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Exploring grapevine canopy management: effects of removing main leaves or lateral shoots before flowering

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ABSTRACT

Over the course of a six-year trial, we investigated the physiological response of the Swiss white cultivar *Vitis vinifera* Petite Arvine, rich in varietal thiols, to the following canopy removal treatments from the cluster area, *i.e.*, from the shoot base to the sixth leaf of each shoot: A) lateral shoots only, B) lateral shoots +50 % main leaves, C) lateral shoots +100 % main leaves or D) main leaves only. All leaf removal (LR) treatments were performed at the pre-flowering stage.

Intensive pre-flowering removal of both lateral shoots +100 % main leaves from the cluster area (C) strongly reduced yield potential (–47 % on average) and tended to reduce the six-year average concentration of 3-mercaptohexanol precursors (Cys-3MH) in the must, but the results were not significant (–21 %; p-value < 0.10). The effect of LR on berry set and must composition were modulated by removing fewer main leaves (–24 % in yield potential and –6 % in Cys-3MH concentration). Climate conditions primarily influenced yield and grape composition.

Main leaves and lateral shoots played different physiological roles: removal of main leaves only (D) resulted in a larger exposed leaf area (+15 %) due to the development of lateral shoots in the cluster area and a lower yield potential (–12 %) due to fewer berries per cluster when compared one-to-one with removal of lateral shoots only (A). In the must at harvest, treatment D had higher concentrations of malic acid (+12 %), yeast-assimilable nitrogen (+10 %) and glutathione (+8 %), but there were no significant trends for TSS, pH, Cys-3MH or Folin index. The overall effects of pre-flowering LR on white wine composition were negligible in the context of this trial.

The study highlighted the different physiological roles of the main leaves and lateral shoots, suggesting that pre-flowering leaf removal should be used cautiously, taking into account the plant's plasticity to environmental conditions. This research is part of a broader project on grapevine canopy management in temperate climates in Switzerland.

KEYWORDS: defoliation, leaf age, glutathione, berry set, aroma precursors, wine composition

INTRODUCTION

Leaf removal (LR) is a common practice in viticulture to limit fungal attacks and improve grape maturation (VanderWeide *et al*., 2021; Zoecklein *et al.*, 1992). The impact of LR is a combination of effects associated with both the loss of important source leaves proximal to the clusters and the modification of the microclimate in the cluster area (Martin *et al.*, 2016). Previous research has shown the importance of LR timing, which is related to regional climatic conditions and should depend on the objective of the viticulturist (Alem *et al.*, 2018). When applied after the berry set, LR usually does not affect the yield at harvest. On the contrary, when applied before flowering, LR has a major impact on carbon allocation to sinks and reproductive activity by decreasing the carbon source needed for the berry set (Frioni *et al.*, 2018). Therefore, pre-flowering LR limits the berry-set rate and subsequently reduces the harvest yield up to 40–50 % yield loss (Komm and Moyer, 2015; Palliotti *et al.*, 2012; VanderWeide *et al.*, 2020). Pre-flowering LR can also affect grape composition and wine sensory profile, increasing the concentrations of must-soluble solids and polyphenols at harvest, making it an appropriate practice to improve colour and structure in red wines (Poni *et al.*, 2009). However, it should be noted that Wang *et al.* (2018) identified inconsistencies in their meta-analysis investigating the impact of LR on grape composition and wine volatiles. These variations suggest that factors such as grape variety, climatic conditions, grape maturity, and the timing/intensity of LR significantly contribute to the variable effects of LR on grape and wine aromatic characteristics. Both pre-flowering LR timing and intensity have significant impacts on the berry set. A delayed LR within the period from 'separated flower buds' to 'flowering' and a smaller quantity of leaves removed both results in a reduced impact on the rate of berry set (Verdenal *et al.*, in press; Verdenal *et al.*, 2019). Pre-flowering LR may also have carry-over effects. An intensive pre-flowering LR affects carbohydrate reserves in dormant wood, potentially decreasing the number of initiated inflorescence primordia, which could affect bud fruitfulness in the subsequent year (Noyce *et al.*, 2016; Risco *et al.*, 2014). Thus, for sustainability reasons, pre-flowering LR is not recommended for young or unhealthy vines.

When adjusting LR intensity, a fundamental question arises: Which leaves should be selectively removed from the cluster area? This decision is crucial, as the impact may vary, given that the function and activity of leaves change with age. At full leaf expansion (35–40 days after appearance), photosynthesis is maximal but decreases thereafter (Intrieri *et al*., 1992). However, leaves older than four months retain 70 % of their maximal assimilation rate (Intrieri *et al*., 1992). Young leaves (1–20 days) exhibit low photosynthetic activity due to their low chlorophyll and nitrogen content, suboptimal concentration of the enzyme Rubisco, and high light reflection; this results in reduced photosynthetic capacity throughout the canopy until fruiting due to the presence of a significant proportion of young leaves unable to reach their maximal photosynthetic capacity and the resulting competition for nutrients (Poni and Intrieri, 2001). Zufferey *et al.* (2000) confirmed that basal leaves on lateral shoots have 20–30 % lower assimilation rates than main leaves from berry set to veraison; however, from veraison to harvest, lateral shoots become more efficient than primary shoots, highlighting the importance of lateral shoots for grape ripening. Additionally, from a practical point of view, lateral shoots may not be sufficiently developed by the time of pre-flowering LR, making their removal time-consuming. In practice, this implies multiple sessions for removing lateral shoots before flowering unless removing the main leaves is considered satisfactory.

An extensive project on grapevine canopy management was carried out by the Swiss research station Agroscope from 2010 to 2021 (Verdenal *et al.*, 2019, Verdenal *et al.*, 2023). The findings indicated that in Switzerland, pre-flowering LR has proven to be an effective mechanisable method for controlling diseases and reducing yield. This practice also mitigates the symptoms of millerandage and sunburn. Regarding grape and wine composition, the findings revealed that pre-flowering LR had a minimal impact on white wines but showed variable effects on red wines, depending on the grape variety. For Pinot noir, pre-flowering LR enhanced wine colour and mouthfeel, whereas Gamay was less influenced by this technique. Moreover, it was determined that mechanical pre-flowering LR was achievable using a low-pressure double-air flow machine.

