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Increasing grapevine canopy height to compensate for pre-flowering basal leaf removal

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ABSTRACT

In viticulture, leaf removal (LR) in the cluster area is an effective practice against fungal diseases. Pre-flowering LR often has additional consequences compared to post-flowering LR, such as yield limitation, higher fruit maturity index and higher anthocyanin concentration in the must at harvest, with positive effects on wine composition. The phenotypic mechanisms behind the accumulation of thiol precursors in grapes remain incompletely understood, as do the ways in which they can be improved by agronomic practices. A six-year field trial on the Swiss white grape variety *Vitis vinifera* Petite Arvine evaluated the effects of 1) timing of LR (*i.e.*, 'separated flower buds' or 'flowering' stages) and 2) compensating for leaf area removed in the bunch area by increasing canopy height (*i.e.*, 100 or 150 cm canopy height) on grape and wine composition, in particular varietal thiols and thiol precursors.

Pre-flowering LR reduced harvest yield by decreasing the number of berries per cluster. Unpredictable climatic conditions prior to flowering, such as cold temperatures and low light levels, significantly influenced fruit set and amplified the negative effects of pre-flowering LR, increasing the risk of excessive yield loss. Adjusting the timing of LR from the 'separated flower buds' stage to the 'flowering' stage provided a means to manage yield loss effectively. Compared to pre-flowering LR, LR applied at the flowering stage decreased acidity and yeast assimilable nitrogen concentrations, increased the concentration of the aroma precursor Cys-3MH in the must at harvest, and slightly enhanced the overall quality of the white wine. Meanwhile increasing trimming height to compensate for leaf removal in the cluster area increased the leaf-to-fruit ratio, and slightly improved both grape ripeness at harvest (higher TSS, lower tartaric acid) and wine sensory profile (higher colour intensity, floral, volume and overall impression). The combination of LR at flowering and increased canopy height (Flow-150) appeared to be a good compromise, mitigating yield loss and slightly improving wine composition in terms of Cys-3MH accumulation in the must. Further research is needed to better understand and optimise the physiological mechanisms underlying the formation of aroma precursors in the fruit.

KEYWORDS: early defoliation, hedge trimming, berry set, aroma precursors, wine quality

INTRODUCTION

Leaf removal (LR) in the cluster area, usually carried out between the phenological stages of berry set and veraison (BBCH 71 and 81, respectively), is an effective practice that makes the microclimate of the cluster area less favourable to fungal attack (Zoecklein *et al.,* 1992). While LR is not recommended after veraison to prevent any delay in fruit ripening, pre-flowering LR is presented as an interesting practice for regulating the yield, thus limiting cluster thinning costs (VanderWeide *et al.,* 2021). Indeed, pre-flowering LR reduces the carbon source required for berry set, limiting the berry set rate and, therefore, the yield at harvest (Frioni *et al.,* 2018). Yield loss following intensive pre-flowering LR can reach up to 40–50 % of initial potential (Komm & Moyer, 2015; Palliotti *et al.,* 2012), but the effect of LR can be mitigated by reducing the quantity of leaves removed (Verdenal *et al.,* 2018; Verdenal *et al.,* 2024). Consequently, the fruit composition is also affected by pre-flowering LR. In their systematic review, VanderWeide *et al.* (2021) explain that following a pre-flowering LR, musts often show higher concentrations of soluble sugars (+5.2 %). Interestingly, berries are often smaller, with thicker skins, which increases the concentration of polyphenols and colour intensity in red wines (Poni *et al.,* 2006; Verdenal *et al.,* 2019).

Both viticulture and winemaking practices strongly influence the development of wine aromas. Fermentation aromas are produced by the metabolisms of both fatty acids and amino acids through yeast activity during fermentation (Styger *et al.,* 2011). Šuklje *et al.* (2014) demonstrated that LR on the Sauvignon blanc variety at the 'pee size' phenological stage increased the concentration of thiols in the corresponding wines, the source of aromas such as passion fruit and grapefruit. Conversely, Sivilotti *et al.* (2017) did not show any significant effect of pre-or post-flowering LR on Cys-3MH in the must at harvest. In a meta-analysis of articles investigating the impact of LR on grape and wine volatiles, Wang *et al.* (2018) identified factors such as grape variety or clone, climatic conditions, grape maturity and LR timing/intensity, that contribute significantly to the variable effects of LR on grape and wine aromatic characteristics. Pre-flowering LR also carries the risk of reduced bud fruitfulness and vigour in the mid-term: pre-flowering LR affects the carbohydrate reserve in the dormant woods and consequently may reduce the number of inflorescence primordia initiated, which would affect bud fruitfulness in the following year (Bennett *et al.,* 2005; Noyce *et al.,* 2016; Risco *et al.,* 2014).

Considering both the risks and the benefits of pre-flowering LR on grape and wine composition, an extensive study was conducted on five cultivars (Chasselas, Doral, Pinot noir, Gamay and Merlot) under Swiss climatic conditions from 2010 through 2021 (Verdenal *et al.,* 2019). In the Swiss context, pre-flowering LR was confirmed as an efficient practice for disease control and yield reduction. It also limited the symptoms of millerandage and sunburn. In terms of grape and wine composition, the effect of pre-flowering LR was negligible for white wines, while it was positive for red wines. Pre-flowering LR had a positive effect on the wine colour and mouthfeel of Pinot noir, while Gamay was more resilient to this practice. Mechanical pre-flowering LR was possible with the use of a low-pressure double air flow machine (Verdenal *et al.,* 2023). For sustainability reasons, this practice is not recommended for young and/or unhealthy vines.

