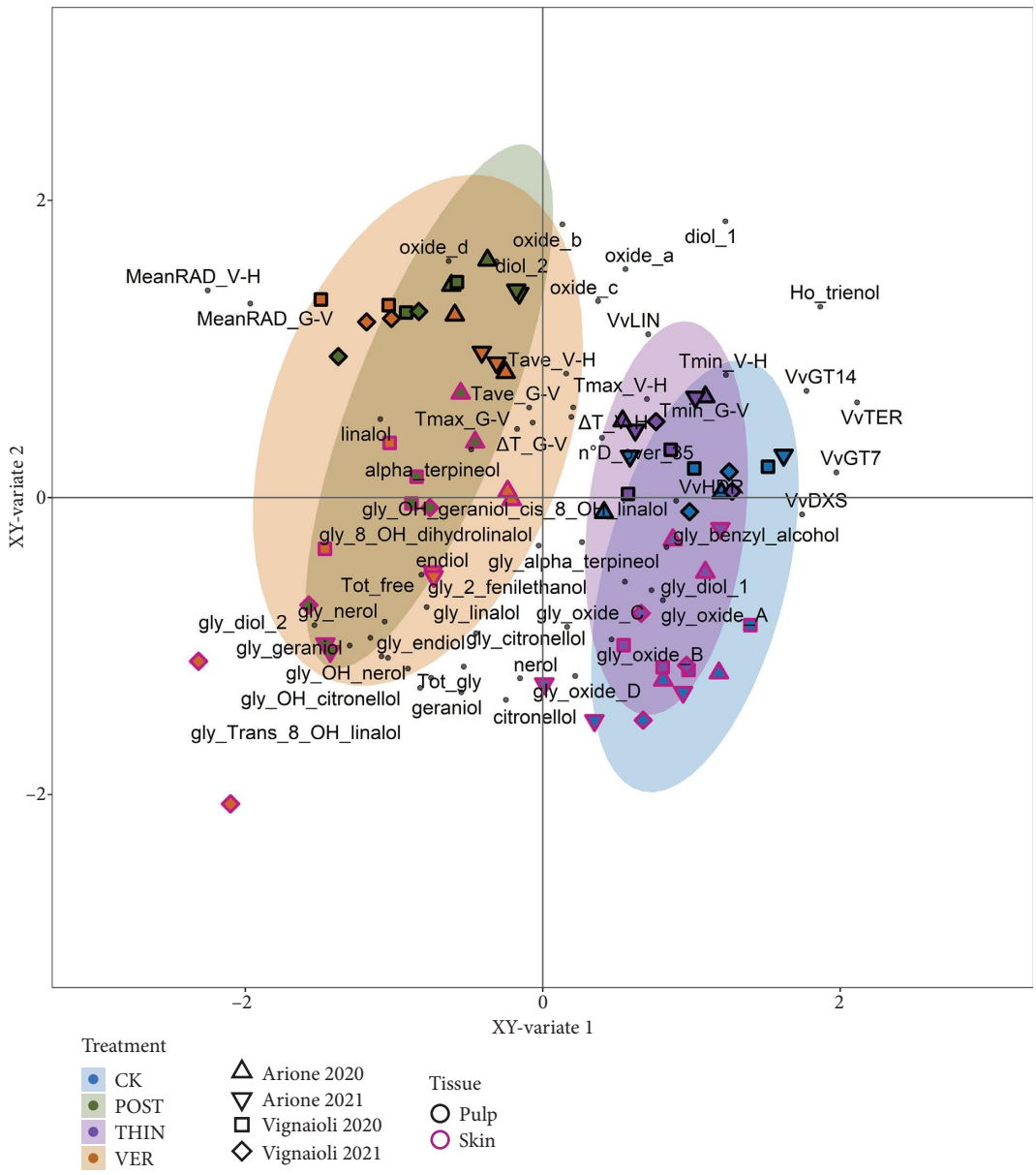

FIGURE 7: Biplot of the PLS-DA built for the seasons 2020 and 2021 considering Arione and Vignaioli vineyards, the skin and pulp tissues and T1 and T2 sampling points. The model was created using the identified monoterpene compounds, the genes and the microclimatic parameters as predictor variables, while treatments (CK, POST, VER and THIN) were employed as response variables. The treatments CK (control), POST (basal leaf removal at post-berry set), VER (basal leaf removal at veraison) and THIN (cluster thinning) are represented by blue, green, orange and purple colours, respectively. Vineyards and years are indicated by triangles for Arione and squares for Vignaioli, with inverted triangles for 2020 and diamonds for 2021 season. Tissue type is indicated by outline colour: purple outlines represent the skin tissue, while black outlines represent pulp tissue.

by the basal leaf removal treatments. For instance, parameters related to temperature—including maximum, minimum, average and temperature delta—were closely linked to the VER samples. In addition, the average irradiation showed a strong association with the POST samples, in alignment with the timing of the early leaf removal. The gene *VvLinNer1* clustered with VER samples, indicating a positive association with this group. In contrast, *VvHDR* and *VvGT7* were located within the THIN treatment ellipse, indicating a closer relationship with the THIN samples. The genes *VvTER*, *VvGT14* and *VvDXS3* fell within the VER quadrant but outside its confidence ellipse, suggesting some variability in their expression relative to this treatment. In addition, no clear clustering of skin and pulp tissue was observed in the biplot of this model. Overall, this analysis highlights the complex interplay between the proposed viticultural practices, monoterpene accumulation, microclimatic parameters and the expression level of the selected key genes involved in terpene biosynthesis. The reliability of the model in distinguishing the impact of the various treatments was confirmed by a validation accuracy of 54.2%.

Table 2: Results of the stepwise regression analysis calculated for the free and glycosylated terpenes in the berry skin/pulp and the microclimatic data.

Tissue	Form	Predictive variables	*B*	*p*-value (*t*-test)	R2	RMSE	*p*-value regression
Berry skin	Glycosylated	Intercept	45.36	0.0066	0.57	81.88	< 0.001
		Tmin_V-H	−47.52	0.0423			
		ΔT_V-H	54.67	0.0173			
	Free	Intercept	117.21	0.0087	0.69	31.79	< 0.001
		Tmax_V-H	32.58	0.0020			
		ΔT_G-V	31.90	0.0005			
		RADmean_V-H	21.64	0.0218			
Berry pulp	Glycosylated	Intercept	−50.47	0.0020	0.29	129.99	0.001
		ΔT_V-H	41.23	0.0081			
		n° days_over_35°C	−1.36	0.0447			
	Free	Intercept	31.49	0.0213	0.54	60.17	< 0.001
		Tmax_V-H	16.53	0.0018			
		RADmean_V-H	0.28	0.0118			

Note: Legend: B, coefficient of the regression; Tmin, Tmax, minimum and maximum daily temperature; ΔT, daily temperature delta between max and min values; *n*°D_over_35 the number of days with maximum temperature above 35°C; MeanRAD the mean daily radiation. These parameters were separately calculated and included in the analysis as before and after veraison (G-V and G-H, where G refers to the green growing stage of the berries, V to veraison and H to harvest, indicating the time intervals over which the variables were calculated). The range of values of the independent variables was as follows: 47–225 μg of 1-heptanol equivalents/kg FW (skin-free terpenes), 163–815 μg of 1-heptanol equivalents/kg FW (pulp-free terpenes), 133–897 μg of 1-heptanol equivalents/kg FW (skin glycosylated terpenes) and 215–2144 μg of 1-heptanol equivalents/kg FW (pulp glycosylated terpenes).