As a continuation of this project, the Swiss federal research station Agroscope conducted two six-year field trials from 2016 to 2021 on the physiological response and the aroma development of the Swiss white cultivar *Vitis vinifera* Petite Arvine, rich in varietal thiols and precursors, to pre-flowering LR. In a second trial not yet published, Verdenal *et al.* demonstrated the negative impact of pre-flowering LR on the concentration of 3-mercaptohexanol precursors (Cys-3MH) in the must and the possibility of reducing its impact by delaying pre-flowering LR closer to the flowering stage. They also highlighted that increasing canopy height to compensate for the removed leaf area had a negative effect on yield. In fact, the prolonged presence of the shoot tips, as a nutrient sink, competed for the limited resources and reduced the berry set rate, while having a negligible effect on the grape composition. The present article adds to the previous findings by highlighting the role of pre-flowering LR intensity and providing further insights into the physiological roles of the main leaves and the lateral shoots of the cluster area, to provide practical advice to grape growers.

MATERIALS AND METHODS

1. Vineyard site and experimental design

The trial took place in Agroscope's experimental vineyard located in Leytron, Switzerland (at coordinates 46° 11' 10.9" N, 7° 13' 16.5" E), spanning 2016–2021. Leytron (480 m above sea level) experiences a continental

climate characterised by an average annual temperature of 9.7 °C, ranging from 0.5 °C in January to 20.7 °C in July and an average rainfall of 580 mm annually with no distinct dry season (Table 1 based on data from Sion, MétéoSuisse). The soil at the site is deep and predominantly gravelly (60–70 % stones), comprising 9 % clay, 30 % silt and 61 % sand. It contains 2.1 % organic matter (considered high), 43.7 % total carbonates (eq. $CaCO_3$, high) and has a pH of 8.0. While phosphorus (P) levels are low at 5.3 mg/kg, potassium (K) levels are high at 45.3 mg/kg and magnesium (Mg) is measured at 13.4 mg/kg. None of these elements is a limiting factor for vine growth. The white grape variety Petite Arvine, grafted on 3309C rootstock, was planted in 2011 across a uniform plot with a density of 6,200 vines/ha (1.8 × 0.9 m) and trained using a Guyot system.

The trial followed a randomised complete block design with four blocks and four treatments (A to D). The treatments consisted of removing the main leaves and lateral shoots depending on the treatment, as outlined in Table 2: A/ lateral shoots only (M0-L100), B/50 % main leaves + lateral shoots (M50-L100), C/100 % main leaves + lateral shoots (M100-L100) and D/ main leaves only (M100-L0). Each treatment had four replicates of 18 vines each. For all treatments, LR was conducted annually at the phenological stage of 'separated flower buds' [BBCH 57 according to the uniform decimal code for growth stages, *i.e.*, on average May 21] (Lancashire *et al.*, 1991) and was done manually in the cluster area, that is, from the base of each shoot up to the sixth leaf. Treatment A was considered the control treatment because it corresponded to the usual local practice. Crop thinning was carried out per treatment before the 'cluster closure' stage (BBCH 77) as a function of yield estimation described hereafter to meet regional production quotas at harvest (10 t/ha) and to remain under real production conditions.

2. Measurements and analyses

Field measurements were taken per replicate, except for leaf mineral composition, which was assessed once per treatment. Vine fruitfulness was determined before thinning and expressed as the average number of clusters per shoot. Potential yield was estimated before cluster thinning in July from a sample of 50 berries, a sample of 10 clusters, and the 10-year average berry weight

TABLE 1. Average temperature and total rainfall per month during the experimental period. Data from Sion, Valais, Switzerland (MétéoSuisse).

	Average temperature (°C)							Total rainfall (mm)						
	2016	2017	2018	2019	2020	2021	1991–2020 norm	2016	2017	2018	2019	2020	2021	1991–2020 norm
jan.	2.3	-2.3	3.9	0.0	1.4	0.1	0.5	127	31	197	25	24	94	54
feb.	4.4	5.3	0.8	4.1	5.7	5.4	2.3	87	40	29	17	75	37	40
mar.	6.6	9.7	6.0	7.9	7.4	7.2	7.2	18	62	33	37	68	32	38
apr.	11.3	11.6	14.3	11.4	13.9	10.0	11.3	39	19	29	28	23	20	33
may	14.7	16.4	16.8	13.2	16.4	12.3	15.4	76	45	38	51	48	108	52
jun.	18.7	21.7	21.1	21.1	18.6	20.4	19.1	44	68	26	53	47	42	49
jul.	21.5	22.1	23.3	22.9	21.4	19.3	20.7	46	51	25	76	38	134	60
aug.	21.0	21.3	21.8	20.9	20.5	18.9	20.0	27	69	82	82	22	42	58
sep.	18.4	14.8	18.7	17.4	16.7	17.0	15.8	14	11	19	5	35	51	38
oct.	10.1	11.8	12.4	12.9	9.7	10.0	10.8	32	12	40	48	67	17	41
nov.	5.9	4.2	6.8	5.8	5.4	4.3	5.0	75	43	7	78	5	16	51
dec.	-0.7	-0.6	2.9	3.2	1.9	-0.9	0.9	0	113	111	107	24	104	67
average	11.2	11.3	12.4	11.7	11.6	10.3	10.8	587	564	634	608	474	696	580

TABLE 2. Pre-flowering treatments applied on the canopy in the cluster area, from the shoot base to the sixth leaf of each shoot. Complete randomised block design with four replicates. Petite Arvine, Leytron, Switzerland.

Treatment	Leaf removal treatment (removed from the cluster area)	
	Main leaves	Lateral shoots
A. M0-L100	–	100 %
B. M50-L100	50 %	100 %
C. M100-L100	100 %	100 %
D. M100-L0	100 %	–

of Petite Arvine at harvest (*i.e.*, 1.2 g), as described in Verdenal *et al.* (2023). Berry weight was determined from 50 berries collected one week before harvest. Cluster weight was estimated from the yield per vine divided by the average number of clusters previously assessed. Pruning weight, an indicator of plant vigour, was assessed in winter by collecting 10 shoots from the penultimate position on the cane; the shoots were then equalised to one metre in length and weighed. Leaf mineral composition (N, P, K, Ca and Mg) was determined at veraison from a sample of 25 whole leaves (petiole + blade) per treatment, taken from the medial part of the canopy and analysed by an external laboratory (Sol-Conseil, Gland, Switzerland). The chlorophyll index was monitored once a month during the vegetative season using an N-Tester (Yara, Paris, France) on the main leaves just above the cluster area. The light-exposed leaf area (m^2/m^2 of soil) was estimated in August by measuring the height and width of the canopy and calculated as in equation (1), where H is the height of the canopy, W is the width, T is the percent estimate of holes in the canopy estimated by one single observer and E is the distance between two rows, as follows.