The extensive literature on pre-flowering LR has left some questions unanswered, such as the following. After intensive LR, the remaining leaf area may be insufficient to ensure grape ripening, and increasing canopy height by higher trimming could help to maintain an equivalent leaf area. Removal of either the main leaves or the lateral shoots, or both, could also have different effects on yield and grape composition. In addition, the effect of pre-flowering LR on the accumulation of aroma precursors in white grape varieties is still unclear, although similar research has been already carried out (Sivilotti *et al.,* 2017; Bubola *et al.,* 2020). To clarify these points, the Swiss Federal Research Station Agroscope conducted two six-year field trials from 2016 to 2021 on the physiological response of the Swiss white cultivar *Vitis vinifera* Petite Arvine, rich in varietal thiols and precursors, to pre-flowering LR. Verdenal *et al.* (2024) described the different physiological roles of main leaves and lateral shoots and the possibility of mitigating yield loss by reducing pre-flowering LR intensity, while this article highlights the effects of compensating for leaves removed from the cluster area by increasing canopy height.

MATERIALS AND METHODS

Besides the research question and the LR treatments tested, the materials and methods used in the present trial were identical to the one published by Verdenal *et al.* (2024) and are detailed hereafter.

1. Vineyard site and experimental design

The trial was carried out in the experimental vineyard of Agroscope in Leytron, Switzerland (46° 11' 10.9" N, 7° 13' 16.5" E), at 485 m above sea level, from 2016 through 2021. The region has a continental climate: the average annual temperature is 9.7 °C (from 0.5 °C in January to 20.7 °C in July), and the average rainfall is 580 mm with no dry season (30-year average, Sion, MétéoSuisse). The climatic conditions during the period of the experiment are presented in Table S1 (MétéoSuisse). The deep and highly gravelly soil (60–70 % stones) of the site was composed of 9 % clay, 30 % silt and 61 % sand. The soil contained 2.1 % organic matter (high), 43.7 % total carbonates (eq. $CaCO_3$, high), and the pH was 8.0. Phosphorus (P, 5.3 mg/kg, low), potassium (K, 45.3 mg/kg, high), and magnesium (Mg, 13.4 mg/kg) were not limiting for vine growth. The white variety Petite Arvine was grafted on 3309C rootstock and planted in 2011 with a density of 6,200 vines/ha on a homogeneous plot and trellised in a single guyot system (seven shoots per vine). Crop thinning was carried out per treatment each year before 'cluster closure' (BBCH 77) to

meet regional production quotas at harvest (10 t/ha) and to remain under real production conditions.

The trial was designed as a randomised complete block design with four blocks and four treatments of 18 vines each, as summarised in Table 1. Two LR timings and two canopy heights were tested. LR was performed each year either at the phenological stage of 'separated flower buds' (BBCH 57, average 21 May, treatments Pref-100 and Pref-150) or at the stage of 'flowering' (BBCH 65, average 9 June, treatments Flow-100 and Flow-150). The LR of the cluster area consisted of the manual removal of the first six basal leaves of each shoot, including the lateral shoots. Canopy height (*i.e.*, shoot length) was maintained by trimming throughout the growing season, either 100 cm (treatments Pref-100 and Flow-100) or 150 cm (treatments Pref-150 and Flow-150).

TABLE 1. Treatments of the trial. Complete randomised block design with four replicates of 18 vines each. Petite Arvine, Leytron, Switzerland.

Treatment	Leaf removal timing	Canopy trimming height
Pref-100	pre-flowering	100 cm
Pref-150	pre-flowering	150 cm
Flow-100	flowering	100 cm
Flow-150	flowering	150 cm

2. Measurements and analyses

Field measurements and part of the must analyses were taken per field replicate (*i.e.*, four times per treatment). For logistical and budgetary reasons, leaf mineral composition, must nitrogen content, wine analysis and sensory analysis were assessed once per treatment (no field replicate). As a consequence, two different statistical analyses were performed on the data, as described in the section on data treatment.

Vine fruitfulness was determined before crop thinning and expressed as the average number of clusters per shoot. The potential yield was estimated before 'cluster closure' (BBCH 77) in July from a sample of 50 berries (berry wt_{july}) and 10 clusters (cluster wt_{july}) per replicate, and the 10-year average berry weight at harvest (berry wt_{harv}), using the Equation (1) as described in Verdenal *et al.* (2023).

$$Yield_{estim} = \frac{\left(\frac{cluster\ wt_{july} \times berry\ wt_{harv}}{berry\ wt_{july}}\right) \times cluster\ nb_{vine}}{plantation\ density \times 1000} \quad (1)$$

Average 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 24 whole leaves per treatment (petiole + blade) collected in the median 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 (BBCH 62 to 89) using an N-tester (Yara, Paris, France) on the main leaves from the median part of the canopy. 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 (2), where H is the height of the canopy, W is the width, T is the percentage of holes in the canopy estimated by one single observer and E is the distance between two rows, as follows.

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

The following analyses were performed on the musts from berry samples collected per treatment just before harvest (no field replicate). The concentrations of ammonium and free alpha-amino acids were assessed using an enzymatic method for ammonium (Methods of Biochemical Analysis and Food Analysis; Boehringer, Mannheim GmbH, 1997) and a spectrophotometric method with a specific kit for free primary amino acids (Primary Amino N; BioSystems, Barcelona, Spain). Yeast assimilable nitrogen (YAN) was calculated as the sum of N (mg N/L) in the form of ammonium and free primary amino acids. 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 Infinity 1290 UHPLC system (Agilent, Santa Clara, US) connected to an Agilent 6460-C Triple Quadrupole LC-MS. Samples were injected into a column and concentrated, and unwanted components were removed. The compounds of interest were then separated on a 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. The grapes per replicate were harvested in one day and the yield was assessed. Must parameters were determined per replicate on samples collected during crushing using an infrared spectrophotometer (WineScan™; FOSS, Hillerød, Denmark),

i.e., soluble sugars (TSS, °Brix), titratable acidity (TA,g/L as tartrate), tartaric and malic acids (g/L) and pH. The four replicates of each treatment were then assembled and approximately 50 kg of grapes per treatment were vinified according to the standard Agroscope protocol, as described in detail by Verdenal *et al.* (2019). The grapes were directly pressurized; 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 stabilized (50 mg SO_2/L), kept for one month at 0 °C, filtrated with 0.45 µm cartridges, and bottled in January. The total phenolic concentration was assessed in the wine using the Folin–Ciocalteu method (Singleton *et al.,* 1999) adapted to a spectrophotometric autoanalyzer (A25; BioSystems, Barcelona, Spain). The results (absorbance at 750 nm corrected by a dilution factor) were expressed as the Folin index. The chromatic characteristics of the wines were described according to the CIELab procedure, following the International Organisation of Vine and Wine (OIV) MA AS2 11 method (OIV, 2016). A sensory analysis was carried out each year, three months after bottling, in a dedicated tasting room; the trained Agroscope panel (12 permanent members) described the wines according to predefined criteria using a 7-point scale (1 = absence/low concentration; 4 = average; 7 = presence/high concentration). The criteria "volume" refers to the texture of the wine in the mouth, a perception that covers the whole of the mouth; "general impression" is a hedonistic criterion to describe the overall appreciation of the wine.