To confirm the findings from the 2020 season, a more complete analysis was performed by constructing a second PLS-DA model which integrated data from both 2020 and 2021 (Figure 7). This enabled us to evaluate the consistency and robustness of the treatment effects on the monoterpene compounds, microclimatic parameters and gene expression across different years. As with the PLS-DA reported in Figure 6, this second model was also subjected to rigorous external cross-validation procedures and achieved a validation accuracy of 58.9%, which indicates its reliability in distinguishing the impacts of the different treatments. A consistent grouping pattern was observed, with CK and THIN samples clustering together on the right side and VER and POST samples on the left one. Although POST and VER samples were not separated in distinct groups in this combined model, the overall clustering supports the consistency and robustness of treatment effects across seasons.

A total of 52% of the dataset variability was explained by the first two components of the model. Across component 1, CK and THIN samples clustered on the right side of the plot, while POST and VER grapes clustered on the left. In addition, a clustering of the different tissue types was observed along component 2, with pulp samples clustering towards the top of the plot and skin samples towards the bottom. The analysis highlighted specific associations between treatments and monoterpene compounds. Basal leaf removal treatments (POST and VER) were closely linked to total free and glycosylated monoterpenes, free oxides, free linalool and several glycosylated monoterpenes, including glycosylated linalool, geraniol, nerol, diol-2, endiol, 2-phenylethanol and 8-OH-dihydrolinalool. However, CK and THIN samples appeared to be associated with free nerol, geraniol, citronellol and glycosylated oxides and diol-1, as well as with the expression level of all the analysed genes. Compared to Figure 6, the microclimatic parameters showed diverse clustering in this model. Average radiation (at both harvest and veraison) was strongly associated with POST and VER samples, along with average temperature and delta temperature at veraison and harvest. The delta temperature at harvest, minimum temperature at both veraison and harvest and the number of days above 35°C were more closely linked to CK and THIN grapes. This combined-season analysis provided additional context to the 2020 findings, allowing for a broader understanding of the relationships among basal leaf removal and cluster thinning treatments on the monoterpene profile, microclimatic parameters and gene expression.

3.3.2. Stepwise Regression Analysis: Impact of Microclimatic Parameters on Glycosylated and Free Monoterpene Compound Concentration. A stepwise regression analysis was used to assess the impact of microclimatic parameters on the accumulation pattern of monoterpenes in skin and pulp tissues (Table 2). The regression results showed that, considering skin tissue, the minimum temperature and daily thermal delta were the strongest predictors for glycosylated monoterpene accumulation, whereas daily maximum temperature, daily thermal delta and the daily mean radiation were the significant variables predicting the accumulation of free compounds in the skin. In pulp tissue, the microclimatic data showed weaker correlations with the accumulation of monoterpenes. For pulp tissue, daily thermal delta from veraison to harvest and the number of days when the maximum temperature exceeded 35°C appeared as significant explanatory variables for glycosylated monoterpene accumulation in the multiple regression. For the prediction of free pulp monoterpenes, daily maximum temperature from veraison to harvest appeared as significant explanatory variable, together with daily mean radiation calculated in the same period.

4. Discussion

This study describes the impact of viticultural practices, namely basal leaf removal at postberry set and veraison, and cluster thinning at veraison, on vineyard microclimatic parameters and aroma compounds (monoterpenes) in Moscato Bianco grapes. A strong modulation in the expression of genes involved in monoterpene biosynthesis, along with their accumulation in Moscato Bianco grapes cultivated under different environmental conditions, was observed in both skin and pulp tissues. These findings could provide an important contribution to improving the aroma profile of *Moscato d'Asti* and *Asti spumante* wines. This is particularly important as, in recent years, a significant loss of aroma due to reduced terpene biosynthesis has been observed by Moscato Bianco growers in the Asti DOCG cultivation area. Canopy management trials were thus conducted in two representative vineyards, which differ in terms of environmental/climatic conditions.

As shown by the PCA in Figure 1, the Arione vineyard was associated with higher VPD and mean radiation. In contrast, the Vignaioli vineyard was characterized by greater thermal variability and higher maximum temperatures. Seasonal variability was also identified as a key factor, with samples from different vintages showing distinct clustering patterns in the PCA. This highlights the influence of year-specific climatic conditions, such as temperature and rainfall patterns, on the efficacy of the treatments. These differences underscore the importance of considering climate variations across different sites when tailoring viticultural practices for specific wine styles, such as *Moscato d'Asti* and *Asti spumante*.

The basal leaf removal treatments (VER and POST) were associated with modifications in key microclimatic parameters, including average irradiation and thermal delta (Table 1), which in turn may have contributed to the grapevine responses observed. Although our study does not demonstrate a direct causal link, these results are consistent with previous research showing that light and temperature conditions can influence the synthesis of VOCs and the expression of related genes. These findings align with the existing literature, which demonstrates how microclimatic parameters, including light and temperature, are influenced by leaf removal [42, 43]. For instance, Feng et al. [44] demonstrated that 100% and 50% leaf removal (from the base of each shoot to the node above the topmost cluster) in Pinot noir at the pea-sized stage effectively altered the percentage of ambient photosynthetically active radiation, which was measured in the cluster zone at 10:00 AM and 2:30 PM, over two consecutive years. Anić et al. [45] also reported that Merlot vines defoliated at berry set in the Croatian hills received greater UV radiation compared to control vines. These modifications in cluster/berry light exposure significantly impact the synthesis of VOCs and the expression of genes involved in their biosynthesis, as shown in previous studies [46, 47]. Shading treatments in Muscat grapes reduced the synthesis of certain volatiles, such as free linalool, geraniol and nerol in the mesocarp. However, they increased the concentration of glycosylated geraniol and nerol in both the mesocarp and the exocarp, thus indicating that light conditions strongly affect the accumulation pattern of these compounds [31]. A recently published paper [48] reported that excluding light from 65 days after anthesis until harvest severely diminished total free monoterpenoid accumulation in Riesling berries at harvest. Friedel et al. [36] re-exposed bunches of Riesling grapes that had been initially shaded at veraison; after 20 days of re-exposure, these grapes showed a significant increase in free and glycosylated monoterpenes compared to non-re-exposed grapes. The re-exposed bunches still exhibited a lower concentration of free and glycosylated monoterpenes compared to the controls (nonshaded), and the expression of genes, such as *VvPNLinNer1* and *VvPNLinNer2*, was higher in re-exposed grapes compared to shaded ones, although their expression level remained lower than in the control grapes.