$$Light\ exposed\ leaf\ area = \frac{[(2\times H)+W]\times(1-T)}{E} \quad (1)$$

Must parameters were determined per replicate at harvest during crushing using an infrared spectrophotometer (WineScan™; FOSS, Hillerød, Denmark), *i.e.*, TSS (Brix), TA (g/L as tartrate), tartaric and malic acids (g/L) and pH. Further analyses were performed on berry samples per treatment, collected from the four replicates and then gathered for analyses. The berry samples were divided into several aliquots for further analysis, all of which are described in detail in Verdenal *et al.* (2023) and are described as follows. One aliquot was used for the determination of total phenolic concentration using the Folin–Ciocalteu method (Singleton *et al.*, 1999) adapted to a spectrophotometric autoanalyser (A25; BioSystems, Barcelona, Spain). The results (absorbance at 750 nm corrected by a dilution factor) were expressed as the Folin index. Another aliquot was used to determine the concentrations of ammonium and free alpha-amino acids using an enzymatic method for ammonium (Methods of Biochemical Analysis and Food Analysis; Boehringer Mannheim, 1997) and a spectrophotometric method with a specific kit for free primary amino acids (Primary Amino Nitrogen; BioSystems, Barcelona, Spain). Yeast assimilable nitrogen (YAN) was calculated as the sum of nitrogen (mg N/L) in the form of ammonium and free primary amino acids. Another aliquot was used to determine the total glutathione concentration using a liquid chromatography-mass spectrometer (LC-MS/MS; Agilent Technologies, Santa Clara, CA, USA) according to the method published by Dienes-Nagy *et al.* (2022). A method adapted from Capone *et al.* (2010) for automated sample preparation was used to analyse cysteine conjugates of 3-mercaptohexanol (Cys-3MH), using an Agilent 1290 Infinity II UHPLC system (Agilent, Santa Clara, US) connected to an Agilent 6460-C Triple Quadrupole LC-MS. Samples were injected onto a column and concentrated, and unwanted components were removed. The compounds of interest were then separated on an Agilent Poroshell 120 SB-C18 column (Agilent N°683975-902) using a solvent gradient. Detection was done using multiple reaction monitoring (MRM) in positive ionisation mode. Specific transitions were used for quantification, and an internal standard was employed for calibration.

Wines were made per treatment each year following the standardised protocol of Agroscope's experimental winery. The grapes per replicate were harvested in one day, and the yield was assessed. The four replicates of each treatment were then assembled, and approximately 80 kg of grapes per treatment were vinified, as described hereafter (Verdenal *et al.*, 2019). The grapes were directly pressurised; 50 mg/L sulfur dioxide (SO_2) was added, and the juices were maintained at 12 °C for 24 hours to settle. They were racked the next day and the six-to-eight-day alcoholic fermentation was started at 20 °C with yeast addition (Zymaflore FX10, 20 g/hL). All wines were centrifuged and lactic bacteria were added (Viniflora CH35, 1 g/hL) to guarantee the completion of malolactic fermentation at 18 to 20 °C. The wines were then stabilised (50 mg SO_2/L), kept for one month at 0 °C, filtrated with 0.65 µm cartridges, and bottled in January. The Folin index was evaluated in the wine with the same method as used for the must, as previously described. The chromatic characteristics of the wines were described according to the CIELab procedure, following the International Organisation of Vine and Wine method (OIV, MA-AS2-11, 2016). A sensory analysis was carried out each year in a dedicated tasting room; the trained Agroscope panel (12 permanent members) described the wines in a comparative blind test series according to predefined criteria using a 7-point scale (1 = absence/low concentration; 4 = average; 7 = presence/high concentration).

3. Data treatment

The data were analysed using XLSTAT (Lumivero©, Paris, France), treating the trial as a randomised complete block design with four treatments and four replicates over six years. Analysis of variances (ANOVA) was conducted according to the following model (equation 2):

$$Y = \mu + \text{year} + \text{treatment} + \text{replicate} + (\text{year} * \text{treatment}) + \varepsilon \quad (2)$$

where µ represents the overall mean response, and ε denotes the model's ANOVA error term. For observations lacking replicates, two-way ANOVA was performed (equation 3):

$$Y = \mu + \text{year} + \text{treatment} + \varepsilon \quad (3)$$

The normality and homoscedasticity of the residuals were confirmed by the Shapiro–Wilk and Levene tests, respectively. Tukey's *post hoc* test was utilised for multiple comparisons, with statistically significant differences denoted by distinct letters ($p < 0.05$).

RESULTS

The data are presented as a function of the LR treatment in Table 3 and as a function of the year in Table 4.

1. Vegetative development and yield parameters

The average bud fruitfulness was 1.7 ± 0.3 clusters per shoot, ranging from 1.4 clusters per shoot in 2021 to 2.0 clusters per shoot in 2019 and 2020, and was unaffected by the LR treatments. Leaves at veraison contained on average (% of dry mass) 2.5 ± 0.2 % N, 0.2 ± 0.0 % P, 1.6 ± 0.2 % K, 3.2 ± 0.3 % Ca and 0.3 ± 0.0 % Mg, within the recommended limits, and therefore the vines did not show mineral deficiencies (Spring and Verdenal, 2017). The chlorophyll index in mid-August (at the veraison stage) confirmed sufficient N nutrition each year, despite intra-year variations due to climatic variations (Spring, 1999). The driest year, 2018, had a lower chlorophyll index. Pre-flowering LR influenced the chlorophyll index, which ranged in August from 516 ± 30 in treatment D (M100-L0) to 534 ± 27 in treatment A (M0-L100). The chlorophyll index seemed to be negatively correlated with the number of main leaves removed, regardless of the lateral shoot removal. The differences were compensated for by the time of harvest, with no differences in September. The light-exposed leaf area measured in August varied from 1.09 ± 0.15 m^2/m^2 of soil in the most defoliated treatment C (M100-L100) to 1.34 ± 0.13 m^2 in treatment D (M100-L0). Early estimated yield was highly influenced by both the year and the LR treatment. It ranged from 0.8 ± 0.6 kg/m^2 in 2016 to 1.7 ± 0.5 kg/m^2 in 2019, and it varied from 0.9 ± 0.4 kg/m^2 in treatment C to 1.7 ± 0.3 kg/m^2 in treatment A. As the interaction year*LR intensity was significant for all yield parameters, the yield potentials are presented year by year in Figure 1A. The 2016 yield potential in treatment C (0.3 kg/m^2; –82 % in comparison with treatment A) was exceptionally low in contrast with the other years (average –37 %). Yield potential was highly correlated with the number of berries per cluster (r = 0.74, p < 0.0001), which was the parameter most affected by the LR treatments. Removal of both main leaves and lateral shoots from the cluster area resulted in a 30 % reduction (C; 160 ± 41 berries) compared with the removal of lateral shoots only (treatment A; 228 ± 31 berries). Berry weight varied significantly, with smaller berries in the most defoliated treatments B (M50-L100) and C (M100-L100) [1.1 ± 0.1 g], in contrast to treatments A (M0-L100) and D (M100-L0) 51.2 ± 0.2 g]. Cluster weight was consequently affected with a 30 % loss in treatment C (155 ± 70 g per cluster) compared with treatment A (221 ± 54 g). Accordingly, the average number of clusters thinned varied from 0.3 clusters per vine in treatment C to 3.3 clusters per vine in treatment A (Figure 1B). The average yield at harvest was 1.0 ± 0.3 kg/m^2, with no differences between treatments and in line with regional quotas, except for 2016, due to excessive yield loss after LR, as explained previously. The leaf-to-fruit ratio had lower values in treatment A (1.1 ± 0.2 m^2/kg) and higher values in treatment C (1.9 ± 1.6 m^2/kg), mainly due to the lower yields.