3. Data treatment

The data were described statistically using XLSTAT (Lumivero©, Paris, France), considering the trial as a randomised complete block design. For observations with replicates, the following model of ANOVA was applied (Equation 3):

$$Y = \mu + \text{year}(6) + \text{LR timing}(2) + \text{trimming height}(2) + \text{replicate}(4) + \text{year} * \text{LR timing} + \text{year} * \text{trimming} + \text{LR timing} * \text{trimming} + \text{LR timing} * \text{trimming} * \text{year} + \varepsilon \quad (3)$$

where µ is the overall mean response, and ε is the error of the model. Years and replicate were considered as random factors, and LR timing and trimming height as fixed factors. For observations without replicates, the following model was applied (Equation 4):

$$Y = \mu + \text{year}(6) + \text{LR timing}(2) + \text{trimming height}(2) + \text{year} * \text{LR timing} + \text{year} * \text{trimming} + \text{LR timing} * \text{trimming} + \varepsilon \quad (4)$$

The normality and homoscedasticity of the residuals were confirmed by the Shapiro–Wilk and Levene tests, respectively. Tukey's post-hoc test was used for multiple comparisons. Numbers followed by different letters are statistically different ($p < 0.05$).

RESULTS

The separate effects of LR timing and trimming height and their interaction are presented in Table 2. The data are also presented as a function of the year (*i.e.*, 2016 to 2021) in Table S2. Complete raw data are available on request.

1. Vegetative development and yield parameters

The effect of the year (*i.e.*, precipitation and temperature) was dominant, strongly affecting most of the observations (Table S2). Grapevines bore 1.7 clusters per shoot on average, varying from 1.4 in 2021 to 2.0 in 2019 and 2020. Bud fruitfulness was slightly reduced by Pref treatments compared to Flow treatments (–0.1 cluster per shoot, p-value < 0.05) while trimming height had no impact (Table 2). The mineral composition of the leaves showed no deficiencies within the thresholds recommended in Switzerland (Spring & Verdenal, 2017) and was not affected by LR timing or canopy height. The chlorophyll index was affected by both LR timing and canopy height (Table 2): the index was reduced by 3 % immediately after pre-flowering LR in June in comparison with flowering LR, but the differences remained physiologically insignificant (Table 2).

LR timing highly affected the yield parameters: the average estimated yield before cluster thinning was only 0.9 kg/m^2 for Pref treatments, which represents a 33 % loss in comparison with Flow treatments (Table 2). The interaction year*LR timing was strong particularly for the yield parameters (*i.e.*, yield estimation, cluster weight, berry number, berry weight), whereas the interactions year*trimming and LR timing*trimming were negligible (Table 2). For this reason, the yield potentials are presented year by year in Figures 1A and 1B: it appears that the yield loss was exceptionally high in 2016 in the Pref treatments (81 %), compared to the Flow treatments and the other years (average 24 %). The yield potential was highly correlated with the bud fruitfulness ($r = 0.72$, $p < 0.0001$), and with the number of berries per cluster ($r = 0.71$, $p < 0.0001$), which was the parameter most affected by LR timing. Cluster thinning was proportional to yield potential, with only 0.4 clusters removed per vine for Pref treatments, while 1.9 clusters had to be removed per vine in Flow treatments (Table 2). Pre-flowering LR affected the berry-set rate and reduced the number of berries per cluster from 198 berries on average in the Flow treatments to 160 berries (–19 %) in the Pref treatments (Table 2). The average cluster weight at harvest varied consequently from 170 g in the Flow treatments to 139 g in the Pref treatments (–18 %) (Table 2 and Figure 2). The average berry weight in the Pref treatments was also lower compared to the Flow treatments (–0.1 g; p-value < 0.05) (Table 2).

A higher trimming height to compensate for the removed leaf area, on the other hand, did not significantly affect the number of berries per cluster or the berry weight (Table 2). A higher trimming induced a smaller cluster weight (–16 %; Table 2 and Figure 2), and the yield estimation tended to be smaller for five of the six years of the trial for a 150 cm trimming height (average –0.1 kg/m^2; $p < 0.10$) in comparison with a

TABLE 2. Vineyard measurements, must analyses and wine analyses and tasting as a function of leaf removal timing and canopy height. Average data for 2016–2021. Petite Arvine, Leytron, Switzerland. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$; •$p < 0.10$; *n.s.*, non-significant (Tukey's test).

