horticulturae

MDPI

Article

Effects of Apical, Late-Season Leaf Removal on Vine Performance and Wine Properties in Sangiovese Grapevines (*Vitis vinifera* L.)

Alberto Vercesi [1], Mario Gabrielli [2], Alessandra Garavani [1] and Stefano Poni [1,*]

[1] Department of Sustainable Crop Production (DiProVeS), Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy; alberto.vercesi@unicatt.it (A.V.); alessandra.garavani@unicatt.it (A.G.)
[2] Department for Sustainable Food Process (DiSTAS), Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy; mario.gabrielli@unicatt.it
* Correspondence: stefano.poni@unicatt.it

Abstract: An urgent challenge posed by climate change in warm grapevine-growing areas is accelerated ripening, which leads to rapid sugar accumulation while phenolics and aroma traits lag behind. Techniques that enable selectively delaying the sugar accumulation process without affecting the accumulation of secondary metabolites are essential. This study aimed to evaluate the effects of apical-to-cluster defoliation, manually applied in 2019 at the onset of veraison (D_1) or 20 days later (D_2), which removed about 30–40% of the pending total leaf area without altering the cluster microclimate compared with a non-defoliated control (C). Ripening trends, vegetative growth, yield components, and the final grape and wine composition, as well as wine sensorial attributes, were assessed. Although both treatments significantly lowered the final leaf area-to-yield ratio (0.80–0.90 m^2/kg) compared with the 1.35 m^2/kg recorded in the C vines, only D_1 reduced the final total soluble solids (TSS) at harvest (2 °Brix less than C). However, the total anthocyanins were similarly limited, and titratable acidity (TA) did not differ from the C vines. The D_1 wine was deemed similar to that made from control plants. Conversely, D_2 failed to delay ripening, yet the D_2 wine was deemed superior in terms of olfactory intensity, body, fruitiness, balance, and overall preference. Although the study was conducted over a single season, the results are robust enough to conclude that the timing of defoliation—i.e., the level of TSS concurrently reached by the C treatment—is crucial to achieving specific effects. Early defoliation appears valid for postponing ripening into a cooler period, making it quite interesting in warm–hot areas with a very long growing season; a much later defoliation, likely due to the interaction between mean canopy age and more light filtering from above the cluster zone, can elevate the quality of and appreciation for the final wine.

Keywords: summer pruning; berry ripening; canopy management; total soluble solids; sensorial analysis

Citation: Vercesi, A.; Gabrielli, M.; Garavani, A.; Poni, S. Effects of Apical, Late-Season Leaf Removal on Vine Performance and Wine Properties in Sangiovese Grapevines (*Vitis vinifera* L.). *Horticulturae* **2024**, *10*, 929. https://doi.org/10.3390/horticulturae10090929

Academic Editors: Arif Atak, Andreia Figueiredo, Inmaculada Pascual and Fermin Morales

Received: 1 August 2024
Revised: 27 August 2024
Accepted: 28 August 2024
Published: 30 August 2024

1. Introduction

In recent decades, several important viticultural regions worldwide have reported a trend toward overly fast grape ripening, causing excessive and/or too rapid sugar accumulation in the berries [1]. In the worst scenario, this is coupled with unacceptably low acidity and high pH, as well as overripe and/or atypical flavors [2,3]. The resulting rise in ethanol concentration in wine has potential negative effects on human health [4], and, presently, an increasing number of consumers prefer wines with moderate alcohol concentration [5]. In all European Union countries and Switzerland, the allowed lowest and highest alcohol concentrations in wines are set at 8.5% *v*/*v* and 15.0% *v*/*v*, respectively, with some derogations (European Commission, 2008) [6]. Additionally, some countries penalize high-alcohol wines by imposing higher import duties, which considerably raise the final price of wine.

The general increase in alcohol concentration in wines worldwide is due to several factors, some of which are undoubtedly related to climate change (i.e., the rise in ambient

CO_2 concentration enhances leaf photosynthesis and berry dehydration, fostered by more frequent temperature peaks) [7,8]. Physiologically, earlier maturity might aggravate the desynchronization between technological and phenolic ripening, causing detrimental effects especially on red wine quality. Typically, while berry sugar concentration reaches very high values (around 25 °Brix), the accumulation of flavonoids lags behind. High grape sugar concentration causes a stress response in yeast, which can lead to stuck and sluggish fermentations [9] and unbalanced wines [10]. High ethanol concentration in wine increases perceptions of hotness and bitterness, reducing the acidity sensations and the perception of some important aroma compounds such as higher alcohols, esters, and monoterpenes [11,12]. Furthermore, high alcohol content can negatively affect malolactic fermentation, due to *Oenococcus oeni* cells losing membrane fluidity and cell viability, leading to longer wine stabilization and changes in sensory profile [13].

Microbiological, physical, or chemical oenological strategies to reduce alcohol content in wines have been proposed, including membrane techniques [14], reverse osmosis [15], supercritical fluid extraction [16], and vacuum distillation [17]. Despite the capability of these post-fermentation techniques to significantly decrease alcohol concentration in wine, they have also been reported to negatively affect wine sensory properties [18,19].

On the other hand, there are several vineyard management techniques to regulate sugar accumulation in the berries and/or decelerate overly fast and unbalanced grape ripening: (i) late winter pruning [20,21]; (ii) minimal pruning [22]; (iii) late shoot trimming [23]; (iv) late apical leaf removal [24]; (v) treatments with auxins [25], brassinazole [26], salicylic acid [2], and cytokinins [27]; (vi) pre-harvest irrigation [28]; (vii) application of anti-transpirants [29]; and (viii) double cropping [30].

Among the above, preference should be given to those ensuring ease of application, economic sustainability (e.g., allowing suitability for full mechanization), and good repeatability of the desired effects. Traditionally, leaf removal involves removing, generally between fruit set and veraison, all or a fraction of the basal leaves to reduce canopy density around clusters, thereby improving the fruit microclimate, cluster light exposure, and air circulation. The lower humidity at the cluster level, associated with better penetration of sprays, might also help decrease the incidence of pests and diseases. Its impact on grape composition, however, is controversial, probably due to the variability in the timing and intensity of the intervention [31], as well as factors including vine variety, irrigation practice, training system, and local microclimate [32–34].

It should also be mentioned that the role of traditional basal leaf removal has changed significantly within the context of global warming [31], where, especially in warm climates, the issues of leaf and berry overheating followed by sunburn and necrosis are increasing concerns [35]. In some sensitive red varieties, excessive temperature leads to poor berry color caused either by supra-optimal temperature for enzymes presiding over the biosynthetic pathway of anthocyanidin formation or by the enhanced degradation of already formed anthocyanins [36]. A standard basal leaf removal is predicted to aggravate such events, and a much more cautious application of the technique, ensuring some leaf cover is always maintained around clusters, is increasingly recommended [31].

However, leaf removal is known as a technique that, depending on the modalities of execution, can lead to entirely different outcomes. For instance, pre-flowering basal leaf removal has proved exceptionally effective for a calibrated reduction in fruit set, leading to looser clusters less susceptible to rotting [37–39]. Conversely, an apical-to-cluster late leaf removal initially proposed by [40] has proved effective for various cultivars and environments [24,40–44] in delaying sugar accumulation with minimal interference on phenolic ripeness and aroma development. The principle behind such results is straightforward: A calibrated source limitation is induced at the onset of the rapid sugar accumulation phase by removing a portion (about 30% of the pending leaf area) of leaves, which, being apical, provide the highest photosynthetic efficiency at that time; meanwhile, the leaf removal, usually mechanically performed, does not significantly alter the microclimate around clusters, explaining the minimal effects on other secondary metabolites. Despite the growing body

of research on late-season leaf removal, there is a lack of comprehensive studies examining both the viticultural and enological impacts of this technique. To the best of our knowledge, only one case [41] extended the survey to the sensorial characteristics of the final Shiraz wines, concluding that minimal changes were observed in major wine volatile compounds between non-defoliation and defoliation treatments. As per Sangiovese, a commercially important Italian grape variety, this study aims to fill this gap by investigating the effects of apical leaf removal on both vine performance and wine properties.

The aims of this study were as follows: (i) to evaluate the impact of apical leaf removal at veraison and post-veraison on the yield components, berry composition, and vine balance of Sangiovese vines; (ii) assess the effects of these treatments on wine chemical composition, with a particular focus on alcohol content and phenolic compounds; and (iii) conduct a comprehensive sensory analysis to determine how defoliation treatments influence the organoleptic properties of Sangiovese wines. By combining viticultural, enological, and sensory analyses, this study provides a holistic understanding of the effects of late-season apical leaf removal on Sangiovese wine production.

2. Materials and Methods

2.1. Experimental Site and Plant Material

The experiment was conducted in 2019 in a vineyard located in Civitella di Romagna (Forlì-Cesena FC Province, Emilia Romagna region, 44°00′ N, 11°56′ E, altitude 330 m a.s.l.), owned by the Dal Nespoli Estate. The vineyard is part of the "Romagna" DOC (Denomination of Origin) wine district, where the planting of the Sangiovese cultivar is predominant.

This area has a temperate-humid climate, classified as Cfa according to Köppen (1936). Weather conditions during the trial year were monitored daily by a nearby meteorological station. Data were retrieved from the regional ARPA-E weather database https://www.arpae.it (accessed on 1 March 2024) and averaged over the 1998–2018 period to indicate an annual mean temperature of 13.6 °C, a maximum mean temperature of 23.9 °C, and a minimum mean temperature of 3.9 °C in January. Mean annual rainfall was 870 mm, with 43% occurring during the growing season from April to September. The average heat summation, given as growing degree days (GDD), amounted to 1889 °C (Figure S1A,C).