In Vignaioli, basal leaf removal at veraison (VER) enhanced aroma compounds at T2 (harvest date for *Moscato d'Asti* production). In Arione, the benefits linked to these treatments were more evident at T1 (optimal harvest timing for *Asti Spumante;* Figures 4 and 5, and Figures S2 and S3, Supporting Information). The effect of the treatment was stronger in Vignaioli than in Arione (Figures 4 and 5, and Figures S2 and S3, Supporting Information). Leaf removal therefore appears to be a promising strategy to positively influence the aromatic profile of *Moscato d'Asti* and *Asti spumante,* depending on the specific vineyard altitude. The effectiveness of the treatments and the timing of the induced effects in grapes depend on the specific microclimatic conditions of each site (Figure 1). The VER treatment induced a general increase in aroma compounds at T2 in Vignaioli. The benefits of VER treatment in Arione at T1 were more evident for specific aroma compounds rather than causing greater accumulation of monoterpenes (Figures 4 and 5, and Figures S2 and S3, Supporting Information).

The more pronounced effects observed in Vignaioli may be due to the synergy between increased light exposure and thermal delta (Table 1), which promoted the synthesis of glycosylated monoterpenes in both the skin and the pulp at T2. Arione's higher altitude and mean radiation (Figure 1 and Table 1) may have altered the response of the vines to leaf removal. A few studies have explored the effects of viticultural treatments in relation to different altitudes on secondary metabolites. Berli et al. [49] highlighted different responses in Merlot grapes at different altitudes, particularly in the accumulation of secondary metabolites when treated with different levels of UV-B radiation. Additionally, variations in aroma compound accumulation have been reported within the same cultivar across different altitudes [50–52].

Basal leaf removal at postberry set (POST) in both vineyards, also with increased light exposure (Table 1), induced inconsistent effects on monoterpene accumulation. It promoted the accumulation of some VOCs and decreased the level of others. To the best of our knowledge, only Kok [53] has analysed the effects of leaf removal at postberry set in Muscat grapes and reported no significant impact on total monoterpene accumulation. Contradictory results have

been described for other grape varieties, with some studies reporting reduced accumulation of 3-isopropyl-2-methoxypyrazine in early stages of berry development in Sauvignon blanc grapes and no significant differences between treatments (defoliated and nondefoliated) at harvest when basal leaf removal was applied at BBCH 73 [54]. Other studies have reported no effects of leaf removal applied at prebloom (~BBCH 57), bloom (BBCH 65) and postbloom (BBCH 75) on monoterpene accumulation in Sauvignon Blanc and Riesling grapes [55]. Positive effects have been described in response to leaf removal at pea-sized stage (EL-31) in increasing varietal thiol precursors in Malvasia [56]. This inconsistency in results highlights the need for cultivar-specific studies and analyses to identify the best canopy management protocols aimed at increasing or preserving specific compounds that define the aromatic profile of grapes and wines.

Cluster thinning (THIN) in the Vignaioli vineyard positively influenced the accumulation of total glycosylated monoterpenes in both tissues at T2 and in pulp at T1, with an increase in the total free fraction observed in the skin at T2 (Figure 4). The pattern we observed can be explained by several physiological mechanisms that have been widely discussed in the literature, but which are still not fully understood. On the one hand, cluster thinning reduces the crop load, and on the other hand, it improves the leaf-to-fruit ratio [57]. This alteration in the source–sink balance plays a key role. It has long been assumed that a reduced crop load enables the vine to channel more resources—such as carbohydrates, nutrients and water—towards the remaining fruit [58]. This re-allocation not only increases the total soluble solids/acidity ratio but also alters the synthesis of secondary metabolites [59]. In fact, beyond primary metabolism, sugars serve as essential precursors for the biosynthesis of monoterpenes through the MEP pathway [32, 60]. As reported by Hemmerlin et al. [61], sugars produced during photosynthesis also play a key role in promoting the synthesis of MEP-derived isoprenoid compounds. Recent work by VanderWeide et al. [48] challenges the assumption that monoterpenoid accumulation is directly coupled to hexose accumulation. Their study demonstrated that free monoterpenoid biosynthesis in Riesling grapes is sensitive to light exposure and uncoupled from grape hexose accumulation. They found that light exclusion significantly reduced free monoterpenoid concentrations, while girdling (which restricted the sugar accumulation) did not alter total free monoterpenoid levels. This suggests that while sugars may play a role in early monoterpenoid biosynthesis, light exposure is a more critical factor in regulating monoterpenoid accumulation during ripening. In our study, the heightened carbohydrate availability early in the season in the THIN samples could have facilitated the monoterpene synthesis at T1. By T2, the difference in monoterpene accumulation between THIN and CK was further increased. The findings of VanderWeide et al. [48] suggest that this relationship may not be universal and that environmental factors, particularly light, play a more significant role in determining monoterpenoid profiles.

In Arione, across both time points and tissues, the THIN treatment appeared to have mostly negative effects on monoterpene accumulation (Figures S2 and S3). At T2, all the treatments applied in Arione resulted in a lower accumulation of several monoterpene compounds compared to the control samples, thus significantly affecting the total content of both free and glycosylated aroma compounds in the pulp (Figure 4). These negative effects underscore the need for a careful consideration of vineyard-specific conditions when implementing viticultural practices.

These differences align with previous research, which suggests that grape berry responses to viticultural practices are strongly influenced by environmental factors, such as climate, season, site characteristics, row orientation, exposure and canopy architecture [62–66]. Sivilotti et al. [67] showed that leaf removal applied before flowering and after flowering did not significantly influence the accumulation of methoxypyrazines and thiol precursors in Sauvignon Blanc grapes. They also observed that in cooler and rainy seasons, under lower solar irradiation, the impact of leaf removal was minimal, whereas during warmer years with increased light and temperature, the response to the treatments appeared to be stronger.

