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ORIGINAL RESEARCH ARTICLE

Mechanisation of pre-flowering leaf removal under the temperate climate conditions of Switzerland

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ABSTRACT

The present trial follows a previous study about pre-flowering leaf removal (LR) (Verdenal *et al.*, 2019) and validates the sustainability of mechanical pre-flowering LR under local Swiss vineyard conditions, using a low-pressure double airflow, to reduce the cost of laborious bunch thinning. In previous studies, pre-flowering LR has shown additional benefits over post-berry-set LR in terms of yield regulation and grape and wine compositions. This trial had two objectives: 1) to test the technical feasibility of mechanical pre-flowering LR, using a low-pressure double airflow and 2) to observe the impact of this practice over five years on yield parameters, grape composition at harvest and wine quality over five years. For this purpose, a trial was conducted on the two cultivars, Doral (white) and Gamay (red), to compare four LR treatments, that is, A) mechanical post-berry-set LR, B) manual pre-flowering LR, C) mechanical pre-flowering LR, and D) double mechanical pre-flowering + post-berry-set LR. More broadly, this work provides practical insights into the consequences of pre-flowering LR on the grapevine, pointing out the advantages and the limits of intensity, timing and mechanisation of this practice. In comparison with the mechanical post-berry-set LR, mechanical pre-flowering LR induced a 7 % loss in bud fruitfulness, a 30 % yield loss and a 3 % gain in total soluble solids (TSS) accumulation in grapes in both cultivars, although the improvement in grape maturity was small and uneven through the years; Gamay anthocyanin concentration remained stable. Pre-flowering LR had no overall impact on the quality of Doral and Gamay wines. Damage was observed on the inflorescences due to the intensity of mechanical pre-flowering LR, which resulted in fewer berries per cluster and a lower yield than with manual LR by the same date. A second LR after berry set was also tested to limit the growth of laterals and clean the clusters from the remaining flower caps to prevent the development of fungal diseases, but it did not show any benefit over a single pre-flowering LR. In conclusion of this trial, a single, moderate mechanical pre-flowering LR is an effective and sustainable practice under temperate climatic conditions, to reduce the costs of laborious manual pre-flowering LR.

KEYWORDS: grapevine, defoliation, pre-flowering stage, mechanisation, wine quality

INTRODUCTION

Grapevine leaf removal (LR) in the cluster area is a common practice under temperate and cool climates, usually done between berry set and cluster closure to create a less favourable microclimate for fungal diseases, such as *Botrytis cinerea* and powdery mildew. Grape growers are now turning their attention to pre-flowering LR, which gives additional benefits under certain conditions. A meta-analysis summarises the extensive literature on this practice, with specific regard to pre-flowering LR (VanderWeide *et al.,* 2021). When applied before flowering, LR strongly affects the berry set and, thus, the number of berries per cluster. It is, therefore, an effective yield-control tool, replacing time-consuming manual cluster thinning (Poni *et al.*, 2005; VanderWeide *et al.*, 2020). It also improves berry structure, that is, skin thickness, skin-to-pulp ratio, and berry composition, compared to non-defoliated grapevines (i.e., total soluble solids [TSS], titratable acidity [TA] and polyphenols) (Komm and Moyer, 2015; Palliotti *et al.*, 2012; Verdenal *et al.*, 2017). By exacerbating competition for assimilates between reproductive and vegetative organs, pre-flowering LR also poses some risks. Excessive yield loss at harvest due to too low of a berry-set rate is the main concern: intensive pre-flowering LR (100 % of the cluster area) can induce up to 50 % yield loss in potted vines (Poni *et al.*, 2005). Other parameters, such as cool climatic conditions during flowering, also affect the berry set and make it difficult to predict potential yield at harvest. Repeating an overly intensive pre-flowering LR can also have repercussions over the years and result in a decline in bud fruiting and plant vigour (Nicolosi *et al.*, 2021; Risco *et al.*, 2014).

The effects of the timing and intensity of LR were experimented on five cultivars (Pinot noir, Merlot, Gamay, Chasselas and Doral) over six years under temperate Swiss climatic conditions and yielded insightful results (Verdenal *et al.*, 2019). In summary, an intensive pre-flowering LR (removal of six basal and lateral leaves) confirmed its huge impact on the agronomic performance of the vine, mainly at the expense of the berry set: the yield was strongly affected, i.e., about -35 % of that of the non-defoliated control treatments. This yield loss was proportional to the initial yield potential of the non-defoliated treatment, which depended on genetics. The intensity of LR modulated its impact on yield (Verdenal *et al.*, 2018). Pre-flowering LR also had a positive impact against millerandage, sunburn symptoms and *Botrytis cinerea* development. In terms of berry structure and composition, skin thickness doubled and polyphenol concentration increased significantly (Verdenal *et al.*, 2019). Due to pre-flowering LR, red wines were often preferred for their colour and mouthfeel. However, this practice had a negligible impact on the composition of white wines. Pre-flowering LR had no negative impact on wine parameters in the context of this trial (Verdenal *et al.*, 2019).