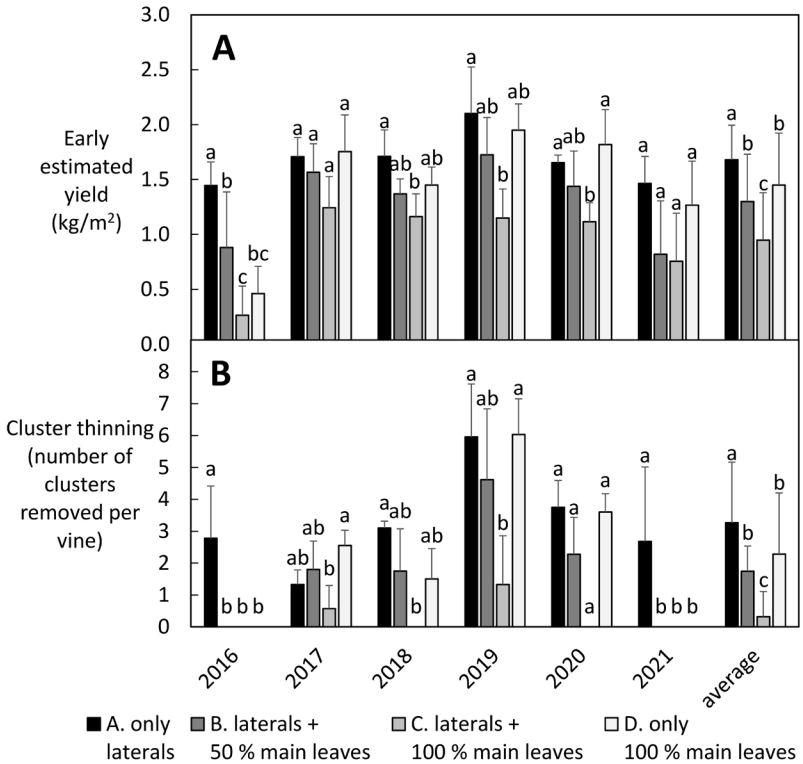


FIGURE 1. Estimated yield before cluster closure (A) and cluster thinning (B) as a function of canopy removal treatment applied in the cluster area from the shoot base to the sixth leaf of each shoot. Different letters within a year, indicate significant differences (Tukey's test, p < 0.05). Error bars are standard deviations, 4 replicates. Data 2016–2021, Petite Arvine, Leytron, Switzerland.

TABLE 3. Agronomic observations, grape must analyses and wine analyses as a function of the canopy removal treatment applied in the cluster area from the shoot base to the sixth leaf of each shoot. Average data 2016–2021. Petite Arvine, Leytron, Switzerland. Numbers on the same line with different letters are statistically different (Tukey's test, $p < 0.05$). $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$; $^{\bullet}p < 0.10$; n.s., non-significant.

	Observations	Canopy removal treatment in the cluster area					
		A. only laterals	B. laterals + 50% leaves	C. laterals + 100% leaves	D. only 100% leaves	*p*-value	Interaction year × canopy treatment
Agronomic observations	Bud fruitfulness (clusters per shoot)	1.7	1.7	1.7	1.7	n.s.	n.s.
	Leaf nitrogen (% dry mass)	2.5	2.5	2.6	2.5	n.s.	—
	Leaf phosphorus (% dry mass)	0.2	0.2	0.2	0.2	n.s.	—
	Leaf potassium (% dry mass)	1.6	1.4	1.6	1.7	·	—
	Leaf calcium (% dry mass)	3.4	3.2	3.3	3.1	n.s.	—
	Leaf magnesium (% dry mass)	0.3	0.3	0.3	0.3	n.s.	—
	Chlorophyll index mid-June	495 a	486 a	461 b	463 b	***	*
	Chlorophyll index mid-July	531 a	525 ab	516 bc	513 c	***	n.s.
	Chlorophyll index mid-August	534 a	532 ab	528 ab	516 b	*	n.s.
	Chlorophyll index mid-September	525	533	522	514	n.s.	n.s.
	Light-exposed leaf area (m^2/m^2 of ground)	1.16 b	1.20 b	1.09 c	1.34 a	***	***
	Early estimated yield (kg/m^2)	1.7 a	1.3 b	0.9 c	1.5 b	***	*
	Cluster thinning (number removed per vine)	3.3 a	1.7 b	0.3 c	2.3 b	***	***
	Berry weight at harvest (g)	1.2 ab	1.1 c	1.1 bc	1.2 a	**	**
	Number of berries per cluster	228 a	205 a	160 b	203 a	***	n.s.
	Cluster weight at harvest (g)	221 a	184 b	155 c	200 ab	***	***
	Yield at harvest (kg/m^2)	1.1	1.0	0.9	1.0	n.s.	***
	Leaf-to-fruit ratio (m^2/kg)	1.1 b	1.3 ab	1.9 a	1.5 ab	*	**
	Pruning weight (g/m)	48	45	44	45	n.s.	n.s.
Grape must analyses	Total soluble sugars (Brix)	23.8 ab	23.6 ab	23.5 b	24.1 a	*	n.s.
	pH	3.03	3.03	3.01	3.03	·	n.s.
	Titratable acidity (g tartrate/L)	10.9 b	11.1 ab	11.1 ab	11.3 a	*	n.s.
	Tartaric acid (g/L)	9.1 b	9.3 b	9.7 a	8.8 c	***	***
	Malic acid (g/L)	4.2 b	4.2 b	4.0 b	4.7 a	***	n.s.
	Ammonium (mg/L)	131 b	140 ab	148 a	144 a	**	—
	Alpha amino N (mg N/L)	147 b	153 ab	149 b	162 a	**	—
	Yeast assimilable nitrogen (mg N/L)	255 b	269 ab	271 a	281 a	**	—
	Folin index must	11.5 ab	12.0 ab	12.7 a	11.0 b	*	—
	Total glutathione (mg/L)	53 b	52 b	51 b	59 a	***	—
	3MH-Cys (µg/L)	21.0	19.7	16.6	21.7	n.s.	—
Wine analyses	Glycerol (g/L)	8.4	8.6	8.7	8.5	n.s.	—
	Folin index wine	6.2 ab	6.5 a	6.6 a	5.9 b	*	—
	Lightness L	99	99	99	99	n.s.	—
	Colour a (red/green)	-0.9	-0.9	-0.9	-0.9	n.s.	—
	Colour b (yellow/blue)	4.8 b	5.2 a	5.2 a	4.8 b	*	—
Wine tasting (score 1 to 7)	Colour intensity	4.0 b	4.1 a	4.1 ab	4.1 ab	*	—
	Fruitiness	4.4	4.4	4.3	4.4	n.s.	—
	Floral	2.6	2.7	2.7	2.7	n.s.	—
	Herbaceous	1.8	1.7	1.7	1.6	n.s.	—
	Lactic	1.2	1.2	1.2	1.2	n.s.	—
	Global nose impression	4.3	4.3	4.3	4.4	n.s.	—
	Volume	4.6	4.5	4.4	4.6	n.s.	—
	Acidity	4.5	4.6	4.6	4.5	n.s.	—
	Bitterness	2.4	2.5	2.5	2.2	n.s.	—
	General impression	4.2	4.2	4.1	4.3	n.s.	—