The vineyard consisted of a 7-year-old planting of *Vitis vinifera* L. cv. Sangiovese (clone VCR 106 grafted onto Kober 5BB rootstock), established at 0.9 m × 2.7 m (within-row and between-row spacing, respectively), resulting in a vine density of 4100/ha. Rows were oriented north–south (NS), and vines were trained to a unilateral spur-pruned cordon with the main wire at 0.85 m above the ground, featuring 5–6 two-node spurs (10–12 buds/vine). Three foliage wires were present above the main wire, for a total row height of about 2.1–2.2 m. Vines were not irrigated during the growing season. Cultural practices followed the protocol for wine production defined by the "Romagna" Sangiovese DOC. No cluster thinning was applied during the growing season.

The soil (5–60 cm depth) was loamy (sand 46%, silt 28%, clay 26%), sub-alkaline (pH = 8.06), very calcareous (10% active and 30% total limestone), and well-endowed with nitrogen (N = 1.74‰), phosphorus (P_2O_5 = 46g/kg), and potassium (K_2O = 571 g/kg), with a cation exchange capacity of 23.5 meq/100 g.

The experiment was conducted during the 2019 growing season and laid out as a randomized block design. A total of 135 vines were divided into 3 blocks, each comprising 3 adjacent rows. Within each block, 3 groups of 15 vines were randomly assigned to 1 of the following treatments: (i) control (C—not defoliated); (ii) leaf removal treatment D_1: leaves (main and lateral) on the 7 nodes above the 1 facing the second clusters were manually removed at the beginning of veraison (stage BBCH 81) according to Lorenz et al. (1995)) [45]; and (iii) leaf removal treatment D_2: the same type of leaf removal as D_1, but 20 days later (stage BBCH 85). Veraison treatment (D_1) was performed on 2 August (Figure S2), while the post-veraison defoliation (D_2) was carried out on 22 August. The timing and

extent of leaf removal treatments were selected based on previous studies [24,40,43,46] and adjusted for the specific phenological development of Sangiovese in this region.

On both leaf removal dates, the main and lateral leaves removed from each test vine per treatment were rapidly taken to the laboratory, and the size of each leaf was measured using a leaf-area meter (LI-COR 3000 Bioscience equipped with the LI-3050C Transparent Belt Conveyer Accessory, Lincoln, NE, USA). The calculated average leaf size was deemed valid for the control treatment. At harvest, three main basal leaves (i.e., node 1–6) per vine were taken to include leaf size variability according to leaf position on the stem and their surface was processed with the same leaf-area meter. After leaf fall, the total number of nodes per vine on the main and lateral canes was counted. Leaf area (LA) per vine was subsequently calculated by combining respective mean leaf sizes and the corresponding node numbers.

2.2. Vegetative Growth, Yield Components, and Grape Composition

The progress of berry growth and ripening was assessed approximately every 10 days, starting from the date of D_1 application (2 August) until harvest on 20 September. At each date, three 200-berry samples per block × treatment combination were taken from a batch of the 15 vines per block. Once taken to the laboratory, each sample was immediately weighed, and manually pressed at room temperature, and the resulting must was used to determine total soluble solids (TSS) as °Brix, pH, and titratable acidity (TA as g/L). TSS was assessed using a temperature-compensated desk refractometer, while pH and titratable acidity (TA) were measured by titration with 0.1 N NaOH to a pH 8.2 endpoint and expressed as g/L of tartaric acid equivalents.

At harvest, cluster number and total grape weight were recorded for four representative vines for each treatment in each block. Concurrently, three representative clusters per vine—usually located on the basal, median, and apical spurs along the cordon—were taken to the laboratory for further processing. From each of the 3 clusters, a 100-berry subsample was taken by carefully cutting each berry at the pedicel with sharp scissors and stored at −20 °C for subsequent phenolic analyses according to [47]. When still frozen, the berries were homogenized at 10,000 rpm with an Ultra-Turrax T25 (Rose Scientific, Edmonton, AB, Canada) homogenizer for 1 min. Then, 2 g of the homogenate was transferred to a pre-tared centrifuge tube, enriched with 10 mL aqueous ethanol (50%, pH 5.0), capped, and mixed periodically for 1 h before centrifugation at 959 g for 5 min. A portion of the extract (0.5 mL) was added to 10 mL of 1 mol/L HCl, mixed, and allowed to stand for 3 h. The absorbance was then measured at 520 and 280 nm on a Jasco V-530 UV spectrophotometer (Jasco Analytical Instruments, Easton, MD, USA). The concentration of total anthocyanins and phenolic substances was given as mg per g of berry fresh mass and mg/berry. All spectrophotometric measurements were performed in triplicate, with a coefficient of variation <5%. Standard curves were prepared fresh for each analysis session to ensure accuracy. The remainder of each cluster sample was crushed, and the resulting musts were analyzed for technological maturity parameters according to the aforementioned methodology.

To assess tartaric and malic acid concentrations, an aliquot of the must was diluted four times, then filtered through a 0.22 µm polypropylene syringe for high-performance liquid chromatography (HPLC) analysis and transferred to autosampler vials. All solvents were of HPLC grade. The chromatographic method was developed using an Agilent 1260 Infinity Quaternary LC (Agilent Technology, Santa Clara, CA, USA) consisting of a G1311B/C quaternary pump with an inline degassing unit, a G1329B autosampler, a G1330B thermostat, a G1316B thermostatic column compartment, and a G4212B diode array detector (DAD) fitted with a 10 mm path and a 1 µL volume Max-Light cartridge flow cell. An Allure Organic Acid column, 300 × 4.6 mm and 5 µm (Restek, Bellefonte, PA, USA) maintained at 30 ± 0.1 °C, was used. Separation was performed under isocratic conditions using water, pH adjusted to 2.5 with orthophosphoric acid, at a flow rate of 0.8 mL/min, and 15 µL of the sample was injected. Mobil phases ranging from 2.5 to 22.5 mM H_2SO_4 in dH_2O with or without acetonitrile (6%) were tested to obtain the

optimal chromatographic separation. The elution was monitored at 200–700 nm and detected by UV–vis absorption with a DAD at 210 nm. Organic acids were identified using authentic standards, and quantification was based on peak areas and performed by external calibration with standards. The retention times (min) of citric, tartaric, and malic acid were 9.3, 10.0, and 11.1, respectively. The must potassium (K^+) concentration was measured by an ion-selective electrode (Model 96–61, Crison Instruments, Barcellona, Spain).

At leaf fall, the two components of one-year pruning weight (main canes and laterals) were recorded on the same vines, and the Ravaz Index was calculated as the yield-to-total pruning weight ratio.

2.3. Winemaking

Experimental wines were produced on a micro-vinification scale (50 L). At harvest, three 40 kg batches of grapes from each treatment were manually harvested and transported to the experimental winery (Università Cattolica del Sacro Cuore, Piacenza, Italy) in 20 kg plastic boxes. Each grape sample was manually destemmed and crushed to obtain approximately 30 L of grape mash (juice, skins, and seeds), which was then transferred to 50 L stainless steel vats (Polsinelli Enologia Srl, Isola dei Liri, Italy). After adding 5 g/hL of potassium metabisulphite, the must was inoculated with 30 g/hL of *Saccharomyces cerevisiae* BO213 strain (Laffort Oenologie, Bordeaux, France). Alcoholic fermentation was performed at 23 ± 1 °C and monitored daily by measuring the must density until the end of fermentation (constant density for three consecutive days). The fermentation temperature was strictly controlled (±0.5 °C) using thermostatically regulated tanks to minimize variation between treatments. Moreover, the temperature of the room containing the fermenters was regulated and maintained constant at 23 °C. Alcoholic fermentation took 10 days to complete. The pomace was manually punched down twice a day during fermentation. At the end of the alcoholic fermentation, the wines were racked off, and the pomace was gently pressed with a hydraulic press (Model W40; Grifo Marchetti, Piadena, Italy). The wines were then placed into 20 L stainless steel vats. They were racked twice, bottled under screw-cap closures in 500 mL dark glass bottles, and stored for 8 weeks at 12.5 °C before chemical and sensory analyses were carried out.

2.4. Wine Analyses

Alcoholic strength at 20 °C (vol.%); density (g/L); titratable acidity (g/L of tartaric acid); volatile acidity (g/L of acetic acid); residual sugars (g/L); pH; and free and total sulfur dioxide (mg/L) were determined according to Organization International de la Vigne et du Vin methods [48]. The organic acids were determined using the RP-HPLC (Agilent 1260 Infinity HPLC) method [43]. The concentration of reducing sugars was assessed using enzymatic assay kits (K-FRUGL, Megazyme International Ltd., Wicklow, Ireland). All analyses were performed in triplicate.

The total polyphenol index was determined using a spectrophotometer (V-730 UV-Vis Jasco, GA, USA) as described by [49]. The results were expressed as gallic acid equivalents (mg/L) via a calibration curve. Total anthocyanins, total flavonoids, proanthocyanidins, color intensity, hue, and flavans reactive with vanillin were analyzed according to [50]. The wine's colorimetric properties were measured using CIELab, as previously described by [51].