		Leaf removal timing			Canopy trimming height			Interactions			
	Observations	Pre-flowering (Pref)	Flowering (Flow)	p-value	100 cm	150 cm	p-value	Year × LR timing	Year × Trimming	LR timing × Trimming	Year × LR timing × Trimming
Vineyard measurements	Bud fruitfulness (clusters per shoot)	1.7	1.8	*	1.7	1.7	*n.s.*	•	*n.s.*	*n.s.*	*n.s.*
	Leaf nitrogen (% dry mass)	2.6	2.5	*n.s.*	2.5	2.6	*n.s.*	*n.s.*	*n.s.*	*n.s.*	–
	Leaf phosphorus (% dry mass)	0.2	0.2	*n.s.*	0.2	0.2	*n.s.*	*n.s.*	*n.s.*	*n.s.*	–
	Leaf potassium (% dry mass)	1.6	1.7	*n.s.*	1.7	1.6	*n.s.*	*n.s.*	*n.s.*	*n.s.*	–
	Leaf calcium (% dry mass)	3.3	3.3	*n.s.*	3.3	3.3	*n.s.*	*n.s.*	*n.s.*	*n.s.*	–
	Leaf magnesium (% dry mass)	0.3	0.3	*n.s.*	0.3	0.3	*n.s.*	*n.s.*	*n.s.*	*n.s.*	–
	Chlorophyll index mid-June	471	487	***	477	480	*n.s.*	***	*n.s.*	*n.s.*	*n.s.*
	Chlorophyll index mid-July	520	522	*n.s.*	517	525	**	•	*n.s.*	*n.s.*	*n.s.*
	Chlorophyll index mid-August	523	530	•	528	525	*n.s.*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
	Early estimated yield (kg/m^2)	0.9	1.4	***	1.2	1.1	•	***	*n.s.*	*n.s.*	*n.s.*
	Cluster thinning (number removed per vine)	0.4	1.9	***	1.4	0.8	**	***	*n.s.*	***	*n.s.*
	Light-exposed leaf area (m^2/m^2 of ground)	1.2	1.2	*n.s.*	1.1	1.3	***	*n.s.*	*n.s.*	*n.s.*	*n.s.*
	Cluster weight at harvest (g)	139	170	***	167	141	***	***	*n.s.*	*n.s.*	*n.s.*
	Number of berries per cluster	160	198	***	182	176	*n.s.*	**	*n.s.*	*n.s.*	*n.s.*
	Berry weight at harvest (g)	1.1	1.0	*	1.1	1.1	*n.s.*	***	*n.s.*	*n.s.*	*n.s.*
	Yield at harvest (kg/m^2)	0.8	1.0	***	1.0	0.8	***	***	*n.s.*	*	*n.s.*
	Leaf-to-fruit ratio (m^2/kg)	2.1	1.3	***	1.5	1.9	•	***	*n.s.*	*n.s.*	*n.s.*
	Pruning weight (g/m)	47	46	*n.s.*	44	49	***	*n.s.*	•	*n.s.*	*n.s.*
Must analyses	Total soluble sugars (°Brix)	23.6	23.6	*n.s.*	23.4	23.7	*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
	pH	3.01	3.01	*n.s.*	3.01	3.02	*	*n.s.*	*n.s.*	*n.s.*	*n.s.*
	Titratable acidity (g tartrate/L)	11.1	10.8	***	11.0	11.0	*n.s.*	**	*n.s.*	*n.s.*	*n.s.*
	Tartaric acid (g/L)	9.6	9.3	***	9.6	9.3	***	***	*n.s.*	*n.s.*	*n.s.*
	Malic acid (g/L)	4.0	3.8	**	3.9	4.0	•	*n.s.*	*n.s.*	*n.s.*	*n.s.*
	Ammonium (mg/L)	143	126	***	137	131	*n.s.*	*n.s.*	*n.s.*	*n.s.*	–
	Alpha-amino N (mg N/L)	148	139	***	142	143	*n.s.*	*	*n.s.*	*	–
	Yeast assimilable nitrogen (mg N/L)	265	242	***	255	252	*n.s.*	**	*	**	–
	Cys-3MH (µg/L)	18	19	***	17	20	***	*n.s.*	*n.s.*	**	–
Wine analyses	Folin index wine	6.6	6.8	*	6.6	6.7	*n.s.*	*n.s.*	*n.s.*	*n.s.*	–
	Lightness L	99	99	*n.s.*	99	99	*n.s.*	*n.s.*	*n.s.*	*n.s.*	–
	Colour a (red/green)	–0.9	–1.0	*	–0.9	–1.0	**	*n.s.*	*n.s.*	*n.s.*	–
	Colour b (yellow/blue)	5.2	5.5	***	5.3	5.5	**	*n.s.*	*n.s.*	*n.s.*	–
Wine tasting (quote 1-7)	Colour intensity	4.06	4.13	***	4.08	4.12	***	*n.s.*	*n.s.*	*n.s.*	–
	Fruitiness	4.4	4.5	*n.s.*	4.4	4.5	*n.s.*	*n.s.*	*n.s.*	*n.s.*	–
	Floral	2.8	2.7	*n.s.*	2.7	2.9	•	*n.s.*	*n.s.*	*n.s.*	–
	Herbaceous	1.7	1.6	*n.s.*	1.7	1.6	*n.s.*	*n.s.*	*n.s.*	*n.s.*	–
	Global nose impression	4.3	4.4	•	4.3	4.4	*n.s.*	*n.s.*	*n.s.*	*n.s.*	–
	Volume	4.5	4.6	*	4.5	4.6	*	*n.s.*	*n.s.*	*n.s.*	–
	Acidity	4.5	4.5	*n.s.*	4.6	4.5	*n.s.*	*n.s.*	*n.s.*	*n.s.*	–
	Bitterness	2.4	2.4	*n.s.*	2.5	2.3	*n.s.*	*n.s.*	*n.s.*	*n.s.*	–
	General impression	4.2	4.3	*	4.1	4.3	**	*n.s.*	*n.s.*	*n.s.*	–