TABLE 4. Agronomic observations, grape must analyses and wine analyses as a function of the year. Average data of the treatments. Petite Arvine, Leytron, Switzerland. Numbers on the same line with different letters are statistically different (Tukey's test, $p < 0.05$). $^{***}p < 0.001$; $^{**}p < 0.01$; $^{*}p < 0.05$; $p < 0.10$; n.s., non-significant.

	Observations	Year						
		2016	2017	2018	2019	2020	2021	*p*-value
Agronomic observations	Bud fruitfulness (clusters per shoot)	1.8 b	1.6 c	1.5 cd	2.0 a	2.0 a	1.4 d	***
	Leaf nitrogen (% dry mass)	2.7 a	2.7 a	2.2 c	2.6 ab	2.4 bc	2.6 abc	**
	Leaf phosphorus (% dry mass)	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	0.2 a	n.s.
	Leaf potassium (% dry mass)	1.6	1.5	1.6	1.6	1.6	1.5	n.s.
	Leaf calcium (% dry mass)	3.1	3.4	3.4	3.3	3.2	3.1	n.s.
	Leaf magnesium (% dry mass)	0.2 b	0.3 a	0.3 a	0.3 a	0.3 ab	0.3 a	***
	Chlorophyll index mid-June	—	—	518 a	456 c	473 b	458 bc	***
	Chlorophyll index mid-July	483 d	542 ab	544 a	533 ab	527 b	499 c	***
	Chlorophyll index mid-August	529 ab	546 a	523 b	547 a	537 ab	485 c	***
	Chlorophyll index mid-September	520 ab	535 a	515 b	—	—	—	*
	Light-exposed leaf area (m^2/m^2 of ground)	1.1 c	1.3 ab	1.2 b	1.3 ab	1.1 c	1.3 a	***
	Early estimated yield (kg/m^2)	0.8 d	1.6 ab	1.4 b	1.7 a	1.5 ab	1.1 c	***
	Cluster thinning (number removed per vine)	0.7 c	1.6 bc	1.6 bc	4.5 a	2.4 b	0.7 c	***
	Berry weight at harvest (g)	1.0 c	1.2 a	1.1 b	1.0 c	1.1 bc	1.3 a	***
	Number of berries per cluster	131 b	220 a	227 a	211 a	203 a	203 a	***
	Cluster weight at harvest (g)	166 b	231 a	260 a	166 b	143 b	174 b	***
	Yield at harvest (kg/m^2)	0.8 b	1.3 a	1.2 a	0.9 b	0.9 b	0.9 b	***
	Leaf-to-fruit ratio (m^2/kg)	2.6 a	1.0 b	1.0 b	1.4 b	1.4 b	1.6 b	***
	Pruning weight (g/m)	54 a	44 b	44 b	45 b	45 b	42 b	***
Grape must analyses	Total soluble sugars (Brix)	23.5 b	23.5 b	25.0 a	23.7 b	23.7 b	23.1 b	***
	pH	2.94 e	3.03 c	3.11 a	2.98 d	3.07 b	3.03 c	***
	Titratable acidity (g tartrate/L)	12.3 a	11.3 b	10.4 c	11.5 b	9.5 d	11.6 b	***
	Tartaric acid (g/L)	10.1 b	9.0 c	10.7 a	9.3 c	8.7 d	7.4 e	***
	Malic acid (g/L)	4.6 b	4.6 b	2.5 c	4.5 b	2.9 c	6.4 a	***
	Ammonium (mg/L)	160 a	155 a	131 b	169 a	112 c	117 bc	***
	Alpha amino N (mg N/L)	167 b	185 a	161 b	162 b	119 c	125 c	***
	Yeast assimilable nitrogen (mg N/L)	299 a	313 a	268 b	302 a	210 c	221 c	***
	Folin index must	10.5 b	11.8 ab	12.3 ab	13.2 a	12.4 a	10.5 b	**
	Total glutathione (mg/L)	43 c	70 a	71 a	56 b	38 d	43 c	***
	3MH-Cys (µg/L)	9.5 c	19.8 bc	12.0 c	23.6 b	15.4 bc	38.2 a	***
Wine analyses	Glycerol (g/L)	7.1 d	8.6 bc	9.6 a	8.0 cd	9.4 ab	8.6 bc	***
	Folin index wine	6.1 ab	6.7 ab	6.9 a	6.9 ab	6.0 b	5.1 c	***
	Lightness L	98 b	100 a	99 ab	99 ab	99 ab	100 ab	*
	Colour a (red/green)	-0.9 b	-1.1 c	-0.9 b	-0.9 b	-0.9 bc	0.6 a	***
	Colour b (yellow/blue)	5.1 b	6.0 a	5.6 ab	5.2 b	5.0 b	3.0 c	***
Wine tasting (score 1 to 7)	Colour intensity	4.1 b	4.3 a	4.2 b	4.1 b	4.0 c	3.7 d	***
	Fruitiness	4.1	4.6	4.5	4.5	4.4	4.3	n.s.
	Floral	2.4 b	2.9 a	2.7 ab	2.8 a	2.6 ab	2.6 ab	**
	Herbaceous	1.7 a	1.6 a	1.7 a	1.8 a	1.8 a	1.7 a	n.s.
	Lactic	1.1 b	1.2 ab	1.1 b	1.2 ab	1.3 a	1.2 ab	*
	Global nose impression	4.2 ab	4.5 ab	4.3 ab	4.6 a	4.1 b	4.3 ab	*
	Volume	4.5 bc	4.8 a	4.7 ab	4.6 ab	4.4 c	4.3 c	***
	Acidity	4.6	4.6	4.4	4.5	4.5	4.6	n.s.
	Bitterness	2.7 a	2.0 c	2.3 abc	2.2 bc	2.5 ab	2.6 ab	**
	General impression	4.0 c	4.6 a	4.3 abc	4.5 ab	4.0 bc	3.9 c	***