Pre-flowering LR is a prophylactic solution that potentially reduces both chemical applications and cluster thinning costs (VanderWeide *et al.*, 2020). However, the considerable time required for its manual implementation limits its popularity among wine growers. Knowing that mechanical LR is delicate before flowering, as shoots and inflorescences are fragile, the choice of the method is essential for achieving optimal results. Mechanical pre-flowering LR by bilateral rotary suction has been tested before flowering and showed interesting results in terms of yield control and grape quality, despite damages due to involuntary shoot/inflorescence trimming (Filippetti *et al.,* 2011). In their trial on the cv. Sangiovese, Intrieri *et al.* (2016) mentioned a high level of damages with this machine, that is, about 10 % loss in shoot number and 17 % loss in visible inflorescence number, thus, considerably lowering the potential yield at harvest. Conversely, LR by low-pressure dual airflow (E 3000 3P, 2003; Collard, Bouzy, France) seems more delicate and appropriate for pre-flowering LR, although it still induces little damage to the inflorescences (VanderWeide *et al.*, 2020).

This article presents the results of a trial conducted in Switzerland over five years on two cultivars to test mechanical pre-flowering LR. This trial had two objectives: 1) to test the technical feasibility of mechanical pre-flowering LR, using a low-pressure double airflow; 2) to observe the impact of this practice over five years on the yield parameters, on the grape composition at harvest and the wine quality over five years. The interest of a second LR after the berry set was tested to limit the growth of laterals and clean the clusters from the remaining flower caps to prevent the development of fungal diseases. More broadly, this work provides practical insights into the consequences of grapevine LR, pointing out the advantages and the limits of intensity, timing and mechanisation of this practice.

MATERIALS AND METHODS

1. Vineyard site and experimental design

The trial was conducted from 2016 to 2020 in the experimental vineyards of Agroscope in Changins, Switzerland (46°23’52.4”N 6°13’48.7”E). Two field-grown *Vitis vinifera* L. cultivars (i.e., Doral and Gamay) were planted in two separate homogeneous plots. The vines were grafted onto rootstock 3309C, planted at a density of 5880 vines/ha and pruned in a single guyot system. The canopy was trimmed to 110 cm in height.

Each trial was structured as a complete randomised block design including four homogeneous blocks (replicates) with four treatments: A) common mechanical post-berry-set LR, B) manual pre-flowering LR, C) mechanical pre-flowering LR and D) double mechanical LR, pre-flowering and post-berry-set. The interest of double mechanical LR was to limit the growth of lateral shoots and blow the flower caps from the cluster before cluster closure to prevent the development of fungal diseases. Pre-flowering LR was done on the same day (31 May, five-year average), between the phenological stages ‘separated floral buds’ (BBCH 57; Lancashire *et al.*, 1991) and ‘full bloom’ (BBCH 65), as soon as the shoots were trained in the trellising; post-berry-set LR was done between ‘berry-set’ (BBCH 71) and ‘pea-size’ (BBCH 75) (23 June, on average).

Manual LR of the cluster area consisted of removing by hand the first six leaves from the base of each shoot, including laterals. Mechanical LR of the equivalent area consisted of using a tractor-mounted compressed-air leaf remover (E 3000 3P, 2003; Collard, Bouzy, France), with different settings for pre-flowering and post-berry-set treatments as described in Table 1. Tractor speed was lower for pre-flowering LR due to the smaller leaf area at that early stage. The rapid air pulses shook and removed the leaves from the foliage around the clusters. Replicates contained 11 plants each for Doral and 20 plants each for Gamay.

TABLE 1. Average date of leaf removal (LR) and settings of the mechanical leaf remover (low-pressure double air flow) for both cultivars Doral and Gamay. 2016–2020, Changins, Switzerland.