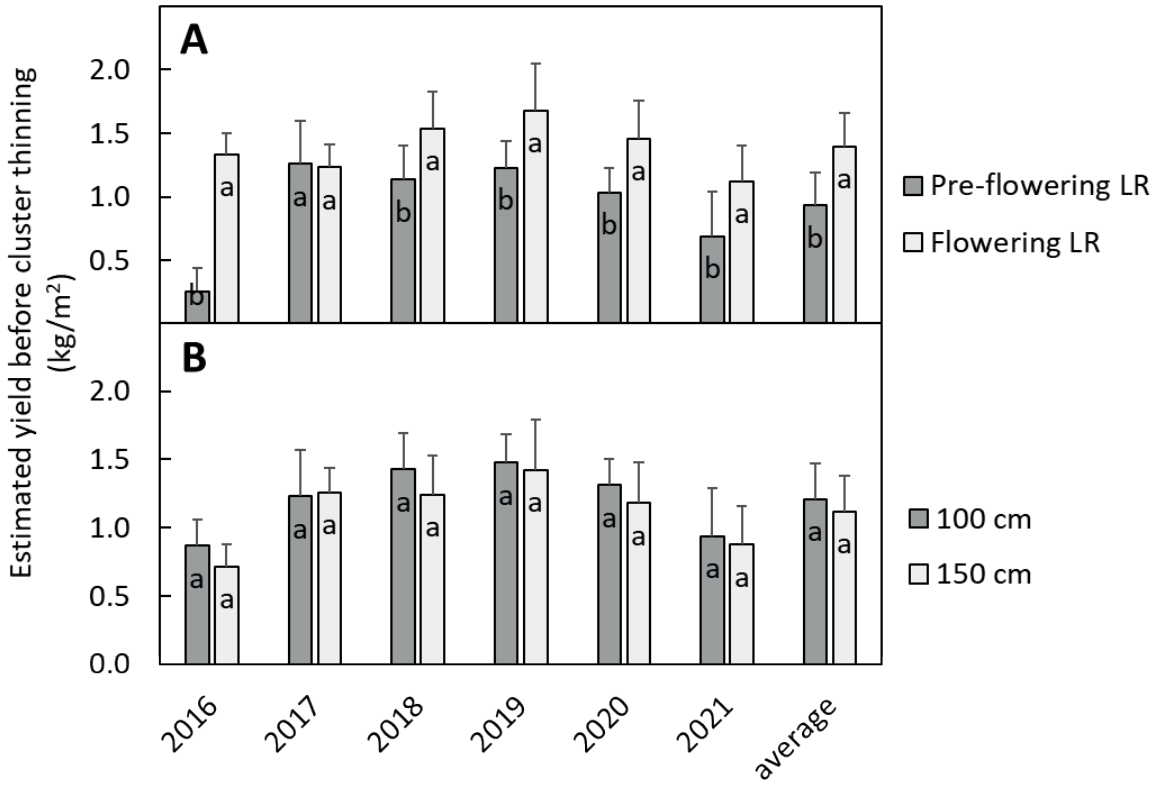

FIGURE 1. Yield estimated before cluster thinning at cluster closure stage per year, as a function of leaf removal timing (A) and canopy height (B). Error bars are standard deviations. Numbers followed by different letters within a year are significantly different (Tukey's test, $p < 0.05$).

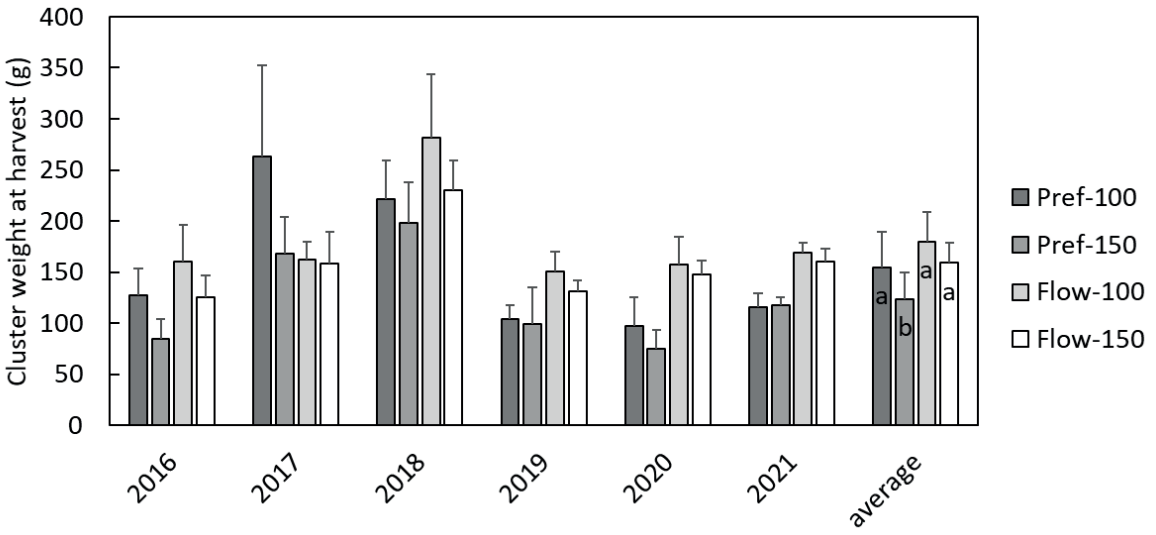

FIGURE 2. Cluster weight at harvest per treatment and per year. Error bars are standard deviations. Leaf removal at "separated flower bud" stage (BBCH 57, Pref) or at the "flowering" stage (BBCH 65, Flow); canopy height at 100 or 150 cm. Numbers followed by different letters within a year are significantly different (Tukey's test, $p < 0.05$).

100 cm trimming height (Table 2 and Figure 1B). Despite a cluster thinning adjusted to the cluster number and weight, the average yield at harvest was 0.2 kg/m² smaller for a 150 cm trimming height in comparison with the treatments with a 100 cm trimming height (Table 2). Increasing the trimming height from 100 cm to 150 cm increased the exposed leaf area by 0.2 m² (Table 2). The leaf-to-fruit ratio increased from 1.9 in the Pref-100 treatment to 2.3 m²/kg in the Pref-150 treatment and from 1.1 in the Flow-100 treatment to 1.4 m²/kg in the Flow-150 treatment (data not shown); all treatments

remained above the minimum threshold recommended in Switzerland to guarantee an optimum fruit ripening (Murisier & Zufferey, 2006). LR timing did not affect the winter pruning weight (average 47 g/m), whereas a higher trimming height increased the average pruning weight by 10 % in comparison with the vines trimmed at 100 cm (Table 2).