Anthocyanin profiles were determined according to [52]. After purification by SPE using a Sep-Pak C_{18} 1 g cartridge (Phenomenex, Torrance, CA, USA), individual anthocyanins were separated using a Phenomenex Luna C18, 250 × 4.6 mm, 5 µm column with gradient elution. The gradient consisted of two eluents: (A) TFA 0.2% (*v*/*v*) and (B) methanol/water/TFA 80/20/0.2 (*v*/*v*/*v*). The gradient conditions were as follows: 0 min 10% B; 15′ 12% B; 25′ 15% B; 33′ 15% B; 38′ 20% B; 42′ 30% B; 45′ 40% B; 48′ 55% B; 49′ 98% B at 0.8 mL/min. The injected volume was 20 µL. The determination was carried out in triplicate.

2.5. Wine Sensory Evaluation

Sensory profiling of wines was conducted using descriptive analysis according to [53]. Descriptive analysis is a two-stage method comprising (1) a lexicon generation process and (2) a set of sensory tests designed to quantify the intensity of the sensory terms established in the lexicon generation phase on a rating scale. Descriptive analysis was performed in a test room designed in accordance with ISO 8529-2007 [54]. The wine samples were monadically served to panelists. Three-digit random numbers were assigned to each sample for tracking purposes prior to service. Wine samples were evaluated in duplicate in two sessions on the same day. The order of presentation was balanced and randomized across samples, panelists, and replicates, according to a rotated tasting plan [55]. Eight assessors were selected based on their extensive experience with sensory evaluation of wines as well as their interest and availability. Panelists were provided with still mineral water and unsalted breadsticks to cleanse their palates between samples.

2.6. Statistical Analysis

Vegetative growth, yield, grape, and wine composition data were subjected to a one-way analysis of variance (ANOVA) using IBM SPSS Statistics 27 (SPSS Inc., Chicago, IL, USA). In the case of significance in the Fisher test, mean separation was performed through the Student–Newman–Keuls test (SNK) at $p < 0.05$. Variation around means was given as the standard error (SE). Data were tested for normality using the Shapiro–Wilk test and for homoscedasticity using Levene's test. Where necessary, data were log-transformed to meet ANOVA assumptions.

Repeated measures of the same parameters (berry fresh weight, TSS, and TA) taken at different dates on the same individuals were analyzed using the Repeated Measures ANOVA routine embedded in the XLSTAT software package 2024-2.2. (Addinsoft, New York, NY, USA). Only in the case of a significant time × treatment interaction was the SNK used for multiple comparisons within dates at $p < 0.05$.

Each descriptor was evaluated by the tasters on a scale from 0 to 10 (0 = absence of perception, 10 = maximum perception), and the data were analyzed using the Friedman test and the evaluation of a least significant difference based on the sum of ranks according to Freund and Wilson (2001).

3. Results

The weather course recorded in 2019 depicted a fairly standard season in terms of cumulated GDD from 1 April to 31 October (1911 °C versus 1889 °C registered over the 21-year historical series), while total rainfall was significantly higher (1096 mm year-round in 2019 vs. 870 mm for the 21-year average) (Figure S1B,D).

The final leaf area per vine was significantly reduced by late defoliation as expected (Table 1). The fraction of removed leaf area over the reference values recorded in control (5.50 m^2) was 35.7% and 39.2% in D_1 and D_2, respectively. The yield per vine averaged across treatments was 4.7 kg, corresponding to a notable 17.4 t/hectare. Consequently, the calculated total leaf area-to-yield ratio at harvest was above the required threshold of 1 m^2/kg in the C treatment (namely 1.35 m^2/kg), whereas both D_1 and D_2 displayed a condition of likely source limitation, with ratios of 0.90 and 0.81 m^2/kg, respectively.

Table 1. Effect of leaf removal treatments on vegetative growth, yield components, vine balance and grape composition of Sangiovese grapevine as compared to a non-defoliated control. C = control, not defoliated; D_1 = defoliated at the beginning of veraison; D_2 = defoliated 20 days after D1.

Variables	C	D_1	D_2	F Defoliation (D)	F Blocks (B)	F (B × D)
Vegetative growth, yield and vine balance						
Nodes/vine (n)	11.7	13.4	12.2	-	-	-
Clusters/vine (n)	17.0	14.92	17.08	0.722 ns	0.928 ns	1.139 ns

Table 1. *Cont.*

Variables	C	D_1	D_2	F Defoliation (D)	F Blocks (B)	F (B × D)
Clusters/shoot (n)	1.49	1.17	1.45	1.432 ns	1.882 ns	2.531 ns
Cluster weight (g)	299	319	260	1.722 ns	0.867 ns	0.590 ns
Berry weight (g)	2.45	2.63	2.54	1.421 ns	1.195 ns	3.131 ns
Yield/vine (kg)	4.98	4.65	4.49	0.272 ns	1.654 ns	2.026 ns
Cluster length (cm)	22.61	23.11	21.11	0.409 ns	2.750 ns	0.730 ns
Compactness index (g/cm)	13.92	14.24	12.73	0.446 ns	1.777 ns	0.536 ns
Total leaf area/vine (m^2)	5.50 ^{b}	3.54 ^{a}	3.31 ^{a}	30.50 **	0.516 ns	1.082 ns
Leaf area to yield (m^2/kg)	1.35 ^{b}	0.90 ^{a}	0.81 ^{a}	3.556 *	1.098 ns	1.256 ns
Wood weight (primary)/vine (g)	635	508	597	1.414 ns	1.266 ns	1.910 ns
Wood weight (lateral)/vine (g)	40.01	50.83	73.33	0.757 ns	0.866 ns	1.221 ns
Total wood weight^2/vine (g)	675	559	670	1.203 ns	1.778 ns	1.034 ns
Ravaz index (kg/kg)	12.59	10.04	10.83	0.403 ns	1.192 ns	0.769 ns
Grape quality						
Sugars (°Brix)	21.27 ^{b}	19.34 ^{a}	21.14 ^{b}	9.935 **	1.755 ns	1.434 ns
pH	3.29	3.28	3.31	0.444 ns	0.634 ns	1.143 ns
Titratable acidity (g/L)	6.09	5.93	5.67	1.064 ns	0.644 ns	2.540 ns
Tartaric acid (g/L)	6.25	6.42	6.24	0.121 ns	0.877 ns	0.752 ns
Malic acid (g/L)	2.91	2.69	2.57	1.007 ns	1.032 ns	3.070 ns
Citric acid (g/L)	0.185	0.169	0.174	1.077 ns	1.424 ns	5.089 **
Tartaric/Malic	2.20	2.50	2.51	2.968 ns	0.790 ns	3.632 *
Anthocyanins (mg/g)	0.541 ^{b}	0.394 ^{a}	0.578 ^{b}	8.393 **	4.688 *	1.726 ns
Anthocyanins (mg/berry)	1.312 ^{b}	1.025 ^{a}	1.459 ^{b}	6.973 **	3.338 *	1.958 ns
Polyphenols (mg/g)	1.560	1.496	1.635	1.484 ns	3.580 *	1.343 ns
Polyphenols (mg/berry)	3.820	3.915	4.135	0.690 ns	1.072 ns	1.404 ns
Berry K+ (mg/L)	1789	1918	1926	0.310 ns	1.277 ns	4.724 **

Different superscript letters within the same row indicate significant differences according to Student–Newman–Keuls test (SNK) ($p < 0.05$). ns = non significant; * significant at $p < 0.05$; ** significant at $p < 0.01$.

In all treatments, the Ravaz Index exceeded the threshold of 10 kg/kg, indicating a status of mild overcropping. None of the recorded yield components were affected by the defoliation practice, and the same was true for the total one-year pruning weight, even when divided between main and lateral canes.

Examining the seasonal dynamics of berry fresh weight (Figure 1A), TSS (Figure 1B), TA (Figure 1C), and total anthocyanin concentration (Figure 1D) revealed that despite some variation over the first two sampling dates, berry growth was not affected by defoliation (Figure 1A). Conversely, late leaf removal had a significant impact on sugar accumulation patterns: while TSS did not differ among treatments over the first sampling date (which corresponded to the imposition of D_1), thereafter, D_1 showed a lagged TSS accumulation at any sampling date compared with C and D_2 (Figure 1B). Conversely, TSS was only temporarily affected by defoliation on DOY 247 when the value was lower than C, and at harvest, D_2 registered a full recovery. TA monitoring indicated that D_1, after defoliation, held higher titratable acidity than the other two treatments until DOY 234; thereafter and until harvest, the values did not differ. To some extent, the dynamics of total anthocyanin concentration (mg/g) closely mirrored those already described for TSS (Figure 1B), and the lag shown by D_1 was never filled (Figure 1D).

Total soluble solids (TSS) at harvest registered a significant decrease in D1 compared with C (−2.0 °Brix) and D_2 (−1.8 °Brix) (Table 1). Interestingly, the lower final TSS in D_1 was coupled with no variation in organic acids content, which proved rather insensitive to the late, apical leaf removal. Conversely, total anthocyanins at harvest, regardless of the unit they were expressed in, were lower in D_1 compared with the remaining treatments (about −22%). However, the same trend was not seen for the total phenolics.