As observed with the variations in the accumulation of aroma compounds, the treatments applied appeared to distinctly modulate the expression of terpene biosynthesis–related genes, depending on the tissue type, timing, season and vineyard site. A few studies have examined the differential expression of these genes in skin and pulp tissues separately. The response of terpene-related genes under different viticultural treatments and light conditions has been reported in the scientific literature [31, 37, 67]. Our findings suggest that *VvLinNer1* and *VvTER* were the most responsive genes to both basal leaf removal and cluster thinning treatments (Figures 2 and 3). This is consistent with other studies investigating the effects of leaf removal, light exposure and cluster thinning on *VvLinNer* expression [29, 68] and other TPS genes [16]. The *VvTER,* which has been previously identified as an α-terpineol synthase [69–71], in our study showed consistent down-regulation across vineyards, harvest dates and tissues. In support of these findings, α-terpineol was generally detected at lower concentrations in samples subjected to canopy management treatments compared to the control (Figures 4 and 5, and Figures S2 and S3, Supporting Information).

The expression level of GT genes is often associated with the accumulation of glycosylated aroma compounds [20, 25, 72]. The selection of the GT genes analysed in this study was based on previous studies that identified their specific involvement in the glycosylation of monoterpene alcohols in Moscato grapes [31]. Other GTs, such as *VvGT1*, *GT3*, *GT5* and *GT6*, have been associated with the glycosylation of flavonols and other flavonoids, and were therefore not included in our gene expression analysis [36].

Although an overall increase in glycosylated compounds was observed after the treatments, especially in the Vignaioli vineyard (Figure 4), a corresponding increase in the expression of the selected GTs was not detected (Figures 2 and 3). Some studies have reported that GTs are not affected by leaf

removal at veraison [65]. Other works suggest that different GT types respond differently to light, and their expression is not directly correlated with the accumulation of glycosylated monoterpenes. Friedel et al. [36] reported that *VvGT7* transcript accumulation in Riesling was related to development and response to light, while the expression of *VvGT14* and *VvGT15* was not influenced by light. In the Muscat grape, Jingxiangyu Zhang et al. [31] observed that the expression of *VvGT14* was significantly promoted by sunlight exclusion from 10 days before veraison to 20 days after maturity. Different studies highlight the complexity of the relationship between the expression of the genes involved in terpene biosynthesis and their accumulation, particularly in relation to viticultural practices and berry development [65, 73, 74]. Overall, these findings suggest that further research is needed to better understand the intricate interplay between gene expression and terpene accumulation.

By employing a broader approach through the multivariate analysis of two different vineyards, two grape tissues and 2 years of experiments, we expanded the dataset and obtained more robust and reliable insights (Figures 6 and 7). This combined analysis strengthens the interpretation of consistent treatment effects across seasons, despite the presence of vintage-specific fluctuations. These analyses show that, beyond the differences induced by vineyard location, seasonal variation, harvest dates and tissue types, the basal leaf removal treatments played a key role in increasing the average irradiation levels of the clusters. This promoted the accumulation of oxides and glycosylated monoterpenes, such as linalool, nerol and geraniol, which significantly contribute to the total aroma profile of Moscato Bianco grapes (Figure 5 and Figure S3, Supporting Information). The positive correlation between increased irradiation and the defoliation treatments is further supported by the microclimatic data (Table 1). The stepwise regression analyses (Table 2) revealed that mean radiation was positively correlated with free terpenes across both skin and pulp tissues, rather than with glycosylated forms. These variations reflect the complex interaction between microclimatic conditions, grapevine physiology and viticultural practices, which can be further explained by the influence of altitude-driven microclimatic factors on grapevine physiology during ripening. Treatments, such as VER and THIN, demonstrated a significant potential in increasing aroma compound accumulation, particularly in Vignaioli, where microclimatic conditions synergized with the treatments applied. The inconsistent effects of POST basal leaf removal treatment, along with the broadly negative impact of all the treatments at T2 in Arione, highlight the need for further research to optimize the timing and application of these practices, particularly under different environmental conditions. While the flotation method used to standardize berry ripeness was effective in reducing variability across treatments for research purposes, this approach may mask potential treatment effects on ripening heterogeneity. Although outside the scope of this study, future research should consider assessing this aspect more directly to better understand the interplay between canopy management and berry ripening dynamics.

5. Conclusions

This study highlights the potential of canopy management strategies, specifically cluster thinning and basal leaf removal at veraison, to enhance the monoterpene profile of Moscato Bianco grapes. By modifying the bunch microclimate, particularly through increased light exposure, these practices showed the ability to enhance the accumulation of free and glycosylated monoterpenes, although their effectiveness varied depending on the vineyard site. In the Vignaioli vineyard, basal leaf removal at veraison (VER) and cluster thinning (THIN) increased the accumulation of glycosylated monoterpenes, particularly at the T2 harvest stage, which is crucial for *Moscato d'Asti* production. In the higher altitude Arione vineyard, the effects were less pronounced, with some treatments reducing monoterpene accumulation. The differential response between the two vineyards emphasizes the importance of site-specific management and the influence of local climatic conditions on grape metabolism. These findings provide additional information for viticulturists to fine-tune canopy management practices, particularly in the face of climate change, to mitigate the potential decline in aromatic intensity in Moscato Bianco grapes.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Disclosure

This manuscript reflects only the authors' views and opinions; neither the European Union nor the European Commission can be considered responsible for them.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

E.L.: investigation, sampling, data analysis, methodology, writing–original draft and review and editing. M.M.: sampling, data collection and analysis, methodology, investigation and writing–review and editing. G.P.: data analysis, methodology, writing–original draft and review and editing. G.B.: conceptualization, sampling, data collection, investigation, methodology and writing–review and editing. G.M.: sampling and data collection. M.R.: sampling and data collection. S.G.: sampling and data collection. D.E.: sampling, methodology, investigation and writing–review and editing. P.T.: conceptualization, methodology, investigation, sampling, writing–original draft, review and editing, resources and supervision. S.B.: conceptualization, methodology, sampling, investigation, data collection and analysis, writing–original draft and review and editing.

E.L. and M.M. have contributed equally to this work and share the first authorship.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. *(Supporting Information)*

Table S1. Harvest dates of 2020 and 2021 in the Arione and Vignaioli vineyards.

Table S2. List of primer sequences used for qPCR analyses.

Table S3. Systematic name (free form), CAS and reference information of each detected volatile aroma compound. The prefix 'gly_' denotes glycosylated forms, while unmodified compound names correspond to the free form of the compounds.

Figure S1. Positioning of temperature and light sensors within the grape cluster.

Figure S2. Heatmap reporting the accumulation of free and glycosylated monoterpenes in Moscato Bianco berry skin at T1 (harvest for *Asti spumante* production) and T2 (harvest for *Moscato d'Asti*) in 2020. The left panel corresponds to Arione vineyard while the right panel corresponds to Vignaioli vineyard. The heatmap shows the z-score values of each compound under the different treatments: CK (control), POST (basal leaf removal at post berry set), VER (basal leaf removal at veraison) and THIN (cluster thinning). Negative z-scores (in red) indicate a decreased accumulation of monoterpenes, while positive z-scores (in blue) indicate an increase. Each value is the average of the three biological replicates and the bars represent the standard error. Asterisks denote statistical significance ($p < 0.05^{*}$, $p < 0.01^{**}$, $p < 0.001^{***}$), assessed using Dunnett's test or Kruskal-Wallis and Dunn's post hoc test, depending on whether the assumptions for parametric or non-parametric tests were met.