2. Must composition

The average TSS concentration in the must at harvest was 23.6 °Brix. It was slightly increased by a higher trimming height (+0.3 °Brix), whereas it was not affected by LR timing (Table 2). The average TA was 11.0 g tartrate/L and was lower in the Flow treatments in comparison with the Pref treatments (–0.3 g tartrate/L; –3 %): it reduced both tartrate (–0.3 g/L) and malate (–0.2 g/L) (Table 2). On the other hand, a higher canopy trimming height did not affect TA, but only decreased tartrate concentration (–0.3 g/L; p-value < 0.001) (Table 2). The average YAN concentration was 253 mg N/L. Flow treatments reduced the YAN concentration in the must in comparison with the Pref treatments (–23 mg N/L; –9 %): both ammonium and amino acid concentrations were reduced (–12 mg/L and –6 mg N/L, respectively) (Table 2). LR timing influenced the effect of canopy height on the YAN (interaction p-value < 0.01), but variations were rather small: YAN concentration tended to be lower in Pref-150 treatment than in Pref-100, while the opposite tendency was observed between the treatments Flow-150 and Flow-100 (Figure 3A). Cys-3MH concentration in the musts exhibited a great variability from year to year (average 18 ± 8 µg/L, varying from 10 to 33 µg/L in 2016 and 2021, respectively) (Figure 3B). Despite this variability, the Cys-3MH concentration of the Pref treatments was lower for five of the six years of the trial (2016, 2017, 2018, 2019, and 2021; Figure 4A), and the six-year average was significantly lower in comparison with the Flow treatments (–6 %, $p < 0.0001$; Table 2). Increasing the canopy height improved the concentration of Cys-3MH in the must of defoliated vines (+18 %; $p < 0.0001$), (Table 2 and Figure 4B). LR timing influenced the effect of canopy height on Cys-3MH accumulation in the grape (interaction p-value < 0.01): increasing canopy height from 100 to 150 cm increased Cys-3MH concentration in the must by 13 % on average in Flow treatments only, while the gain was only 3 % in Pref treatments. The positive effect of the combination of both LR at the flowering stage and higher canopy (Flow-150) on Cys-3MH accumulation was particularly strong in the years 2017, 2019 and 2021, *i.e.*, +47 % over the three years in the Flow-150 treatment compared to the Flow-100 treatment (Figure 3B).

3. Wine composition and tasting

The wines from the Flow treatments had a higher Folin index, which was 6.8 on average (+3 %; p-value < 0.05) in comparison with the Pref treatments while trimming height had no effect (Table 2). The higher Folin index due to the Flow treatment affected the wine colour giving a yellower tone to the wine (higher colour b, CIELab) in comparison with the Pref treatments (Table 2). The effects of LR timing and trimming height on the wine colour and bouquet were small but significant (Table 2): the wine from the Flow treatments had a higher colour intensity, a higher volume in the mouth and a better overall impression of the wine (hedonistic criteria) when compared to the Pref treatments. Increasing the canopy height from 100 to 150 cm also increased the colour intensity, the volume in the mouth and the overall impression (Table 2).

DISCUSSION

The discussion is organised in two parts, with the effects of LR timing (Pref or Flow) and canopy height (100 or 150 cm) considered separately.

1. The benefits and risks of pre-flowering LR

Removing six basal leaves per shoot at the pre-flowering stage significantly limited carbohydrate supply to developing inflorescences, which reduced fruit set and altered cluster morphology (*i.e.*, berry number, skin thickness, composition) (Harner *et al.,* 2024). The yield was reduced by 36 % on average compared to LR at the flowering stage. Consequently, laborious cluster-thinning work to respect the production quotas was significantly reduced in comparison with that required for vines defoliated at the flowering stage and later (–62 %, Table 2), saving time and money. There was no bunch rot attack during the period of this trial, so we could not confirm the strong efficiency of pre-flowering LR against fungal attacks (–60 %) as summarised by VanderWeide *et al.* (2021) in their review. Delaying LR from pre-flowering to the flowering stage had no impact on the exposed leaf area or the vigour of the plant. However, Bennett *et al* (2005) showed that the earlier LR occurs (*i.e.*, starting four weeks after flowering in their trial), the greater the reduction in carbohydrate reserves in the wood and roots, which may explain the lower bud fertility observed in the Pref treatments compared to the Flow treatments.

Regarding grape composition at harvest, the differences between treatments were relatively small and meaningless in terms of wine composition despite some statistical differences. Pref treatments had no effect on TSS concentration compared to the Flow treatments, but they induced a higher acidity and higher YAN concentration. Increasing the acidity of the must without affecting the TSS content could be of interest to winemakers, knowing that the current global warming induces lower acidity. Increasing the concentration of YAN is also beneficial for wine quality, limiting the risk of sluggish or stuck fermentations and increasing the source of amino acids, which are precursors to aromatic molecules such as higher alcohols and esters (Bell & Henschke, 2005). In the context of this trial, the concentrations of YAN in the musts were close to or above 200 mg N/L, which is sufficient and non-restrictive for fermentation (Bell & Henschke, 2005). However, pre-flowering LR had a negative impact on the Cys-3MH concentration in the must compared to LR at the flowering stage, potentially reducing the accumulation of thiols in the wine (Figure 4). On the contrary, the treatment Flow-150 seemed very beneficial to the accumulation of Cys-3MH in