2. Must composition and wine tasting

The average TSS content in the must at harvest varied significantly from 23.5 ± 1.2 Brix in treatment C (M100-L100) to 24.1 ± 1.3 Brix in treatment D (M100-L0). The hot and dry climatic conditions in 2018 were favourable for grape ripening, resulting in a higher TSS content (25.0 ± 0.9 Brix), higher pH (3.11 ± 0.03), lower TA (10.4 ± 0.6 g /L) and lower yeast-assimilable N concentration (268 ± 20 mg N/L) compared with the other years. However, 2016 had a lower pH (2.94 ± 0.05) and the highest TA (12.3 ± 0.8 g/L) due to unfavourable cold and wet climatic conditions, especially in spring (May, June). Due to the significant interaction year*LR intensity for tartaric acid ($p < 0.0001$), the results are presented separately year by year in Figure 2A. Apart from 2016, the concentration of tartaric acid in the must was higher in the most intensively defoliated treatment C. Treatment C also exhibited the lowest pH (3.01 ± 0.05, $p < 0.10$) and the highest Folin index (12.7 ± 1.2; $p < 0.05$) (Table 3). In contrast, the highest concentrations of malic acid were regularly found in treatment D (only 100 % main leaves; Figure 2B). The concentration of yeast-assimilable nitrogen in the must was higher when more main leaves were removed, regardless of the removal of lateral shoots, ranging from 255 ± 42 mg N/L in treatment A (M0-L100) to 281 ± 48 mg N/L in treatment D. In any case, the yeast-assimilable nitrogen concentration remained above the deficiency threshold (140 mg N/L) for proper fermentation conditions (Bell and Henschke, 2005). Intensive pre-flowering LR induced a higher Folin index (+15 %), indicating a higher concentration of polyphenols in the musts of treatment C compared with treatment D. The musts of treatment D had a higher concentration of total glutathione (59 mg/L) in contrast to the other treatments. Concerning aroma precursors, the average concentration of Cys-3MH in the musts was primarily influenced by the years, varying from 9.5 µg/L (2016) to 38.2 µg/L (2021), without significant differences between the LR treatments. The six-year average of the concentration of Cys-3MH tended to be lower in the musts of treatment C, but the results were not significant (in average –21 %; p-value < 0.10) [Figure 3].

The wines from intensively defoliated treatments B (M50-L100) and C (M100-L100) had higher Folin indexes, following the same trend as in the musts and indicating higher concentrations of polyphenols than in treatments A and D. The colours of the wines of treatments B and C were consequently affected and had a higher colour b (CIELab), indicating a visible yellower taint, as confirmed by the tasting criterion of 'colour intensity' (Table 3). The wines from treatment C were devalued in 2016 for their lower global nose impression and again in 2017 because of their lower general impression (results not shown). Treatment D gave a better nose impression and general impression in 2017, perhaps due to its higher concentration in Cys-3MH, although no correlation could be established between the Cys-3MH concentrations in the musts and the aromas 'fruity' and 'floral in the wines over the years of the trial. No other tasting criteria were affected by LR treatments, revealing an overall small effect of pre-flowering LR on wine composition and quality.

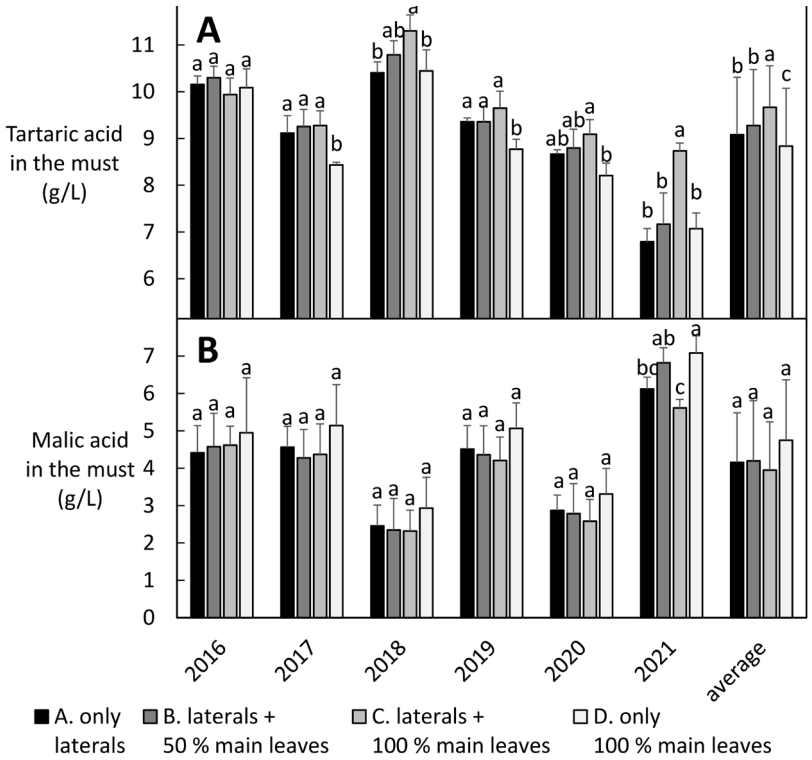


FIGURE 2. Concentration of tartaric and malic acids in the must at harvest as a function of canopy removal treatment applied in the cluster area from the shoot base to the sixth leaf of each shoot. Different letters within a year, indicate significant differences (Tukey's test, $p < 0.05$). Error bars are standard deviations, 4 replicates. Data 2016–2021, Petite Arvine, Leytron, Switzerland.