Figure S3. Heatmap reporting the accumulation of free and glycosylated monoterpenes in Moscato Bianco in berry pulp at T1 (harvest for *Asti spumante* production) and T2 (harvest for *Moscato d'Asti*) in 2020. The left panel corresponds to Arione vineyard while the right panel corresponds to Vignaioli vineyard. The heatmap shows the z-score values of each compound under the different treatments: CK (control), POST (basal leaf removal at post berry set), VER (basal leaf removal at veraison), and THIN (cluster thinning). Negative z-scores (in red) indicate a decreased accumulation of monoterpenes, while positive z-scores (in blue) indicate an increase. Each value is the average of the three biological replicates and bars represent the standard error. Asterisks denote statistical significance ($p < 0.05^{*}$, $p < 0.01^{**}$, $p < 0.001^{***}$), assessed using Dunnett's test or Kruskal-Wallis and Dunn's post hoc test, depending on whether the assumptions for parametric or non-parametric tests were met.

Figure S4. Terpenes profile of free and glycosylated compounds in skin and pulp of grapes harvested in Arione vineyard, season 2020.

Figure S5. Terpenes profile of free and glycosylated compounds in skin and pulp of grapes harvested in Vignaioli vineyard, season 2020.

Figure S6. Biplot of PLS-DA model built for season 2020. Vineyards (Arione and Vignaioli), grape berry tissues (skin and pulp) and time points (T1 and T2) were considered together. The model was created using the identified monoterpenes, the gene expression levels and the microclimatic parameters as predictor variables, while treatments (CK, POST, VER and THIN) were employed as response variables. The treatments CK (control), POST (basal leaf removal at post berry-set), VER (basal leaf removal at veraison), and THIN (cluster thinning) are represented by the blue, green, orange and purple colours, respectively. The vineyards Arione and Vignaioli are represented by triangles and squares, respectively. Empty shapes indicate skin tissue, while filled shapes represent pulp tissue.

References

[1] Consorzio per la Tutela dell'Asti, *Disciplinare di Produzione Della Denominazione di Origine Controllata e Garantita dei Vini «Asti»* (2021), https://www.astidocg.it/asti-docg-denominazione/.

[2] D. Eberle, G. Bezzo, and G. Malerba, *Il Vigneto Moscato e le Denominazioni "Asti" e 'Moscato d'Asti'*, no. 9 (L'Enologo, 2015).

[3] C. D'Onofrio, F. Matarese, and A. Cuzzola, "Study of the Terpene Profile at Harvest and During Berry Development of Vitis vinifera L. Aromatic Varieties Aleatico, Brachetto, Malvasia di Candia Aromatica and Moscato Bianco," *Journal of the Science of Food and Agriculture* 97, no. 9 (2017): 2898–2907, https://doi.org/10.1002/jsfa.8126.

[4] R. Di Stefano, S. Bottero, R. Pigella, D. Borsa, G. Bezzo, and L. Corino, "Precursori d'aroma Glicosilati Presenti Nelle Uve di Alcune Cultivar a Frutto Colorato," *Enotecnico* 34 (1998): 63–76.

[5] M. Ugliano and L. Moio, "Free and Hydrolytically Released Volatile Compounds of Vitis vinifera L. cv. Fiano Grapes as Odour-Active Constituents of Fiano Wine," *Analytica Chimica Acta* 621, no. 1 (2008): 79–85, https://doi.org/10.1016/j.aca.2008.03.002.

[6] J. Crespo, P. Rigou, V. Romero, M. García, T. Arroyo, and J. M. Cabellos, "Effect of Seasonal Climate Fluctuations on the

Evolution of Glycoconjugates During the Ripening Period of Grapevine cv. Muscat à Petits Grains Blancs Berries," *Journal of the Science of Food and Agriculture* 98, no. 5 (2018): 1803–1812, https://doi.org/10.1002/jsfa.8656.

[7] A. Palliotti, M. Gatti, and S. Poni, "Early Leaf Removal to Improve Vineyard Efficiency: Gas Exchange, Source-to-Sink Balance, and Reserve Storage Responses," *American Journal of Enology and Viticulture* 62, no. 2 (2011): 219–228, https://doi.org/10.5344/ajev.2011.10094.

[8] S. Poni, M. Gatti, A. Palliotti, et al., "Grapevine Quality: A Multiple Choice Issue," *Scientia Horticulturae* 234 (2018): 445–462, https://doi.org/10.1016/j.scienta.2017.12.035.

[9] Y. Wang, L. He, Q. Pan, C. Duan, and J. Wang, "Effects of Basal Defoliation on Wine Aromas: A Meta-Analysis," *Molecules* 23, no. 4 (2018): 779, https://doi.org/10.3390/molecules23040779.

[10] J. VanderWeide, T. Frioni, Z. Ma, M. Stoll, S. Poni, and P. Sabbatini, "Early Leaf Removal as a Strategy to Improve Ripening and Lower Cluster Rot in Cool Climate (Vitis vinifera L.) Pinot Grigio," *American Journal of Enology and Viticulture* 71, no. 1 (2020): 70–79, https://doi.org/10.5344/ajev.2019.19042.

[11] E. Cataldo, L. Salvi, F. Paoli, M. Fucile, and G. B. Mattii, "Effect of Agronomic Techniques on Aroma Composition of White Grapevines: A Review," *Agronomy* 11, no. 10 (2021): 2027, https://doi.org/10.3390/agronomy11102027.

[12] C. A. Black, M. Parker, T. E. Siebert, D. L. Capone, and I. L. Francis, "Terpenoids and Their Role in Wine Flavour: Recent Advances," *Australian Journal of Grape and Wine Research* 21 (2015): 582–600, https://doi.org/10.1111/ajgw.12186.

[13] W. Han, N. Han, X. He, and X. Zhao, "Berry Thinning to Reduce Bunch Compactness Improves Fruit Quality of Cabernet Sauvignon (Vitis vinifera L.)," *Scientia Horticulturae* 246 (2019): 589–596, https://doi.org/10.1016/j.scienta.2018.11.037.

[14] M. Gatti, F. Bernizzoni, S. Civardi, and S. Poni, "Effects of Cluster Thinning and Preflowering Leaf Removal on Growth and Grape Composition in cv. Sangiovese," *American Journal of Enology and Viticulture* 63, no. 3 (2012): 325–332, https://doi.org/10.5344/ajev.2012.11118.