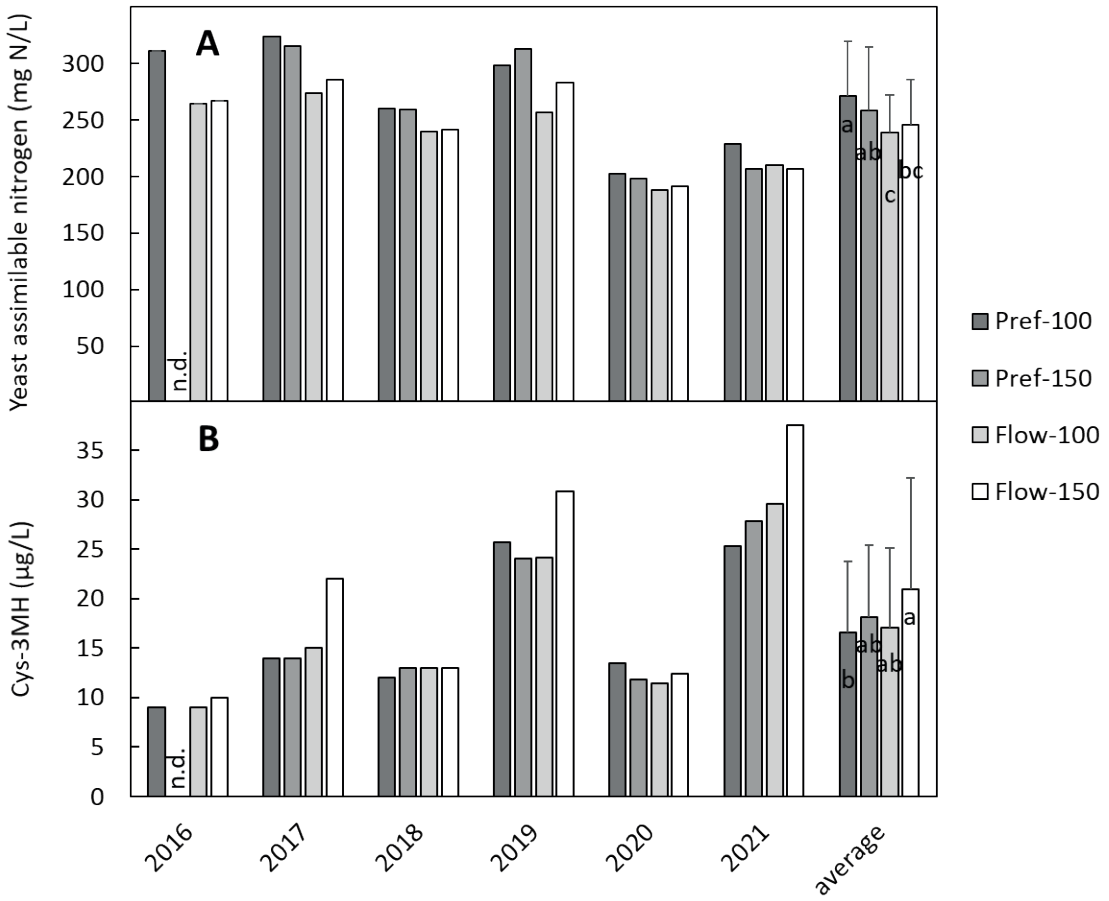

FIGURE 3. Concentrations of yeast assimilable nitrogen (A) and aromatic precursor Cys-3MH in the must at harvest (B) as a function of the year. Error bars are standard deviations. Numbers followed by different letters are significantly different (Tukey's test, $p < 0.05$). n.d. = non-determined.

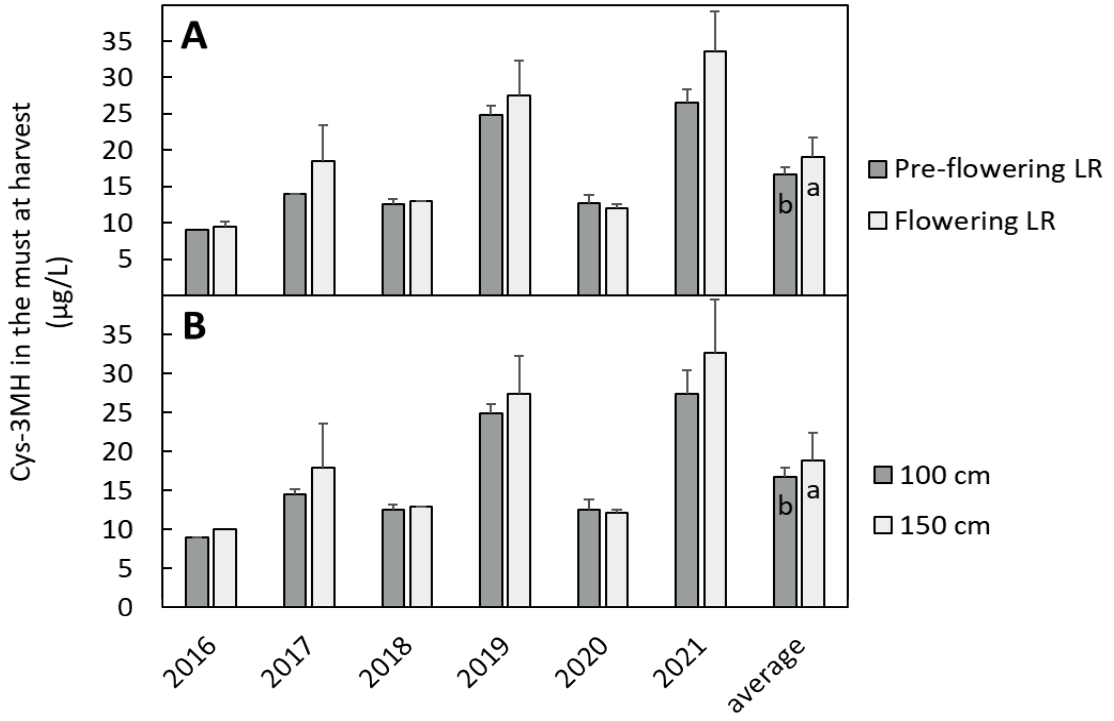

FIGURE 4. Concentration of aroma precursor Cys-3MH in the must at harvest per year, as a function of leaf removal timing (A) and canopy height (B). Error bars are standard deviations. Numbers followed by different letters are significantly different (Tukey's test, $p < 0.05$).

the must, compared to the three other treatments (Figure 3B). This could be explained by both the lower constraint of a later LR at the flowering stage and a higher photosynthetic activity due to the larger canopy. Šuklje *et al.* (2014) demonstrated for Sauvignon blanc that the chemical composition and sensory attributes of the wine can be significantly altered by changes in the fruit microclimate, resulting from the combined effects of light quantity (leaf removal) and light quality (UV radiation). In the course of the tasting, a few criteria were discriminating, but not consistently over the years: the treatment Pref-100 was depreciated in 2016 (lower global nose impression), in 2017 (lower general overall impression) and 2018 (smaller volume) (results not shown).