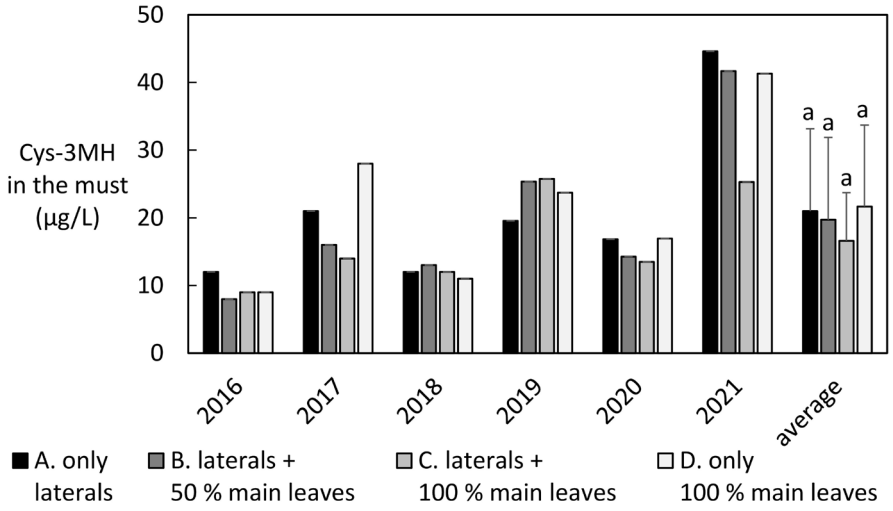


FIGURE 3. Concentration of the aroma precursor Cys-3MH in the must at harvest as a function of canopy removal treatment applied in the cluster area from the shoot base to the sixth leaf of each shoot. Same letters within a year, indicate no significant difference (Tukey's test, $p > 0.05$). No Tukey's test for individual years, since there was no replicate. Error bars are standard deviations for the six-year averages. Data 2016–2021, Petite Arvine, Leytron, Switzerland.

DISCUSSION

This article highlights the role of pre-flowering LR intensity and provides further relevant insights into the physiological roles of the main leaves and the lateral shoots of the cluster area.

1. The intensity of pre-flowering LR

Intensive pre-flowering LR significantly impacted the agronomic performance of the vines, mainly to the detriment of the berry set and potential yield. Compared with the removal of only lateral shoots (A, M0-L100), which is a common practice in Switzerland, the total removal of both lateral shoots and main leaves from the cluster area (C, M100-L100) resulted in an average yield loss of 37 % over the period 2017–2021, which confirmed the results from other trials (Frioni *et al.*, 2018; VanderWeide *et al*., 2021; Verdenal *et al.*, 2019). The positive correlation between the yield loss and the number of main leaves removed before flowering from the cluster area (0 %, 50 % and 100 %; treatments A, B and C, respectively) confirmed the role of main leaves as the major contributor of plant assimilates during the weeks before berry set, as explained in the next section (Lopes *et al*., 2020; Palliotti *et al*., 2012). Reducing LR intensity (50 % main leaves; treatment B) allowed modulation of the effect on yield and limited the yield loss to 5 % and 21 % in treatments B and D, respectively, in comparison with treatment A over the period 2017–2021, in line with the results of Verdenal *et al.* (2019) on the cultivar Chasselas. The lower yield potential resulting from pre-flowering LR allowed for a significant reduction in the time and cost related to production control by manual grape thinning (Figure 1).

In terms of risks, potential excessive yield loss must be considered. Yield formation occurs over two growing seasons and environmental factors (*i.e*., soil, water, nutrient availability, and climate) interact to determine the final crop (Keller, 2015). As a perfect illustration, over the 10 days leading up to flowering in 2016, the temperature was 2.7 °C lower than the 30-year average of 19.1 °C, and sunlight was 2.0 % less than the average of 21.6 Mj/m^2. We suppose that these adverse weather conditions, combined with limited resources for berry set due to decreased leaf area and photosynthesis activity, may account for the exceptionally low yield potential observed that year. In other words, the unfavourable weather conditions at flowering time exacerbated the effect of pre-flowering LR on the berry-set rate. Specifically, there was a yield loss of 82 % in the intensive pre-flowering LR treatments C and a loss of 68 % in treatment D (M100-L100 and M100-L0, respectively; all main leaves removed), compared with treatment A (M0-L100), due to some cluster necrosis and low berry-set rate. Otherwise, the vines were healthy and vigorous throughout the trial, and no long-term carry-over effects of the pre-flowering LR treatments, such as reductions in bud fruitfulness or pruning wood weight, were observed, contrary to the results of other trials (Harner *et al.,* 2024; Lopes *et al.*, 2020; Noyce *et al.*, 2016; Risco *et al.*, 2014). Removing 100 % of the main leaves from the cluster area (treatments C and D) resulted in a lower leaf chlorophyll index from June to August, indicating a lower chlorophyll concentration in the leaves. Despite this result, there were no negative consequences for vegetative growth in this trial, since the chlorophyll index remained above the minimum threshold recommended to guarantee sufficient nitrogen nutrition of the plant (Spring and Verdenal, 2017).

Pre-flowering LR increased the leaf-to-fruit ratio, mainly due to yield loss, which is in line with the results of Poni *et al.* (2006). Pre-flowering LR also probably stimulated the growth of lateral shoots, as suggested by other authors, but this was not measured in this trial (Palliotti *et al.*, 2012; Tardaguila *et al.*, 2010). Maintaining a balance between the

vegetative and reproductive organs is more important than considering the crop load alone to determine the physiological threshold for overcropping (Kliewer and Dokoozlian, 2005; Murisier and Zufferey, 2006). In this trial, the leaf-to-fruit ratio was above the recommended threshold of 1.0–1.2 m^2/kg in all the treatments. Consequently, removing the main leaves from the cluster area (B 50 % and C 100 %) had a minor effect on TSS accumulation and tartaric acid degradation in the must during grape ripening in comparison with treatment A (0 % main leaves removed). The higher concentration of tartaric acid in the must from intensive pre-flowering LR (C, M100-L100) supported the hypothesis that greater exposure to light could lead to greater synthesis of tartaric acid (Poni *et al.*, 2006; Tardaguila *et al.,* 2010). Pre-flowering LR impact on wines was negligible and no consistent effects were observed, except for an increase in Folin index and colour intensity. This higher concentration of polyphenols observed after an intensive pre-flowering LR (B and C) was probably due to three factors, all contributing to greater extraction of the polyphenols from the skin to the must: first, the higher skin-to-pulp ratio resulting from the smaller berry size (Palliotti *et al*., 2012; Poni *et al.*, 2006); second, the thicker berry skin (Verdenal *et al*., 2019); and third, the higher light exposure of clusters as a mechanism inducing higher polyphenols concentration in berry skins (Berli *et al.*, 2011). In terms of winemaking, increasing the concentration of phenols, such as catechin, can increase the bitterness of white wines (Fischer and Noble, 1994). Volatile thiols, responsible for the exotic flavours in Petite Arvine wines, are present in grapes in a non-volatile form, bound to glutathione (Glut-3MH) or cysteine (Cys-3MH). Spring *et al.* (2014) demonstrated a significant positive correlation between the concentration of Cys-3MH in the must and the quality of the aromas of Petite Arvine wines. In the present study, intensive pre-flowering LR (C, M100-L100) tended to reduce the concentrations of Cys-3MH ($p < 0.10$) and total glutathione in the must, with no significant consequences on wine aromas. This result contrasts with the impact of post-flowering LR, which increased Cys-3MH concentration in a trial on Sauvignon blanc (Šuklje *et al.*, 2014). In the context of our trial, Cys-3MH and glutathione concentrations in the must were primarily affected by the conditions of the year: the cooler conditions of 2021 seemed to enhance the Cys-3MH concentration in the must. In contrast, Sivilotti *et al*. (2017) observed for Sauvignon blanc in the climate of northeastern Italy that the years characterised by more rainfall events, lower temperature and solar radiation limit the effect of pre-flowering LR on the microclimatic conditions of the cluster and thus the accumulation of thiol precursors.