Treatment	Pre-flowering LR	Post berry-set LR
Average LR date	31 May	23 June
Air pressure (bar)	0.8	0.9
Tractor speed (km/h)	0.6	2.0

2. Field measurement

The field measurements were performed per replicate (i.e., four times per treatment), except for the leaf mineral composition, which was assessed once per treatment. Phenological differences between treatments were assessed on Gamay at veraison, by estimating the percentage of coloured berries on 25 clusters per replicate at one date during veraison. Bud fruitfulness was estimated (average number of clusters per shoot). The potential yield (Yield$_{estim}$) was estimated in July (before cluster thinning) from a sample of 50 berries and 10 clusters per replicate using the following formula:

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

Berry wt$_{July}$ and cluster wt$_{July}$ are the average berry and cluster weights in July at the cluster closure stage (physiological stage BBCH 75–77), respectively; berry wt$_{harv}$ is the 10-year average berry weight at harvest (BBCH 89) for each cultivar; cluster nb$_{vine}$ is the number of clusters per vine. The weights are expressed in grams and the yield is in kg/m^2. Cluster thinning was performed per treatment each year prior to phenological cluster closure, based on Yield$_{estim}$, to produce 10 t/ha at harvest in accordance with the regional practice. The objective of cluster thinning was to meet production quotas and remain under real production conditions to answer a relevant industry question. The average berry weight was assessed from 200 berries collected one week before harvest. The cluster weight was estimated from the yield per vine divided by the average cluster number previously assessed. Pruning weight, an indicator of plant vigour, was assessed during winter by removing 10 shoots from the second-to-last position on the cane; the shoots are then equalized to one meter in length and weighed; pruning weight is expressed in grams per meter (g/m).

3. Leaf and grape analyses

Leaf mineral composition (N, P, K, Ca and Mg) was assessed at veraison from a sample of 25 entire leaves per treatment and analysed by an external laboratory, Sol-Conseil (Gland, Switzerland).

Must samples were collected per replicate at harvest during crushing. The general must parameters were determined using an infrared spectrophotometer (FOSS WineScan^{TM}, Hillerød, Denmark), i.e., (TSS, °Brix), TA (g/L as tartrate), tartaric and malic acids (g/L) and pH. Further analyses were performed per treatment (i.e., no replicate) on grape extracts: from 2017 to 2020, a sample of berries with pedicels per treatment was collected the week before harvest (i.e., 200 berries per treatment for Doral and 300 for Gamay). The berry samples were divided into several aliquots for further analyses, all detailed in Verdenal *et al.* (2017) and described as follows. One aliquot was used for the determination of the 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; an enzymatic method was used for ammonium (Methods of Biochemical Analysis and Food Analysis, Boehringer Mannheim GmbH, 1997), and a spectrophotometric method with a dedicated kit was used for free primary amino acids (Primary Amino Nitrogen; from BioSystems, Barcelona, Spain). The yeast assimilable nitrogen (YAN) was calculated as the sum of nitrogen (mg N/L) in the form of ammonium and free primary amino acid. Another aliquot was used to determine the total glutathione concentration using a liquid chromatography-mass spectrometer (LC-MS/MS, Agilent Technologie, Santa Clara, CA, U.S. A.) as per the method published by Dienes-Nagy *et al.* (2022). Exclusively for Gamay, a final aliquot allowed for assessing the total free anthocyanins and the anthocyanin profile, as detailed in Verdenal *et al.* (2017). Anthocyanins were detected at 520 nm, and the profile was expressed in percentage of the total peak area. The results were expressed in mg of malvidin-3-*O*-glucoside per litre. The acetylated forms and the coumaroylated forms of anthocyanins were given as groups.

4. Wine analyses and tasting

For each cultivar, the harvest date was determined as a function of the TSS concentration. The grapes were harvested each year per replicate in one day, and the yield was assessed. The four replicates of each treatment were then gathered and approximately 50 kg of grapes were vinified per treatment following the standard Agroscope protocol, as detailed by Verdenal *et al.* (2019). Finished wines were analysed using an infrared spectrophotometer (FOSS WineScan^{TM}, Hillerød, Denmark) for the following parameters: alcohol, dry extract, pH, volatile acid, titratable acidity, tartaric, malic and lactic acids, glycerol, proline and succinic acid. The total free anthocyanins and the anthocyanin profile were assessed in Gamay wines, as previously described for the grape extracts.

Folin Index and total glutathione were assessed as previously described in the must. 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 completed every year in a dedicated tasting room; the trained Agroscope panel (12 permanent members) described the wines according to the following pre-defined criteria using a 7-point scale. All the wines were tasted again in 2021 using the same method to evaluate ageing potential. The sensory data were analysed with the FIZZ program (Biosystems©, Courtenon, France).

5. Data treatment

The data were described statistically using XLSTAT (Addinsoft©, Paris, France). The analyses were conducted for each cultivar separately, as two distinct, randomised, complete block designs, considering leaf-removal treatment (four levels) as a fixed factor, and year (five levels) and replicate (four levels) as random factors. The description and significance of differences among treatments were evaluated using analysis of variance as follows: for data with replicates, we applied a three-way ANOVA considering year, replicate, LR treatment and year*treatment interaction. For data without replicates, we applied a two-way ANOVA considering the only year and LR treatment as the two factors of variability. Tukey's post hoc test was used for multiple comparisons.