In terms of risks, potential excessive yield loss should be considered, especially if not offset by relevant improvements in fruit chemistry and/or reductions in rot (Harner *et al.,* 2024). Swiss climatic conditions at flowering are unpredictable and can strongly influence fruit set and the impact of LR on it. This trial pointed out the strong relationship between the climatic conditions of the year and the impact of pre-flowering LR and confirmed the results of a two-year trial on Cabernet franc (Frioni *et al.,* 2017). The interaction year*LR timing was indeed significant for the yield parameters (*i.e.*, estimated yield, cluster thinning, cluster weight and berry weight) and a few parameters in the must composition (*i.e.*, acids and N compounds). This strong influence of the year on the effect of pre-flowering LR is in contradiction with the results of Poni *et al.* (2006) who observed very few interactions on the cultivar Trebbiano. As an example in the present trial, the 2016 weather conditions during flowering were exceptionally cold and cloudy: during the 10 days before flowering, the temperature was 2.7 °C below the 30-year norm of 19.1 °C, and the sunlight was 2.0 % below the norm of 21.6 Mj/m^2. These exceptionally bad weather conditions and the lack of resources for berry-set due to a low photosynthesis activity could explain the extremely low yield potential in 2016, particularly with respect to the Pref treatments which resulted in an 81 % yield loss compared to the Flow treatments, due to a lower berry number per cluster and entire cluster necrosis (Figure 1A). For the other years, the average 35 % yield loss in the Pref treatments was relatively constant and confirmed the results from other studies (Frioni *et al.,* 2019; Verdenal *et al.,* 2019).

2. Compensating LR by increasing canopy height

Compensating for leaves removed from the cluster area with a higher canopy has been proposed to avoid excessive yield loss by maintaining a sufficient leaf area. The compensation was basically equivalent to a "trade-off" between the adult leaves + lateral shoots from the cluster area and the young leaves from the upper part of the canopy. However, this did not produce the expected results. Increasing canopy height decreased the cluster weight independently from LR timing (Figure 2). This observation could be explained by the competition between vegetative and reproductive developments: the first trimming was applied at the flowering stage (BBCH 65) in the 100 cm treatments and 10 days later in the 150 cm treatments. Growing a taller canopy required trimming the vines later and therefore keeping the apexes active longer during the "berry-set" stage (BBCH 71), as they are powerful nutrient sinks. Frioni *et al.* (2018) demonstrated that the reduction of carbon assimilation per shoot was linearly correlated with the priority that shoot apex gained in terms of sink destination. Functionally, Poni and Intrieri (2001) showed that from bud burst to fruit set, the photosynthetic activity of the whole canopy suffers from the fact that a significant part of the leaf area is 'young' and therefore unable to reach maximum photosynthetic capacity. Maximum photosynthetic activity is reached at about full leaf expansion (35–40 days old) and declines thereafter, but leaves older than four months maintain 70 % of their maximum assimilation rate (Intrieri *et al.,* 1992). Other authors (Palliotti *et al.,* 2012; Poni *et al.,* 2006; Tardaguila *et al.,* 2010) suggested that pre-flowering LR probably also stimulated lateral shoot growth, but this was not observed in this experiment and exposed leaf area was not affected by pre-flowering LR compared to the Flow treatments. The differences in yield between treatments could be partially compensated by cluster thinning.

Compensating for leaves removed from the cluster area with a higher canopy increased the exposed leaf area (+0.2 m^2/m^2 of soil) and enhanced grape ripening (*i.e.*, higher concentration of TSS and lower concentration of tartaric and malic acids). The leaf-to-fruit ratios of all the treatments were above the recommended minimum threshold of 1.0–1.2 m^2/kg to ensure proper fruit ripening (Kliewer & Dokoozlian, 2005; Murisier & Zufferey, 2006). Therefore, the gains in terms of must composition remained small: over a six-year average, increasing canopy height enhanced TSS accumulation by only 0.3 °Brix (+1 %) and decreased the concentration of both tartaric acid and malic acid, which represents a rather small effect compared to other trials, as summarised by VanderWeide *et al.* (2021). Empirically, we know that Petite Arvine needs a greater leaf-to-fruit ratio, that is, 1.2–1.5 m^2/kg, to fully ripen its grapes under Swiss climatic conditions, which reveals variability between varieties. In terms of wine tasting, the better quote of volume was probably related to the higher °Brix and higher pH. This could be explained by the larger canopy which allowed a higher total photosynthetic activity.

Increasing canopy height did not significantly affect bud fruitfulness or leaf N content but it enhanced the pruning wood weight: this potentially increased the nutritional reserves of the plant, which could bring a better resilience to the variability of the climatic conditions in the long term.

Finally, the combination of both LR at the flowering stage and higher canopy (treatment Flow-150) appeared as an interesting compromise between LR timing and canopy height, mitigating the yield loss, promoting Cys-3MH accumulation in the fruits and slightly enhancing wine composition.

CONCLUSIONS

Pre-flowering LR reduced yield at harvest by reducing the number of berries per cluster. The yield loss could be mitigated by applying LR later during the flowering stage.

Thus, adjusting the timing of LR within the period from the 'separated flower buds' stage to the 'flowering' stage allowed mitigation of yield loss. Compared to the pre-flowering stage, LR at the flowering stage reduced acidity and YAN concentrations increased the concentration of the aroma precursor Cys-3MH in the must at harvest and slightly improved the overall wine quality of the white variety Petite Arvine. The unpredictable climatic conditions just before flowering played an important role in fruit formation, with cold conditions and low light levels having a negative effect on fruit set, thus increasing the impact of pre-flowering LR and the risk of excessive yield loss. LR at flowering, combined with a higher canopy, limited yield loss and was beneficial for the accumulation of the aroma precursor cys-3MH in the must. Further research is required to promote the physiological mechanisms involved in the formation of aroma precursors in the fruit.

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