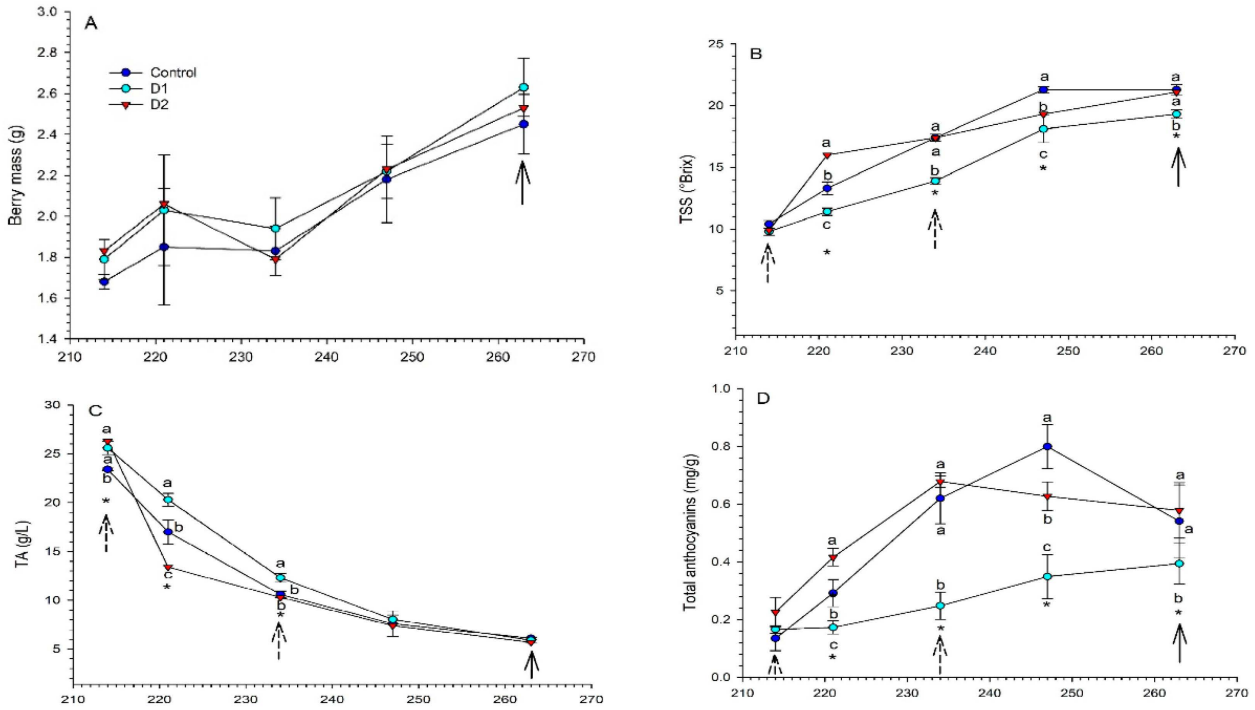

Figure 1. Seasonal variation in berry fresh mass (**A**), total soluble solids (TSS) (**B**), titratable acidity (**C**), and total anthocyanin concentration (**D**) recorded in 2019 from the onset of veraison until harvest in C, D_1, and D_2 treatments. The two broken arrows indicate dates of leaf removal, whereas the solid arrow indicates the harvest date. Repeated measures analysis resulted in the following: for (**A**), between-subject (treatment) effects and time × treatment interaction were non-significant; in (**B**), treatment and time × treatment effects were significant at Pr > F = 0.0001; in (**C**), treatment effect was significant at Pr > F = 0.015 and time × treatment interaction was significant at Pr > F = 0.0001; in (**D**), treatment effect was significant at Pr > F = 0.0001 and time × treatment interaction was significant at Pr > F = 0.023. Mean separation within single dates using lowercase letters was performed by SNK test at $p = 0.05$ level only when a significant time × treatment interaction was found (indicated with an asterisk). Within single dates, lack of separation implies ns.

All alcoholic fermentations were completed within 9–10 days. The final wines had a residual sugar level < 1 g/L, while ethanol concentration was significantly lower (10.4°) than in C (12.1°) and D_2 wines (12.5°). TA and pH were again not affected (Table 2). However, the profile of organic acids was slightly modified by the leaf removal treatments. A small yet significant decrease in tartaric acid was measured in D_1 compared with the control and D_2 samples, whereas the lowest concentrations of malic acids were found in the D_1 and D_2 wines. The volatile acidity values ranged from 0.08 ± 0.02 to 0.11 ± 0.01, which fell within the agreeable limits. The differences in total SO_2 content were due to different additions made during winemaking; however, the values never exceeded maximum legal limits.

Table 2. Effect of leaf removal treatments on chemical attributes of the final Sangiovese wines as compared to a non-defoliated control. C = control, not defoliated; D_1 = defoliated at the onset of veraison; D_2 = defoliated 20 days after D_1.

Attributes	C	D_1	D_2	F sig.
Density	0.9930 ^{b}	0.9943 ^{a}	0.9927 ^{b}	5.443 **
Ethanol (% vol)	12.15 ^{a}	10.41 ^{b}	12.49 ^{a}	16.875 **
Total acidity (g tartaric acid/L)	5.64	5.59	5.63	0.090 ns
pH	3.50	3.46	3.54	2.717 ns

Table 2. *Cont.*

Attributes	C	D_1	D_2	F sig.
Total SO_2 (mg/L)	44.37 ^{b}	49.92 ^{b}	61.44 ^{a}	8.439 **
Free SO_2 (mg/L)	9.39	11.95	11.95	4.000 ns
Combined SO_2 (mg/L)	34.99 ^{b}	37.97 ^{b}	49.49 ^{a}	5.655 **
Volatile Acidity (g acetic acid/L)	0.11	0.08	0.11	4.200 ns
Tartaric acid (g/L)	2.62 ^{a}	2.50 ^{b}	2.67 ^{a}	6.259 **
Malic acid (g/L)	2.23 ^{a}	2.06 ^{b}	2.07 ^{b}	5.131 **
Acetic acid (g/L)	0.06	0.06	0.07	0.855 ns

Different superscript letters within the same row indicate significant differences according to Student–Newman–Keuls test (SNK) ($p < 0.05$). ns = non significant; ** significant at $p < 0.01$.

When examining specific phenolic classes and color parameters, the D_1 wine samples had a more than halved total anthocyanin concentration (75 $\pm$ 6 mg L^{-1}) compared with the D_2 (178 $\pm$ 55 mg L^{-1}) and control wines (161 $\pm$ 36 mg L^{-1}), confirming data recorded on the must samples (Table 3). The D_1 wines received the highest scores in brightness (L*) and tint compared with the controls and the D_2 wines. However, the red-green color contribution (a*) and the intensity of red color (%Red) were significantly higher in C and D_2 compared to the D_1 wines.

Table 3. Effect of leaf removal treatments on phenolic composition and concentration of monomeric anthocyanins of Sangiovese wines. C = control, not defoliated; D_1 = defoliated at the beginning of veraison; D_2 = defoliated 20 days after D_1.

Variables	C	D_1	D_2	F sig.
Total Polyphenols (mg gallic acid/L)	1989	1975	2168	0.764 ns
Total Anthocyanins (mg malvidin-3-glucoside/L)	161 ^{a}	75 ^{b}	178 ^{a}	6.188 **
Total Flavonoids (mg (+)-catechin/L)	806	721	799	0.592 ns
Proanthocyanidins (mg cyanidin chloride/L)	996	831	1105	2.096 ns
Flavans Reactive Vanillin (mg (+)-catechin/L)	1080	993	1106	0.682 ns
L* (brightness)	52.9 ^{b}	67.4 ^{a}	49.3 ^{b}	6.799 **
a* (red/green)	47.6 ^{a}	34.9 ^{b}	52.3 ^{a}	11.560 **
b* (yellow/blue)	9.2	7.4	11.5	2.521 ns
ΔE	-	19.3	25.4	-
% Yellow	38.5 ^{ab}	41.3 ^{a}	37.5 ^{b}	5.780 **
% Red	52.1 ^{ab}	48.4 ^{b}	53.7 ^{a}	6.277 **
% Blu	9.5	10.3	8.8	1.241 ns
IC	3.26	2.24	3.81	3.985 ns
Tint	0.74 ^{b}	0.86 ^{a}	0.70 ^{b}	6.443 **
Delphinidin-3-glucoside	6.61	5.31	8.55	2.388 ns
Cyanidin-3-glucoside	2.77	1.83	3.65	1.550 ns
Petunidin-3-glucoside	17.78 ^{a}	11.45 ^{b}	18.45 ^{a}	6.469 **
Peonidin 3-glucoside	8.26	3.74	9.06	3.084 ns
Malvidin-3-glucoside	82.53 ^{ab}	42.25 ^{b}	110.54 ^{a}	6.065 **
Cyanidin-3-(6-acetyl)-glucoside	1.51 ^{a}	0.14 ^{b}	0.51 ^{b}	19.479 **
Malvidin-3-(6-acetyl)-glucoside	0.29 ^{b}	0.71b	1.58 ^{a}	10.379 **
Petunidin3-(6-p-coumaryl)-glucoside	0.24	0.19	0.68	3.007 ns
Malvidin-3-(6-p-coumaryl)-glucoside	0.48	0.44	0.54	0.226 ns
Total	120.46 ^{ab}	66.06 ^{b}	153.57 ^{a}	6.053 **

Different superscript letters within the same row indicate significant differences according to Student–Newman–Keuls test (SNK) ($p < 0.05$). ns = non significant; ** significant at $p < 0.01$.