[15] W. Wang, Y. Liang, G. Quan, X. Wang, and Z. Xi, "Thinning of Cluster Improves Berry Composition and Sugar Accumulation in Syrah Grapes," *Scientia Horticulturae* 297 (2022): 110966, https://doi.org/10.1016/j.scienta.2022.110966.

[16] X. Yue, Y. Ju, Y. Fang, and Z. Zhang, "Transcriptomics Integrated With Metabolomics Reveals the Effect of Cluster Thinning on Monoterpene Biosynthesis in 'Muscat Hamburg' Grape," *Foods* 10, no. 11 (2021): 2718, https://doi.org/10.3390/foods10112718.

[17] C. Conduruso, F. Cincotta, G. Tripodi, et al., "Effects of Cluster Thinning on Wine Quality of Syrah Cultivar (Vitis vinifera L.)," *European Food Research and Technology* 242, no. 10 (2016): 1719–1726, https://doi.org/10.1007/s00217-016-2671-7.

[18] Y. Wang, Y. N. He, L. He, et al., "Changes in Global Aroma Profiles of Cabernet Sauvignon in Response to Cluster Thinning," *Food Research International* 122 (2019): 56–65, https://doi.org/10.1016/j.foodres.2019.03.061.

[19] Y. Romboli, S. F. Di Gennaro, S. Mangani, et al., "Vine Vigour Modulates Bunch Microclimate and Affects the Composition of Grape and Wine Flavonoids: an Unmanned Aerial Vehicle Approach in a Sangiovese Vineyard in Tuscany," *Australian Journal of Grape and Wine Research* 23, no. 3 (2017): 368–377, https://doi.org/10.1111/ajgw.12293.

[20] P. Piombino, E. Pittari, A. Genovese, A. Bellincontro, O. Failla, and L. Moio, "Effects of Leaf Removal on Free and Glycoconjugate Aromas of Skins and Pulps of Two Italian Red Grapevine Varieties," *Foods* 12, no. 19 (2023): 3661, https://doi.org/10.3390/foods12193661.

[21] E. C. Georgiadou, M. Mina, V. Neoptolemou, et al., "The Beneficial Effect of Leaf Removal During Fruit Set on Physiological, Biochemical, and Qualitative Indices and Volatile Organic Compound Profile of the Cypriot Reference Cultivar 'Xynisteri'," *Journal of the Science of Food and Agriculture* 103, no. 8 (2023): 3776–3786, https://doi.org/10.1002/jsfa.12268.

[22] I. Ghiglieno, S. Carlin, G. Cola, et al., "Impact of Meteorological Conditions, Canopy Shading and Leaf Removal on Yield, Must Quality, and Norisoprenoid Compounds Content in Franciacorta Sparkling Wine," *Frontiers in Plant Science* 14 (2023): 1125560, https://doi.org/10.3389/fpls.2023.1125560.

[23] Z. Li, D. Yang, X. Guan, Y. Sun, and J. Wang, "Changes in Volatile Composition of Cabernet Sauvignon (Vitis vinifera L.) Grapes Under Leaf Removal Treatment," *Agronomy* 13, no. 7 (2023): 1888, https://doi.org/10.3390/agronomy13071888.

[24] X. Yue, X. Ma, Y. Tang, et al., "Effect of Cluster Zone Leaf Removal on Monoterpene Profiles of Sauvignon Blanc Grapes and Wines," *Food Research International* 131 (2020): 109028, https://doi.org/10.1016/j.foodres.2020.109028.

[25] W. Schwab and M. Wüst, "Understanding the Constitutive and Induced Biosynthesis of mono-and Sesquiterpenes in Grapes (Vitis Vinifera): a Key to Unlocking the Biochemical Secrets of Unique Grape Aroma Profiles," *Journal of Agricultural and Food Chemistry* 63, no. 49 (2015): 10591–10603, https://doi.org/10.1021/acs.jafc.5b04398.

[26] J. Battilana, L. Costantini, F. Emanuelli, et al., "The 1-Deoxy-d-Xylulose 5-Phosphate Synthase Gene Co-Localizes With a Major QTL Affecting Monoterpene Content in Grapevine," *Theoretical and Applied Genetics* 118, no. 4 (2009): 653–669, https://doi.org/10.1007/s00122-008-0927-8.

[27] X. R. Sun, X. Q. Xu, G. J. Zhang, et al., "Monoterpene Accumulation and Its Biosynthesis: Gene Transcript Profiles of Two Grape Cultivars During Berry Development," *XI International Conference on Grapevine Breeding and Genetics* 1082 (July 2014): 37–42, https://doi.org/10.17660/actahortic.2015.1082.2.

[28] D. M. Martin, A. Chiang, S. T. Lund, and J. Bohlmann, "Biosynthesis of Wine Aroma: Transcript Profiles of Hydroxymethylbutenyl Diphosphate Reductase, Geranyl Diphosphate Synthase, and Linalool/Nerolidol Synthase Parallel Monoterpenol Glycoside Accumulation in Gewürztraminer Grapes," *Planta* 236, no. 3 (2012): 919–929, https://doi.org/10.1007/s00425-012-1704-0.

[29] X. Xi, Q. Zha, Y. He, Y. Tian, and A. Jiang, "Influence of Cluster Thinning and Girdling on Aroma Composition in 'Jumeigui' Table Grape," *Scientific Reports* 10, no. 1 (2020): 6877, https://doi.org/10.1038/s41598-020-63826-7.

[30] J. D. Dunlevy, C. M. Kalua, R. A. Keyzers, and P. K. Boss, "The Production of Flavour & Aroma Compounds in Grape Berries," in *Grapevine Molecular Physiology & Biotechnology* (Springer Netherlands, 2009), 293–340.

[31] E. Zhang, F. Chai, H. Zhang, S. Li, Z. Liang, and P. Fan, "Effects of Sunlight Exclusion on the Profiles of Monoterpene Biosynthesis and Accumulation in Grape Exocarp and Mesocarp," *Food Chemistry* 237 (2017): 379–389, https://doi.org/10.1016/j.foodchem.2017.05.127.

[32] J. Lin, M. Massonnet, and D. Cantu, "The Genetic Basis of Grape and Wine Aroma," *Horticulture Research* 6, no. 1 (2019): 81, https://doi.org/10.1038/s41438-019-0163-1.

[33] A. Banerjee and T. D. Sharkey, "Methylerythritol 4-Phosphate (MEP) Pathway Metabolic Regulation," *Natural Product*

Reports 31, no. 8 (2014): 1043–1055, https://doi.org/10.1039/c3np70124g.

[34] A. K. Hjelmeland and S. E. Ebeler, "Glycosidically Bound Volatile Aroma Compounds in Grapes and Wine: A Review," *American Journal of Enology and Viticulture* 66, no. 1 (2015): 1–11, https://doi.org/10.5344/ajev.2014.14104.