Pre-flowering LR is an interesting tool for regulating yield and reducing grape-thinning work. However, we found that its effect on the grape and wine compositions of the white cultivar Petite Arvine was negligible, despite an increasing LR intensity, that is, 0 % (A), 50 % (B) and 100 % (C) of the main leaves from the cluster area. The interaction of the climate in Switzerland may also affect the berry set and make it difficult to predict the impact of pre-flowering LR on both the yield at harvest and the grape composition. As suggested by Harner *et al.* (2024), given both the risk of not reaching the production target and the limited impact on white wine composition, we do not recommend intensive pre-flowering LR (*i.e*., more than 50 % LR in the cluster area) due to the unpredictable berry-set rate related to the climate condition at the flowering stage in the same year.

2. Removing either main leaves or lateral shoots from the cluster area

The removal of only the main leaves (D, [M100-L0] resulted in a larger exposed leaf area (+15 %) in comparison with the removal of all the lateral shoots (A, M0-L100), mainly due to the growth of the lateral shoots in the cluster area. This induced a lower yield potential (–14 %), mainly due to fewer berries per cluster (–11 %). Keeping in mind that the peak photosynthetic activity occurs when the leaves are fully expanded, at around 35–40 days old, the canopy's overall photosynthetic activity is reduced until berry set due to the higher proportion of young leaves and young lateral shoots, which have not yet achieved their maximum photosynthetic capacity (Intrieri *et al.*, 1992; Poni and Intrieri, 2001). In treatment D, the removal of a significant portion of photosynthetically active leaves caused a substantial reduction in carbon assimilation per shoot (main leaves = carbon source), and this reduction was directly correlated with the increased priority of the shoot apexes as a sink destination, which is inversely correlated with berry set (Frioni *et al.,* 2018). However, the removal of only the lateral shoots in treatment A (apexes = carbon sink + low photosynthetic activity) had two consequences: 1) the reduction of the number of active apexes, inducing a smaller sink strength of the developing canopy in favour of reproductive development, and 2) the preservation of a large photosynthetically active leaf area, inducing a smaller carbon assimilation depression compared with treatment D and resulting in a higher berry-set rate. As summarised by Frioni *et al.* (2018), the berry set is linked to the relative sink strength of developing vegetation alongside the total decrease in carbon assimilation caused by LR.

The musts from treatment D (M100-L0) contained more malic acid (+14 %), more yeast-assimilable nitrogen (+10 %) and more glutathione (+10 %) in comparison with those from treatment A (M0-L100) [Table 3]. The substantial variation in the tartaric-to-malic ratio (1.9 *versus* 2.2 in treatments D and A, respectively; Figure 2) increased the overall titratable acidity. Increasing must acidity, particularly malic acid, may be appropriate in the current context of global warming, which highly influences the TTS-to-TA ratio (Petrie and Sadras, 2008). Removing only the main leaves (treatment D) contributed to the growth of the lateral shoots in the cluster area and resulted in a final larger leaf area and a subsequent cooler microclimate in the cluster area. As suggested by Lakso and Kliewer (1978), the lower exposure and temperature of the clusters, representing less abiotic stress, contributed to the higher concentration of malic acid and glutathione in the musts of treatment D than in those of treatment A (removal of lateral shoots). Having higher

levels of glutathione in wine contributes to the preservation of aroma and colour (Nikolantonaki *et al.*, 2018). The larger size and higher sink strength of the canopy in treatment D may explain the higher concentration of ammonium and amino acids in the must at harvest (Table 3). Otherwise, there was no significant trend for TSS, pH, Cys-3MH or Folin index when comparing treatments, A and D. The subsequent differences in wine composition were small but significant. The removal of the main leaves resulted in more colour intensity and fewer vegetable aromas than the removal of the lateral shoots only. The climate of the region is relatively dry, with less than 600 mm of rainfall per year. In that specific context, there was no fungal attack during the six years of the trial, and removing only the main leaves instead of the lateral shoots seemed to be an interesting low-risk practice with a moderate effect on both yield potential and grape composition.

This trial highlighted the physiological implications of removing the main leaves or lateral shoots from the cluster area before flowering. Traditionally, in cool and temperate climate regions, such as Switzerland, LR from the cluster area is an efficient prophylactic practice against fungal attacks on grapes. The traditional removal of lateral shoots between the 'berry set' and 'cluster closure' stages is labour-intensive and has a negligible impact on grape composition. In comparison, the pre-flowering removal of main leaves is faster, reduces cluster thinning work and potentially improves grape composition. Further research focusing on this issue is needed.

CONCLUSION

The trial confirmed the significant impact of pre-flowering LR of the cluster area on the berry-set rate and the potential yield at harvest. The berry-set rate was related to the LR intensity and the unpredictable climate conditions at the flowering stage in the same year.

The effect of pre-flowering LR on the composition of musts and wines of the white cultivar Petite Arvine was negligible. Intensive pre-flowering tended to reduce the concentrations of Cys-3MH and glutathione in the must at harvest, with no significant effect on wine aromas on average over six years.

In view of both the risk of not reaching the production target and the limited impact on white wine composition, we do not recommend intensive pre-flowering LR (*i.e.*, more than 50 % LR in the cluster area).

Observing the separate impacts of either removing the main leaves or the lateral shoots over six years provided insights into the physiological mechanisms influencing fruit development and aroma formation.

Removing only the main leaves seems to be a viable practice with a moderate effect on both the yield potential and the must composition at harvest, that is, higher concentrations of malic acid, yeast-assimilable nitrogen and glutathione. Further trials should focus on this practice.

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