Nine different anthocyanins were identified and quantified. Five of them corresponded to the group substituted with a glycoside molecule, two were from the acetyl group, and two were from the coumarate group. The anthocyanins present in the greatest amounts in the

Sangiovese wines for all treatments were malvidin-3-glucoside, followed by petunidin-3-glucoside, peonidin-3-glucoside, and delphinidin-3-glucoside. The D_1 wines had the lowest concentrations of petunidin-3-glucoside and malvidin-3-glucoside compared with control wines, while no differences were found in D_2 wines for those parameters (Table 3). Neither of the defoliations affected delphinidin-3-glucoside, cyanidin-3-glucoside, peonidin-3-glucoside, petunidin-3-(6-p-coumaryl)-glucoside, or malvidin-3-(6-p-coumaryl)-glucoside contents. On the other hand, the concentration of the acylated anthocyanins was inversely affected by the defoliation treatments (D_1 and D_2), causing an increase in malvidin-3-(6-acetyl)-glucoside and a decrease in cyanidin-3-(6-acetyl)-glucoside.

Wine sensory evaluation (Table 4, Figure 2) showed significant differences for six descriptors: body, bitterness, fruitiness, balance, aftertaste preference, and global preference, with the highest scores obtained through the later defoliation (D_2). Overall, descriptor evaluations were similar between the C and D_1 wines, except for higher acidity and a green (veggie) taste in the presence of defoliation.

Table 4. Effect of leaf removal treatments on sensory descriptors evaluated on Sangiovese wines. C = control, not defoliated; D_1 = defoliated at the beginning of veraison; D_2 = defoliated 20 days after D_1.

Descriptors	C	D1	D2	T (Friedman) sig.
Olfactory intensity	3.50	3.25	4.50	3.47 *
Body	3.75 ^{a}	3.50 ^{a}	5.37 ^{b}	7.17 **
Acidity	3.50	5.01	4.75	1.75 ns
Bitter	1.75 ^{a}	2.50 ^{ab}	2.75 ^{b}	4.59 *
Astringency	2.50	2.88	3.63	1.92 ns
Fruits	3.87 ^{a}	4.00 ^{a}	4.75 ^{b}	3.26 *
Flowers	2.29	2.38	2.88	1.62 ns
Vegetables	2.38	3.63	3.38	2.39 ns
Spicy	2.50	2.75	3.00	0.68 ns
Balance	3.38 ^{a}	3.37 ^{a}	5.00 ^{b}	16.06 **
Olfactory preference	3.38	3.25	4.13	1.67 ns
Aftertaste preference	3.75 ^{a}	3.37 ^{a}	4.75 ^{b}	4.45 *
Global preference	3.50 ^{a}	3.13 ^{a}	5.13 ^{b}	30.77 **

Different superscript letters within the same row indicate significant differences according to the non-parametric Friedman test ($p < 0.05$). ns = non significant; * significant at $p < 0.05$; ** significant at $p < 0.01$.

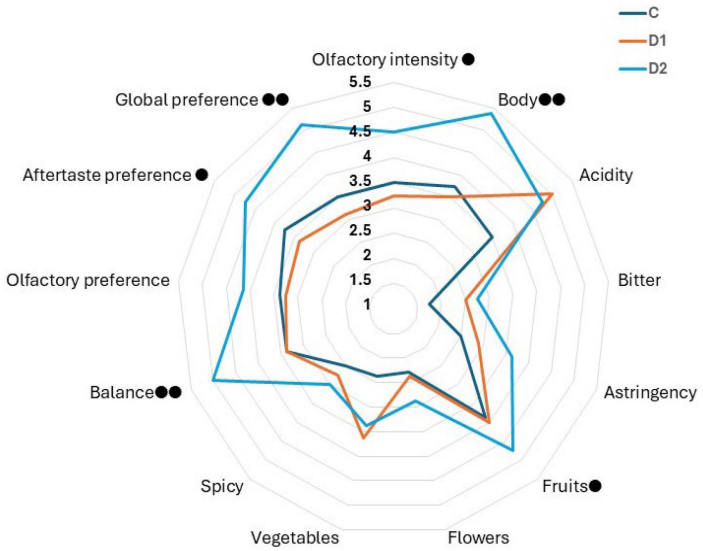

Figure 2. Aroma spider graph of the sensory characteristics of Sangiovese wines, obtained using 12 panelists with wines analyzed in triplicate. Black dots indicate significance at ● $p < 0.1$, ●● $p < 0.05$.

4. Discussion

Although the study was conducted on a single season, the results obtained from late, apical-to-cluster leaf removal were consistent in terms of final grape composition and wine traits. Notably, the work discovered that D_1 induced a general and expected ripening delay without significantly altering the sensory attributes of the final wine. However, D_2 had little impact on berry maturation compared with the non-defoliated vines, yet it significantly changed six wine descriptors. The D_2 wines were described as more bodied and intense, with a greater red fruit aroma and overall higher appreciation than the other two wines.

Focusing on the main objective of apical late leaf removal—slowing down the sugaring process with minimal alteration of acidity and accumulation of main flavonoid compounds [56], D_1 was highly effective at delaying sugar accumulation in the berry compared with the C treatment. Concurrently, TA remained decoupled, showing no difference at harvest among treatments. However, D_1 failed to maintain a similar total anthocyanin concentration to that of C, resulting in consistently reduced pigmentation in both the must (Table 1) and the final wines (Table 3).

A survey of published work on late-season apical leaf removal shows that ripening delay is generally consistent, although variability exists depending on the timing of defoliation or the cultivar [23,24,40–43,46,57–59]. Indeed, the technique has been shown to be overall incapable of affecting the ripening pace [60].

In addition to the genotype, pedoclimatic factors, and the timing of application, the success of the technique in delaying ripening depends on two additional variables: (i) the extent and duration of photosynthetic compensation by the remaining foliage and (ii) the change in the leaf-to-fruit ratio after treatment. Regarding the former, apical defoliation treatments on Sangiovese pre- and post-veraison triggered about a 35% higher net CO_2 gas exchange per unit of leaf area per day [40]. This means that even if the post-treatment leaf area-to-fruit ratio is significantly decreased compared with the control treatment, the calculated seasonal carbon/yield ratio might not differ between treatments due to the high capacity for photosynthetic compensation.

In our study, photosynthetic compensation could not be determined, yet the LA/Y was significantly lowered in both treatments compared with the C treatment. This might help explain why sugar ripening was delayed, at least in D_1. The relationship between leaf area and fruit weight (LA/Y) has been widely discussed in [61]. The authors state that the rate of sugar accumulation in berries depends on the LA/Y and that values between 0.8 and 1.2 m^2/kg are needed to mature fruit on a single-canopy training system. Some authors have found that to delay sugar accumulation, a reduction in the LA/Y is necessary [62,63]. However, examining our ripening curve dynamics (Figure 1A–D) revealed that defoliation applied at the onset of veraison caused a source limitation strong enough to decrease the initial slope of the fast-sugaring process, which continued until harvest, reaching 2 Brix less than the C treatment. On the other hand, it should also be explained why, in D_2, despite the same significant reduction in the final LA/Y ratio, ripening was only mildly retarded and full recovery was observed at harvest in terms of TSS and berry pigmentation (Table 1). An obvious candidate for such a response is the timing of leaf removal, which occurred when C vines were already close to 17 Brix. Moreover, at the time of the rapid post-veraison TSS surge, D_2 still had a non-limiting LA/Y, likely playing an important role in assisting fast sugar intake from the berries.

Regarding the alteration of phenolic maturity, several studies using post-veraison manual or mechanical apical-to-cluster zone leaf removal have demonstrated the potential to delay sugar accumulation from a few days to a maximum of a couple of weeks, with little to no effect on the accumulation of anthocyanins and phenolics or on the replenishment of reserves stored in canes and roots [24,40,44]. A good reason to explain such decoupling is that while the potential for sugar accumulation in berries is overall controlled by the amount and quality of the photosynthesizing leaf area, the ability to form and preserve color is more genotype dependent and significantly affected by the local microclimate around the clusters [64]. Such a desirable decoupling was not fully confirmed in our work,

as the final total anthocyanin concentration in the grapes and final wines was diminished by the D_1 treatment. One hypothesis to explain this undesirable result is that when a source limitation is imposed between veraison and ripening, according to mathematical analysis of carbon balance [63,65], berries use a higher proportion of fixed carbon for sugar accumulation (76.9%) under carbon limitation (only 3 leaves per cluster) than under carbon sufficiency (48% recorded with 12 leaves per cluster). Thus, under carbon limitation, the grape berry manages the metabolic fate of carbon in such a way that sugar accumulation is maintained at the expense of secondary metabolites.

Considering the composition of the three wines, it should be noted that anthocyanins with more hydroxyl groups in the B rings contribute more blueness, whereas the degree of methylation of the B rings increases redness [66]. Thus, malvidin-3-*O*-glucoside and its derivatives, by far the most abundant, are the reddest anthocyanins. The control wine had 52.5% malvidin-3-*O*-glucoside over total monomeric anthocyanins, aligning well with data reported for Sangiovese grown in the Romagna Region [67]. The D_1 wine had a decrease in malvidin-3-*O*-glucoside (47.5% of total anthocyanins) and petunidin malvidin-3-*O*-glucoside, which accords well with lower %red and a* (red/green) and higher brightness. Nevertheless, the panelists' appreciation of the D_1 wine was not significantly different from that of the C wine.