[35] L. Costantini, C. D. Kappel, M. Trenti, et al., "Drawing Links From Transcriptome to Metabolites: The Evolution of Aroma in the Ripening Berry of Moscato Bianco (Vitis vinifera L.)," *Frontiers in Plant Science* 8 (2017): 780, https://doi.org/10.3389/fpls.2017.00780.

[36] M. Friedel, J. Frotscher, M. Nitsch, et al., "Light Promotes Expression of Monoterpene and Flavonol Metabolic Genes and Enhances Flavour of Winegrape Berries (Vitis vinifera L. cv. Riesling)," *Australian Journal of Grape and Wine Research* 22, no. 3 (2016): 409–421, https://doi.org/10.1111/ajgw.12229.

[37] D. H. Lorenz, K. W. Eichhorn, H. Bleiholder, R. Klose, U. Meier, and E. Weber, "Growth Stages of the Grapevine: Phenological Growth Stages of the Grapevine (Vitis vinifera L. Ssp. vinifera)—Codes and Descriptions According to the Extended BBCH Scale," *Australian Journal of Grape and Wine Research* 1, no. 2 (1995): 100–103, https://doi.org/10.1111/j.1755-0238.1995.tb00085.x.

[38] R. Di Stefano, "Proposition D'une Methode de Preparation de L'echantillon Pour la Determination des Terpenes Libres et Glycosides des Raisins et des Vins [Methode D'analyse Rapide, Reproductibilite; Vins Aromatiques]," *Bulletin de L'oiv* 64 (1991).

[39] M. Pollon, F. Torchio, S. Giacosa, S. R. Segade, and L. Rolle, "Use of Density Sorting for the Selection of Aromatic Grape Berries With Different Volatile Profile," *Food Chemistry* 276 (2019): 562–571, https://doi.org/10.1016/j.foodchem.2018.10.040.

[40] K. J. Livak and T. D. Schmittgen, "Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2– ΔΔCT Method," *Methods* 25, no. 4 (2001): 402–408, https://doi.org/10.1006/meth.2001.1262.

[41] F. Rohart, B. Gautier, A Singh, and K. A. Lê Cao, "mixOmics: An R Package for 'Omics Feature Selection and Multiple Data Integration," *PLoS Computational Biology* 13, no. 11 (2017): e1005752.

[42] S. Poni and C. Intrieri, "Grapevine Photosynthesis: Effects Linked to Light Radiation and Leaf Age," *Advances in Horticultural Science* 15 (2001): 5–15.

[43] A. Baiano, A. De Gianni, M. A. Previtali, M. A. Del Nobile, V. Novello, and L. de Palma, "Effects of Defoliation on Quality Attributes of Nero di Troia (Vitis vinifera L.) Grape and Wine," *Food Research International* 75 (2015): 260–269, https://doi.org/10.1016/j.foodres.2015.06.007.

[44] H. Feng, F. Yuan, P. A. Skinkis, and M. C. Qian, "Influence of Cluster Zone Leaf Removal on Pinot Noir Grape Chemical and Volatile Composition," *Food Chemistry* 173 (2015): 414–423, https://doi.org/10.1016/j.foodchem.2014.09.149.

[45] M. Anić, M. Osrečak, Ž. Andabaka, et al., "The Effect of Leaf Removal on Canopy Microclimate, Vine Performance and Grape Phenolic Composition of Merlot (Vitis vinifera L.) Grapes in the Continental Part of Croatia," *Scientia Horticulturae* 285 (2021): 110161, https://doi.org/10.1016/j.scienta.2021.110161.

[46] J. M. Gambetta, V. Romat, L. M. Schmidtke, and B. P. Holzapfel, "Secondary Metabolites Coordinately Protect Grapes From Excessive Light and Sunburn Damage During Development," *Biomolecules* 12, no. 1 (2021): 42, https://doi.org/10.3390/biom12010042.

[47] Z. Ma, S. Yang, J. Mao, et al., "Effects of Shading on the Synthesis of Volatile Organic Compounds in 'Marselan' Grape Berries (Vitis vinifera L.)," *Journal of Plant Growth Regulation* 40, no. 2 (2021): 679–693, https://doi.org/10.1007/s00344-020-10123-2.

[48] J. Vander Weide, J. Pico, M. Petersen, Y. Yan, W. F. Zandberg, and S. D. Castellarin, "Free Monoterpenoid Accumulation in 'Riesling' (Vitis vinifera L.) Is Light-Sensitive and Uncoupled From Grape Hexose Accumulation," *Plant Physiology and Biochemistry* 219, no. 2025 (2025): 109212, https://doi.org/10.1016/j.plaphy.2024.109212.

[49] F. Berli, J. D'Angelo, B. Cavagnaro, R. Bottini, R. Wuilloud, and M. F. Silva, "Phenolic Composition in Grape (Vitis vinifera L. cv. Malbec) Ripened With Different Solar UV-B Radiation Levels by Capillary Zone Electrophoresis," *Journal of Agricultural and Food Chemistry* 56, no. 9 (2008): 2892–2898.

[50] T. X. Yue, M. Chi, C. Z. Song, et al., "Aroma Characterization of Cabernet Sauvignon Wine From the Plateau of Yunnan (China) With Different Altitudes Using SPME-GC/MS," *International Journal of Food Properties* 18, no. 7 (2015): 1584–1596, https://doi.org/10.1080/10942912.2014.923442.

[51] M. Alessandrini, F. Gaiotti, N. Belfiore, F. Matarese, C. D'Onofrio, and D. Tomasi, "Influence of Vineyard Altitude on Glera Grape Ripening (Vitis vinifera L.): Effects on Aroma Evolution and Wine Sensory Profile," *Journal of the Science of Food and Agriculture* 97, no. 9 (2017): 2695–2705, https://doi.org/10.1002/jsfa.8093.

[52] J. B. de Oliveira, R. Egipto, O. Laureano, R. de Castro, G. E. Pereira, and J. M. Ricardo-da-Silva, "Climate Effects on Physicochemical Composition of Syrah Grapes at Low and High Altitude Sites From Tropical Grown Regions of Brazil," *Food Research International* 121 (2019): 870–879, https://doi.org/10.1016/j.foodres.2019.01.011.

[53] D. Kok, "Variation in Total Phenolic Compounds, Anthocyanin and Monoterpene Content of 'Muscat Hamburg' Table Grape Variety (V. vinifera L.) as Affected by Cluster Thinning and Early and Late Period Basal Leaf Removal Treatments," *Erwerbs-Obstbau* 58, no. 4 (2016): 241–246, https://doi.org/10.1007/s10341-016-0283-9.

[54] D. Mosetti, J. C. Herrera, P. Sabbatini, et al., "Impact of Leaf Removal After Berry Set on Fruit Composition and Bunch Rot In 'Sauvignon Blanc'," *Vitis* 55, no. 2 (2016): 57–64.