The behavior of the vines subjected to apical defoliation (D_2) performed when grapes on the C vines had already crossed the 16 °Brix threshold (Figure 1B) led to no relevant changes in grape and wine composition compared with the non-defoliated treatment. Undoubtedly, D_2 was superior to the other two wines in terms of olfactory intensity, body, fruitiness, balance, aftertaste, and global preference. Explaining such a preference as a function of the specific treatment the vines received is problematic. However, a similar overall picture was described for Shiraz [60], where despite finding no significant variation in berry maturity patterns between a control treatment and a post veraison-apical leaf removal treatment, the latter produced wines that were more intense, with greater body and a very different aroma profile characterized by greater grassy and red fruit aromas.

A direct comparison between the results obtained in our study by leaf removal in terms of grape and wine composition with those corresponding to basal leaf removal performed at different timings is inappropriate. Regardless of the timing, a basal defoliation always causes short- and long-term changes in cluster microclimate [68,69], where a sudden re-exposure of previously shaded clusters to high light and temperature conditions is common. Despite such drastic manipulation, specific effects due to basal leaf removal on berry flavor and wine sensory properties are not easy to disentangle [31,70,71].

The aforementioned conditions do not occur or occur minimally when apical leaf removal is applied, since there is no direct interaction with the fruiting area. Moreover, since the treatment is typically applied at veraison or later, the vegetative reaction to the operation is almost nil, confirming a quite static situation. In terms of canopy microclimate, the main variation that can be conceived is that, at high sun angles, basal leaves and clusters are deprived of the cap made by the surmounting leaves; hence, light penetration to the lower part of the canopy can be somewhat improved. Indeed, the literature supports that changing the timing of defoliation [59,72,73] can greatly impact the final wine. In Grenache, it was shown that late (veraison) leaf removal was much less effective than early (pre-flowering) leaf removal at modifying the final wine's composition and quality [59]. The wine made using the early defoliation treatment was rated the most preferred in terms of global value by the panelists.

The two defoliation treatments in our study shared the same intensity, confirmed by the actual amounts of removed leaf area (Table 1). Therefore, the timing of application is again the most likely candidate for the observed differentiation in the final wine style. The main difference was that, in D_1, the whole ripening process was mostly managed by the basal and already senescing leaves, whereas in D_2, the first part of the sugaring process (from 6 to about 16.5 Brix) was still assisted by median and still highly functional leaves. It also appears that an overall younger canopy in D_2 from veraison until harvest played

a significant role in terms of wine sensory attributes compared with the D_1 treatment. It has already been reported in Sauvignon blanc [74] that the highest leaf area-to-yield ratio resulted in the best overall sensorial quality of wine; however, this does not apply to our case where D_1 and D_2, despite having very similar LA/Y ratios, greatly differed in the final wine attributes. It can be speculated that the better sensorial quality of the D_2 wine stems from either a less limited LA/Y or a younger canopy than those associated with the D1 treatment. In such a direction, the work from Šuklje, K., et al. [74] is indirectly supportive of the previous hypothesis: when assessing the aroma potential of Sauvignon blanc in terms of thiols, the worst performance was shown by a summer pruning treatment envisaging severe shoot trimming performed about two weeks before veraison which was not followed by any significant lateral regrowth. This form of suddenly aged canopy (trimming typically removes the youngest part of the shoot) is something that approximates what we had in D1, where from veraison onward, ripening was primarily in charge of the retained basal leaves.

5. Conclusions

Although this study could not be corroborated by a second trial season due to impediments related to the COVID-19 pandemic, the results statistically support that the timing of application of late-season apical-to-cluster leaf removal was crucial in inducing specific patterns in terms of berry ripening and composition, as well as wine sensory attributes.

Leaf removal at the onset of veraison by stripping median and apical leaves inserted at nodes 6–12 resulted in consistently delayed ripening (−2 Brix less than C at the same harvest date) and final wines having significantly moderated alcohol content (−1.6° less than C). D_1 was unable to decouple total anthocyanin accumulation from sugar accumulation, resulting in limited pigmentation in berries and final D_1 wine. Therefore, the D_1 modality is interesting as a potential ripening delayer, which might avoid subsequent actions of wine de-alcoholization. Since the post-harvest ripening trend of this treatment was not followed, we cannot judge its recovery capacity, which under the progressively longer growing season triggered by climate change, is deemed highly probable.

Somewhat surprisingly, D_2 acted in the opposite way compared with D1. Ripening dynamics, the grapes, and wine composition were not significantly altered versus C vines, yet the final wine received the highest appreciation. The reasons for such a differentiation against the C wine are still unclear; indeed, in D_2, the source limitation was set much later in the season compared with D_1 and, when compared with C, removing the leaves hanging above the cluster zone might have created a better environment for aroma components. It is likely that a transcriptomic and metabolomic approach should be associated with further investigation into the technique, to unveil the role of gene expression when sudden and permanent changes in canopy demography and, albeit to a lesser extent, cluster microclimate are caused by canopy manipulations.

Finally, it is worth noting that the proposed apical late-season leaf removal is easily mechanizable, as the canopy portion that needs to be defoliated is totally cluster-free. This is greatly reassuring for the driver, who does not have to worry about damaging clusters. Under such ideal configuration, machine speed can easily reach 1.5–2.0 km/h and, due to the double passage needed, one hectare can be de-leafed in about 4–5 h.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10090929/s1, Figure S1: Cumulated monthly rainfall (mm) and mean monthly air temperature (°C); Figure S2: A detail of a row section of Sangiovese where the hand leaf removal has just been performed at D1 timing.

Author Contributions: Conceptualization, S.P.; Methodology, A.V., M.G., A.G. and S.P.; Software, M.G.; Validation, A.V. and M.G.; Formal analysis, A.V. and M.G.; Investigation, A.V. and A.G.; Resources, A.V.; Data curation, A.V., M.G., A.G. and S.P.; Writing—original draft, A.V., M.G. and S.P.; Writing—review & editing, S.P.; Project administration, A.G.; Funding acquisition, A.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Emilia Romagna Region grant number [5050344].

Data Availability Statement: The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Gutiérrez-Gamboa, G.; Zheng, W.; Martinez de Toda, F. Strategies in vineyard establishment to face global warming in viticulture: A mini review. *J. Sci. Food Agric.* **2021**, *101*, 1261–1269. [CrossRef]
2. Palliotti, A.; Tombesi, S.; Silvestroni, O.; Lanari, V.; Gatti, M.; Poni, S. Changes in vineyard establishment and canopy management urged by earlier climate-related grape ripening: A review. *Sci. Hortic.* **2014**, *178*, 43–54. [CrossRef]
3. Varela, C.; Dry, P.; Kutyna, D.; Francis, I.; Henschke, P.; Curtin, C.; Chambers, P. Strategies for reducing alcohol concentration in wine. *Aust. J. Grape Wine Res.* **2015**, *21*, 670–679. [CrossRef]
4. Hendriks, H.F. Alcohol and human health: What is the evidence? *Annu. Rev. Food Sci. Technol.* **2020**, *11*, 1–21. [CrossRef] [PubMed]
5. Liguori, L.; Albanese, D.; Crescitelli, A.; Di Matteo, M.; Russo, P. Impact of dealcoholization on quality properties in white wine at various alcohol content levels. *J. Food Sci. Technol.* **2019**, *56*, 3707–3720. [CrossRef]
6. European Commission. *Council Regulation 479/2008 on the Common Organisation of the Market in Wine*; European Commission: Brussels, Belgium, 2008; p. 61.
7. Kirschbaum, M. Direct and indirect climate change effects on photosynthesis and transpiration. *Plant Biol.* **2004**, *6*, 242–253. [CrossRef] [PubMed]
8. Wohlfahrt, Y.; Smith, J.; Tittmann, S.; Honermeier, B.; Stoll, M. Primary productivity and physiological responses of *Vitis vinifera* L. cvs. under Free Air Carbon dioxide Enrichment (FACE). *Eur. J. Agron.* **2018**, *101*, 149–162. [CrossRef]
9. Coulter, A.; Henschke, P.; Simos, C.; Pretorius, I. When the heat is on, yeast fermentation runs out of puff. *Aust. N. Z. Wine Ind. J.* **2008**, *23*, 26–30.
10. Steakley, J.; Steakley, B.; Steakley, J.; Steakley, B. Red Wine Imbalance Problem Solving with Cause and Effect Analysis. In *A Quest for Quality Wine, Every Time. A Guide for Root Cause Analysis*; Springer: Cham, Switzerland, 2020; pp. 133–189.
11. Robinson, A.L.; Ebeler, S.E.; Heymann, H.; Boss, P.K.; Solomon, P.S.; Trengove, R.D. Interactions between wine volatile compounds and grape and wine matrix components influence aroma compound headspace partitioning. *J. Agric. Food Chem.* **2009**, *57*, 10313–10322. [CrossRef]
12. Villamor, R.R.; Ross, C.F. Wine matrix compounds affect perception of wine aromas. *Annu. Rev. Food Sci. Technol.* **2013**, *4*, 1–20. [CrossRef]
13. Chu-Ky, S.; Tourdot-Marechal, R.; Marechal, P.-A.; Guzzo, J. Combined cold, acid, ethanol shocks in Oenococcus oeni: Effects on membrane fluidity and cell viability. *Biochim. Biophys. Acta (BBA)-Biomembr.* **2005**, *1717*, 118–124. [CrossRef] [PubMed]
14. Liguori, L.; Russo, P.; Albanese, D.; Di Matteo, M. Production of low-alcohol beverages: Current status and perspectives. In *Food Processing for Increased Quality and Consumption*; Elsevier: Amsterdam, The Netherlands, 2018; pp. 347–382.
15. Pham, D.-T.; Ristic, R.; Stockdale, V.J.; Jeffery, D.W.; Tuke, J.; Wilkinson, K. Influence of partial dealcoholization on the composition and sensory properties of Cabernet Sauvignon wines. *Food Chem.* **2020**, *325*, 126869. [CrossRef]
16. Fornari, T.; Hernández, E.J.; Ruiz-Rodriguez, A.; Señorans, F.J.; Reglero, G. Phase equilibria for the removal of ethanol from alcoholic beverages using supercritical carbon dioxide. *J. Supercrit. Fluids* **2009**, *50*, 91–96. [CrossRef]
17. Petrozziello, M.; Panero, L.; Guaita, M.; Prati, R.; Marani, G.; Zinzani, G.; Bosso, A. Effect of the extent of ethanol removal on the volatile compounds of a Chardonnay wine dealcoholized by vacuum distillation. In *BIO Web of Conferences, Proceedings of the 41st World Congress of Vine and Wine, Punta del Este, Uruguay, 19–23 November 2018*; EDP Sciences: Ulis, France, 2019; p. 02020.
18. Lisanti, M.T.; Gambuti, A.; Genovese, A.; Piombino, P.; Moio, L. Partial dealcoholization of red wines by membrane contactor technique: Effect on sensory characteristics and volatile composition. *Food Bioprocess Technol.* **2013**, *6*, 2289–2305. [CrossRef]
19. Diban, N.; Arruti, A.; Barceló, A.; Puxeu, M.; Urtiaga, A.; Ortiz, I. Membrane dealcoholization of different wine varieties reducing aroma losses. Modeling and experimental validation. *Innov. Food Sci. Emerg. Technol.* **2013**, *20*, 259–268. [CrossRef]
20. Frioni, T.; Tombesi, S.; Silvestroni, O.; Lanari, V.; Bellincontro, A.; Sabbatini, P.; Gatti, M.; Poni, S.; Palliotti, A. Postbudburst spur pruning reduces yield and delays fruit sugar accumulation in Sangiovese in central Italy. *Am. J. Enol. Vitic.* **2016**, *67*, 419–425. [CrossRef]
21. Gatti, M.; Pirez, F.J.; Chiari, G.; Tombesi, S.; Palliotti, A.; Merli, M.C.; Poni, S. Phenology, canopy aging and seasonal carbon balance as related to delayed winter pruning of *Vitis vinifera* L. cv. Sangiovese grapevines. *Front. Plant Sci.* **2016**, *7*, 659. [CrossRef] [PubMed]
22. Zheng, W.; del Galdo, V.; García, J.; Balda, P.; de Toda, F.M. Use of minimal pruning to delay fruit maturity and improve berry composition under climate change. *Am. J. Enol. Vitic.* **2017**, *68*, 136–140. [CrossRef]
23. Valentini, G.; Allegro, G.; Pastore, C.; Colucci, E.; Filippetti, I. Post-veraison trimming slow down sugar accumulation without modifying phenolic ripening in Sangiovese vines. *J. Sci. Food Agric.* **2019**, *99*, 1358–1365. [CrossRef] [PubMed]
24. Palliotti, A.; Panara, F.; Silvestroni, O.; Lanari, V.; Sabbatini, P.; Howell, G.S.; Gatti, M.; Poni, S. Influence of mechanical postveraison leaf removal apical to the cluster zone on delay of fruit ripening in Sangiovese (*Vitis vinifera* L.) grapevines. *Aust. J. Grape Wine Res.* **2013**, *19*, 369–377. [CrossRef]

25. Dal Santo, S.; Tucker, M.R.; Tan, H.-T.; Burbidge, C.A.; Fasoli, M.; Böttcher, C.; Boss, P.K.; Pezzotti, M.; Davies, C. Auxin treatment of grapevine (*Vitis vinifera* L.) berries delays ripening onset by inhibiting cell expansion. *Plant Mol. Biol.* **2020**, *103*, 91–111. [CrossRef] [PubMed]
26. Parada, F.; Espinoza, C.; Arce-Johnson, P. Phytohormonal control over the grapevine berry development. In *Phytohormones: Signaling Mechanisms and Crosstalk in Plant Development and Stress Responses*; IntechOpen: London, UK, 2017.
27. Poni, S.; Intrieri, C.; Silvestroni, O. Interactions of leaf age, fruiting, and exogenous cytokinins in Sangiovese grapevines under non-irrigated conditions. I. Gas exchange. *Am. J. Enol. Vitic.* **1994**, *45*, 71–78. [CrossRef]
28. Christensen, P. Response of 'Thompson Seedless' Grapevines to the Timing of Preharvest Irrigation Cut-Off. *Am. J. Enol. Vitic.* **1975**, *26*, 188–194. [CrossRef]
29. Gatti, M.; Galbignani, M.; Garavani, A.; Bernizzoni, F.; Tombesi, S.; Palliotti, A.; Poni, S. Manipulation of ripening via antitranspirants in cv. Barbera (*Vitis vinifera* L.). *Aust. J. Grape Wine Res.* **2016**, *22*, 245–255. [CrossRef]
30. Poni, S.; Del Zozzo, F.; Santelli, S.; Gatti, M.; Magnanini, E.; Sabbatini, P.; Frioni, T. Double cropping in *Vitis vinifera* L. cv. Pinot Noir: Agronomical and physiological validation. *Aust. J. Grape Wine Res.* **2021**, *27*, 508–518. [CrossRef]
31. Poni, S.; Frioni, T.; Gatti, M. Summer pruning in Mediterranean vineyards: Is climate change affecting its perception, modalities, and effects? *Front. Plant Sci.* **2023**, *14*, 1227628. [CrossRef]
32. Gatti, M.; Bernizzoni, F.; Civardi, S.; Poni, S. Effects of cluster thinning and preflowering leaf removal on growth and grape composition in cv. Sangiovese. *Am. J. Enol. Vitic.* **2012**, *63*, 325–332. [CrossRef]
33. Verdenal, T.; Zufferey, V.; Dienes-Nagy, A.; Bourdin, G.; Gindro, K.; Viret, O.; Spring, J.-L. Timing and intensity of grapevine defoliation: An extensive overview on five cultivars in Switzerland. *Am. J. Enol. Vitic.* **2019**, *70*, 427–434. [CrossRef]
34. Sternad Lemut, M.; Trost, K.; Sivilotti, P.; Arapitsas, P.; Vrhovsek, U. Early versus late leaf removal strategies for Pinot Noir (*Vitis vinifera* L.): Effect on colour-related phenolics in young wines following alcoholic fermentation. *J. Sci. Food Agric.* **2013**, *93*, 3670–3681. [CrossRef]
35. Gambetta, J.M.; Holzapfel, B.P.; Stoll, M.; Friedel, M. Sunburn in grapes: A review. *Front. Plant Sci.* **2021**, *11*, 2123. [CrossRef]
36. Mori, K.; Goto-Yamamoto, N.; Kitayama, M.; Hashizume, K. Loss of anthocyanins in red-wine grape under high temperature. *J. Exp. Bot.* **2007**, *58*, 1935–1945. [CrossRef]
37. Poni, S.; Casalini, L.; Bernizzoni, F.; Civardi, S.; Intrieri, C. Effects of early defoliation on shoot photosynthesis, yield components, and grape composition. *Am. J. Enol. Vitic.* **2006**, *57*, 397–407. [CrossRef]
38. VanderWeide, J.; Schultze, S.R.; Nasrollahiazar, E.; Poni, S.; Sabbatini, P. Impacts of pre-bloom leaf removal on wine grape production and quality parameters: A systematic review and meta-analysis. *Front. Plant Sci.* **2021**, *11*, 621585. [CrossRef]
39. Frioni, T.; Acimovic, D.; Tombesi, S.; Sivilotti, P.; Palliotti, A.; Poni, S.; Sabbatini, P. Changes in within-shoot carbon partitioning in Pinot noir grapevines subjected to early basal leaf removal. *Front. Plant Sci.* **2018**, *9*, 1122. [CrossRef]
40. Poni, S.; Gatti, M.; Bernizzoni, F.; Civardi, S.; Bobeica, N.; Magnanini, E.; Palliotti, A. Late leaf removal aimed at delaying ripening in cv. Sangiovese: Physiological assessment and vine performance. *Aust. J. Grape Wine Res.* **2013**, *19*, 378–387. [CrossRef]
41. Zhang, P.; Wu, X.; Needs, S.; Liu, D.; Fuentes, S.; Howell, K. The influence of apical and basal defoliation on the canopy structure and biochemical composition of *Vitis vinifera* cv. Shiraz grapes and wine. *Front. Chem.* **2017**, *5*, 48. [CrossRef] [PubMed]
42. Lucchetta, V.; Volta, B.; Tononi, M.; Zanotelli, D.; Andreotti, C. Effects of pre-harvest techniques in the control of berry ripening in grapevine cv. Sauvignon blanc. In *BIO Web of Conferences*; EDP Sciences: Ulis, France, 2019; p. 04016.
43. Gatti, M.; Garavani, A.; Krajecz, K.; Ughini, V.; Parisi, M.G.; Frioni, T.; Poni, S. Mechanical mid-shoot leaf removal on Ortrugo (*Vitis vinifera* L.) at pre-or mid-veraison alters fruit growth and maturation. *Am. J. Enol. Vitic.* **2019**, *70*, 88–97.
44. Lanari, V.; Lattanzi, T.; Borghesi, L.; Silvestroni, O.; Palliotti, A. Post-veraison mechanical leaf removal delays berry ripening on 'Sangiovese' and 'Montepulciano' grapevines. In Proceedings of the I International Workshop on Vineyard Mechanization and Grape and Wine Quality 978, Piacenza, Italy, 27–29 June 2012; pp. 327–333.
45. Lorenz, D.H.; Eichhorn, K.W.; Bleiholder, H.; Klose, R.; Meier, U.; Weber, E. 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. *Aust. J. Grape Wine Res.* **1995**, *1*, 100–103.
46. Buesa, I.; Caccavello, G.; Basile, B.; Merli, M.C.; Poni, S.; Chirivella, C.; Intrigliolo, D.S. Delaying berry ripening of Bobal and Tempranillo grapevines by late leaf removal in a semi-arid and temperate-warm climate under different water regimes. *Aust. J. Grape Wine Res.* **2019**, *25*, 70–82. [CrossRef]
47. Iland, P.G.; Cynkar, W.; Francis, I.; Williams, P.; Coombe, B.G. Optimisation of methods for the determination of total and red-free glycosyl glucose in black grape berries of *Vitis vinifera*. *Aust. J. Grape Wine Res.* **1996**, *2*, 171–178. [CrossRef]
48. Camin, F.; Bontempo, L.; Larcher, R.; Grando, M.S.; Sanz, P.M.; Fauhl-Hassek, C.; Hajslova, J.; Hurkova, K.; Uttl, L.; Thomas, F. Wine and must. In *Foodintegrity Handbook: A Guide to Food Authenticity Issues and Analytical Solutions*; Eurofins Analytics France: Nantes, France, 2018; pp. 205–228.
49. Glories, Y. La couleur des vins rouges. *Conn. Vigne Vin* **1984**, *18*, 253–271.
50. Di Stefano, R.; Cravero, M. Metodi per lo studio dei polifenoli dell'uva. *Riv. Vitic. Enol.* **1991**, *44*, 37–45.
51. CIE Colorimetry. *Official Recommendations of the International Commission on Illumination*; Publication CIE No. 15.2; CIE Colorimetry: Vienna, Austria, 1986.
52. Fracassetti, D.; Gabrielli, M.; Corona, O.; Tirelli, A. Characterisation of Vernaccia Nera (*Vitis vinifera* L.) grapes and wine. *S. Afr. J. Enol. Vitic.* **2017**, *38*, 72–81.