[55] B. L. Komm and M. M. Moyer, "Effect of Early Fruit-Zone Leaf Removal on Canopy Development and Fruit Quality in Riesling and Sauvignon Blanc," *American Journal of Enology and Viticulture* 66, no. 4 (2015): 424–434, https://doi.org/10.5344/ajev.2015.15007.

[56] M. Bubola, I. Lukić, S. Radeka, et al., "Enhancement of Istrian Malvasia Wine Aroma and Hydroxycinnamate Composition by Hand and Mechanical Leaf Removal," *Journal of the Science of Food and Agriculture* 99, no. 2 (2019): 904–914, https://doi.org/10.1002/jsfa.9262.

[57] W. M. Kliewer and R. J. Weaver, "Effect of Crop Level and Leaf Area on Growth, Composition, and Coloration of Tokay'Grapes," *American Journal of Enology and Viticulture* 22, no. 3 (1971): 172–177, https://doi.org/10.5344/ajev.1971.22.3.172.

[58] A. J. Winkler, "Effects of Overcropping," *American Journal of Enology and Viticulture* 5, no. 1 (1954): 4–12, https://doi.org/10.5344/ajev.1954.5.1.4.

[59] J. Martínez-Lüscher and S. K. Kurtural, "Source–Sink Manipulations Have Major Implications for Grapevine Berry and Wine Flavonoids and Aromas That Go Beyond the Changes in Berry Sugar Accumulation," *Food Research International* 169 (2023): 112826, https://doi.org/10.1016/j.foodres.2023.112826.

[60] M. Rodrıguez-Concepción and A. Boronat, "Elucidation of the Methylerythritol Phosphate Pathway for Isoprenoid Biosynthesis in Bacteria and Plastids. A Metabolic Milestone Achieved Through Genomics," *Plant Physiology* 130, no. 3 (2002): 1079–1089, https://doi.org/10.1104/pp.007138.

[61] A. Hemmerlin, J. L. Harwood, and T. J. Bach, "A Raison D'Être for Two Distinct Pathways in the Early Steps of Plant Isoprenoid Biosynthesis?" *Progress in Lipid Research* 51, no. 2 (2012): 95–148, https://doi.org/10.1016/j.plipres.2011.12.001.

[62] S. Poni and F. Bernizzoni, "A Three-Year Survey on the Impact of Pre-Flowering Leaf Removal on Berry Growth Components and Grape Composition in cv. Barbera Vines," *OENO One* 44, no. 1 (2010): 21–30, https://doi.org/10.20870/oeno-one.2010.44.1.1458.

[63] M. S. Lemut, K. Trost, P. Sivilotti, and U. Vrhovsek, "Pinot Noir Grape Colour Related Phenolics as Affected by Leaf Removal Treatments in the Vipava Valley," *Journal of Food Composition and Analysis* 24, no. 6 (2011): 777–784, https://doi.org/10.1016/j.jfca.2011.03.003.

[64] S. Zhuang, L. Tozzini, A. Green, et al., "Impact of Cluster Thinning and Basal Leaf Removal on Fruit Quality of Cabernet Franc (Vitis vinifera L.) Grapevines Grown in Cool Climate Conditions," *HortScience* 49, no. 6 (2014): 750–756, https://doi.org/10.21273/hortsci.49.6.750.

[65] L. He, X. Q. Xu, Y. Wang, et al., "Modulation of Volatile Compound Metabolome and Transcriptome in Grape Berries Exposed to Sunlight Under Dry-Hot Climate," *BMC Plant Biology* 20 (2020): 1–18, https://doi.org/10.1186/s12870-020-2268-y.

[66] D. Škrab, P. Sivilotti, P. Comuzzo, et al., "Cluster Thinning and Vineyard Site Modulate the Metabolomic Profile of Ribolla Gialla Base and Sparkling Wines," *Metabolites* 11, no. 5 (2021): 331, https://doi.org/10.3390/metabo11050331.

[67] P. Sivilotti, R. Falchi, J. C. Herrera, et al., "Combined Effects of Early Season Leaf Removal and Climatic Conditions on Aroma Precursors in Sauvignon Blanc Grapes," *Journal of Agricultural and Food Chemistry* 65, no. 38 (2017): 8426–8434, https://doi.org/10.1021/acs.jafc.7b03508.

[68] K. Sasaki, H. Takase, S. Matsuyama, et al., "Effect of Light Exposure on Linalool Biosynthesis and Accumulation in Grape Berries," *Bioscience, Biotechnology, and Biochemistry* 80, no. 12 (2016): 2376–2382, https://doi.org/10.1080/09168451.2016.1217148.

[69] D. M. Martin and J. Bohlmann, "Identification of Vitis vinifera (−)-α-terpineol Synthase by in Silico Screening of Full-Length cDNA ESTs and Functional Characterization of Recombinant Terpene Synthase," *Phytochemistry* 65, no. 9 (2004): 1223–1229, https://doi.org/10.1016/j.phytochem.2004.03.018.

[70] K. Geng, D. Li, J. Zhang, Y. Zhang, Z. Zhan, and Z. Wang, "Evolution of Volatile Aroma Compounds and Amino Acids in Cabernet Gernischt Grape Berries (Vitis vinifera L.): Comparison of Different Training Systems for Mechanical Soil Burial," *Foods* 11, no. 11 (2022): 1568, https://doi.org/10.3390/foods11111568.

[71] R. N. Bosman and J. G. Lashbrooke, "Grapevine Mono-and Sesquiterpenes: Genetics, Metabolism, and Ecophysiology," *Frontiers in Plant Science* 14 (2023): 1111392, https://doi.org/10.3389/fpls.2023.1111392.

[72] Y. Q. Wen, G. Y. Zhong, Y. Gao, Y. B. Lan, C. Q. Duan, and Q. H. Pan, "Using the Combined Analysis of Transcripts and Metabolites to Propose Key Genes for Differential Terpene Accumulation Across Two Regions," *BMC Plant Biology* 15 (2015): 1–22, https://doi.org/10.1186/s12870-015-0631-1.

[73] F. Matarese, G. Scalabrelli, and C. D'Onofrio, "Analysis of the Expression of Terpene Synthase Genes in Relation to Aroma Content in Two Aromatic Vitis vinifera Varieties," *Functional Plant Biology* 40, no. 6 (2013): 552–565, https://doi.org/10.1071/fp12326.

[74] R. N. Bosman, J. A. M. Vervalle, D. L. November, P. Burger, and J. G. Lashbrooke, "Grapevine Genome Analysis Demonstrates the Role of Gene Copy Number Variation in the Formation of Monoterpenes," *Frontiers in Plant Science* 14 (2023): 1112214, https://doi.org/10.3389/fpls.2023.1112214.