53. Stone, H.; Bleibaum, R.N.; Thomas, H.A. *Sensory Evaluation Practices*; Academic Press: Cambridge, MA, USA, 2020.
54. *ISO 8529-2007*; Nuclear Energy—Reference Neutron Radiations—Part 1: Characteristics and Methods of Measurement. International Organization for Standardization: Geneva, Switzerland, 2007.
55. MacFie, H.J.; Bratchell, N.; Greenhoff, K.; Vallis, L.V. Designs to balance the effect of order of presentation and first-order carry-over effects in hall tests. *J. Sens. Stud.* **1989**, *4*, 129–148. [CrossRef]
56. Poni, S.; Gatti, M.; Palliotti, A.; Dai, Z.; Duchêne, E.; Truong, T.-T.; Ferrara, G.; Matarrese, A.M.S.; Gallotta, A.; Bellincontro, A. Grapevine quality: A multiple choice issue. *Sci. Hortic.* **2018**, *234*, 445–462. [CrossRef]
57. Caccavello, G.; Giaccone, M.; Scognamiglio, P.; Forlani, M.; Basile, B. Influence of intensity of post-veraison defoliation or shoot trimming on vine physiology, yield components, berry and wine composition in Aglianico grapevines. *Aust. J. Grape Wine Res.* **2017**, *23*, 226–239. [CrossRef]
58. Filippetti, I.; Movahed, N.; Allegro, G.; Valentini, G.; Pastore, C.; Colucci, E.; Intrieri, C. Effect of post-veraison source limitation on the accumulation of sugar, anthocyanins and seed tannins in *Vitis vinifera* cv. Sangiovese berries. *Aust. J. Grape Wine Res.* **2015**, *21*, 90–100. [CrossRef]
59. Diago, M.-P.; de Toda, F.M.; Vilanova, M. Effects of timing of leaf removal on yield, berry maturity, wine composition and sensory properties of CV. grenache grown. *J. Int. Sci. Vigne Vin* **2008**, *42*, 221–229.
60. De Bei, R.; Wang, X.; Papagiannis, L.; Cocco, M.; O'Brien, P.; Zito, M.; Ouyang, J.; Fuentes, S.; Gilliham, M.; Tyerman, S. Postveraison leaf removal does not consistently delay ripening in Sémillon and Shiraz in a hot Australian climate. *Am. J. Enol. Vitic.* **2019**, *70*, 398–410. [CrossRef]
61. Kliewer, W.M.; Dokoozlian, N.K. Leaf area/crop weight ratios of grapevines: Influence on fruit composition and wine quality. *Am. J. Enol. Vitic.* **2005**, *56*, 170–181. [CrossRef]
62. Bledsoe, A.; Kliewer, W.; Marois, J. Effects of timing and severity of leaf removal on yield and fruit composition of Sauvignon blanc grapevines. *Am. J. Enol. Vitic.* **1988**, *39*, 49–54. [CrossRef]
63. Bobeica, N.; Poni, S.; Hilbert, G.; Renaud, C.; Gomès, E.; Delrot, S.; Dai, Z. Differential responses of sugar, organic acids and anthocyanins to source-sink modulation in Cabernet Sauvignon and Sangiovese grapevines. *Front. Plant Sci.* **2015**, *6*, 142952. [CrossRef] [PubMed]
64. Downey, M.O.; Dokoozlian, N.K.; Krstic, M.P. Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: A review of recent research. *Am. J. Enol. Vitic.* **2006**, *57*, 257–268. [CrossRef]
65. Zhu, J.; Génard, M.; Poni, S.; Gambetta, G.A.; Vivin, P.; Vercambre, G.; Trought, M.C.; Ollat, N.; Delrot, S.; Dai, Z. Modelling grape growth in relation to whole-plant carbon and water fluxes. *J. Exp. Bot.* **2019**, *70*, 2505–2521. [CrossRef] [PubMed]
66. He, F.; Liang, N.-N.; Mu, L.; Pan, Q.-H.; Wang, J.; Reeves, M.J.; Duan, C.-Q. Anthocyanins and their variation in red wines I. Monomeric anthocyanins and their color expression. *Molecules* **2012**, *17*, 1571–1601. [CrossRef] [PubMed]
67. Giacosa, S.; Parpinello, G.P.; Segade, S.R.; Ricci, A.; Paissoni, M.A.; Curioni, A.; Marangon, M.; Mattivi, F.; Arapitsas, P.; Moio, L. Diversity of Italian red wines: A study by enological parameters, color, and phenolic indices. *Food Res. Int.* **2021**, *143*, 110277. [CrossRef]
68. Reynolds, A.G.; Wardle, D.A.; Naylor, A.P. Impact of training system, vine spacing, and basal leaf removal on Riesling. Vine performance, berry composition, canopy microclimate, and vineyard labor requirements. *Am. J. Enol. Vitic.* **1996**, *47*, 63–76. [CrossRef]
69. Mosetti, D.; Herrera, J.; Sabbatini, P.; Green, A.; Alberti, G.; Peterlunger, E.; Lisjak, K.; Castellarin, S.D. Impact of leaf removal after berry set on fruit composition and bunch rot in 'Sauvignon blanc'. *Vitis* **2016**, *55*, 57–64.
70. Martin, D.; Grose, C.; Fedrizzi, B.; Stuart, L.; Albright, A.; McLachlan, A. Grape cluster microclimate influences the aroma composition of Sauvignon blanc wine. *Food Chem.* **2016**, *210*, 640–647. [CrossRef] [PubMed]
71. Kozina, B.; Karoglan, M.; Herjavec, S.; Jeromel, A.; Orlic, S. Influence of basal leaf removal on the chemical composition of Sauvignon Blanc and Riesling wines. *J. Food Agric. Environ.* **2008**, *6*, 28.
72. Kemp, B. The Effect of the Timing of Leaf Removal on Berry Ripening, Flavour and Aroma Compounds in Pinot Noir Wine. Doctoral Dissertation, Lincoln University, Lincoln, PA, USA, 2010.
73. Scheiner, J.J.; Sacks, G.L.; Pan, B.; Ennahli, S.; Tarlton, L.; Wise, A.; Lerch, S.D.; Heuvel, J.E.V. Impact of severity and timing of basal leaf removal on 3-isobutyl-2-methoxypyrazine concentrations in red winegrapes. *Am. J. Enol. Vitic.* **2010**, *61*, 358–364. [CrossRef]
74. Šuklje, K.; Česnik, H.B.; Janeš, L.; Kmecl, V.; Vanzo, A.; Deloire, A.; Sivilotti, P.; Lisjak, K. The effect of leaf area to yield ratio on secondary metabolites in grapes and wines of *Vitis vinifera* L. cv. Sauvignon blanc. *OENO ONE* **2013**, *47*, 83–97. [CrossRef]