RESULTS

The results for both cultivars are summarised as a function of LR treatments in Table 2 (vineyard observations and must composition) and Table 3 (wine analysis and tasting data). The results are also summarised as a function of the year in Supplementary Table S1 (vineyard observations and must composition) and Table S2 (wine analysis and tasting data).

1. Vegetative development and yield parameters

The five-year average of bud fruitfulness was slightly lower for the two pre-flowering mechanical treatments (i.e., C and D) for both cultivars (1.8 and 2.1 clusters per shoot, respectively, for Doral and Gamay). Doral bud fruitfulness was globally lower in 2020 (1.6 clusters per shoot), while it remained stable for Gamay (Table 2 and Supplementary Figure S1). Leaf mineral composition did not vary among LR treatments, except for calcium in Gamay, which was slightly lower (-0.1 % d.m.) in the pre-flowering mechanical LR. Variations in exposed leaf area between treatments were small, all treatments had approximately 1.0 m^2 of leaf area per m^2 of soil. The average winter pruning weights were 53 and 43 g/m for Doral and Gamay, respectively, without differences among the treatments (Table 2). The pruning weight of Doral was smaller for all the treatments in the two last years of the trial (i.e., 2019 and 2020), while it remained unchanged for Gamay. Early estimated yield also showed a decrease in all treatments in 2019 and 2020 for Doral (i.e., 0.6 kg/m^2, compared with an average of 1.4 kg/m^2 in 2016–2018), but not for Gamay (Figure 2). The estimated yield was strongly influenced by LR for both cultivars, with the lowest estimates in the two intensive mechanical LR treatments before flowering (average of 0.9 and 1.3 kg/m^2 for Doral and Gamay, respectively) (Table 2); mechanical LR after fruit set had the highest estimate (average of 1.5 and 1.9 kg/m^2); and the manual LR estimate was intermediate (1.1 and 1.6 kg/m^2) (Figure 2). This variation among treatments was due to the number of berries per cluster (i.e., -27 % and -21 % for the pre-flowering mechanical LR of Doral and Gamay, respectively, compared with the post-berry set LR) (Figure 1), and consequently to the cluster weight (-25 % and -18 %, respectively) (Table 2). In both cultivars, the average yield at harvest varied only from 0.8 to 1.0 kg/m^2, due to homogenisation by cluster thinning. A year*treatment interaction was observed on most yield parameters, i.e., the number of berries per cluster, cluster weight (Gamay only), early estimated yield (Doral only), cluster thinning and yield at harvest. All the yield parameters are correlated to the berry-set rate. The number of berries per cluster, per year and per treatment, is detailed in Figure 1 to illustrate the variation of the yield parameters over the years: the yield loss was usually bigger in the years with higher yield estimation.

2. Must composition at harvest

Post-berry-set LR treatment had the lowest TSS concentration at harvest for both cultivars (i.e., 22.8 and 23.5 °Brix, for Doral and Gamay, respectively), although the extent of variability between LR treatments was relatively small (less than 1.0 °Brix) and primarily related to the year and the cultivar (Figure 1). This treatment also had the highest TA among the treatments (i.e., 8.3 and 10.2 g tartrate/L, respectively), mostly related to more tartaric acid (8.3 and 8.9 g/L, respectively) (Table 2). A year*treatment interaction was observed on grape maturity at harvest (i.e., TSS and acidity), although the range of variation did not induce any significant difference in wine composition and tasting (Table 2). The variation per year and per treatment of the TSS is detailed in Figure 1. The post-berry-set LR treatment had the lower pH only in Gamay. Grape nitrogen concentration was influenced by cultivar: for Doral, YAN concentration was lower in the two pre-flowering (i.e., both manual and mechanical) treatments compared with the double mechanical LR treatment. Conversely, the YAN concentration in Gamay tended ($p < 0.10$) to be higher in the mechanical pre-flowering LR treatment, due to a higher concentration of amino nitrogen. The Folin Index tended ($p < 0.10$) to be higher in the double mechanical LR treatment for both cultivars. Glutathione concentration was slightly higher for Doral in the manual LR treatment (50.3 mg/L versus an average of 47.8 mg/L for the other treatments). A similar trend was observed for Gamay (25.7 mg/L versus an average of 19.4 mg/L for the other treatments, $p < 0.10$) (Table 2). The total anthocyanin concentration as a function of LR treatments was unchanged in Gamay grapes (mean 629 mg/L), whereas the proportions of anthocyanins varied as follows: the double mechanical LR had the highest proportions of delphinidol-3-glucoside, cyanidol-3-glucoside and petunidol-3-glucoside, while the pre-flowering mechanical LR had the highest proportion of malvidol-3-glucoside.

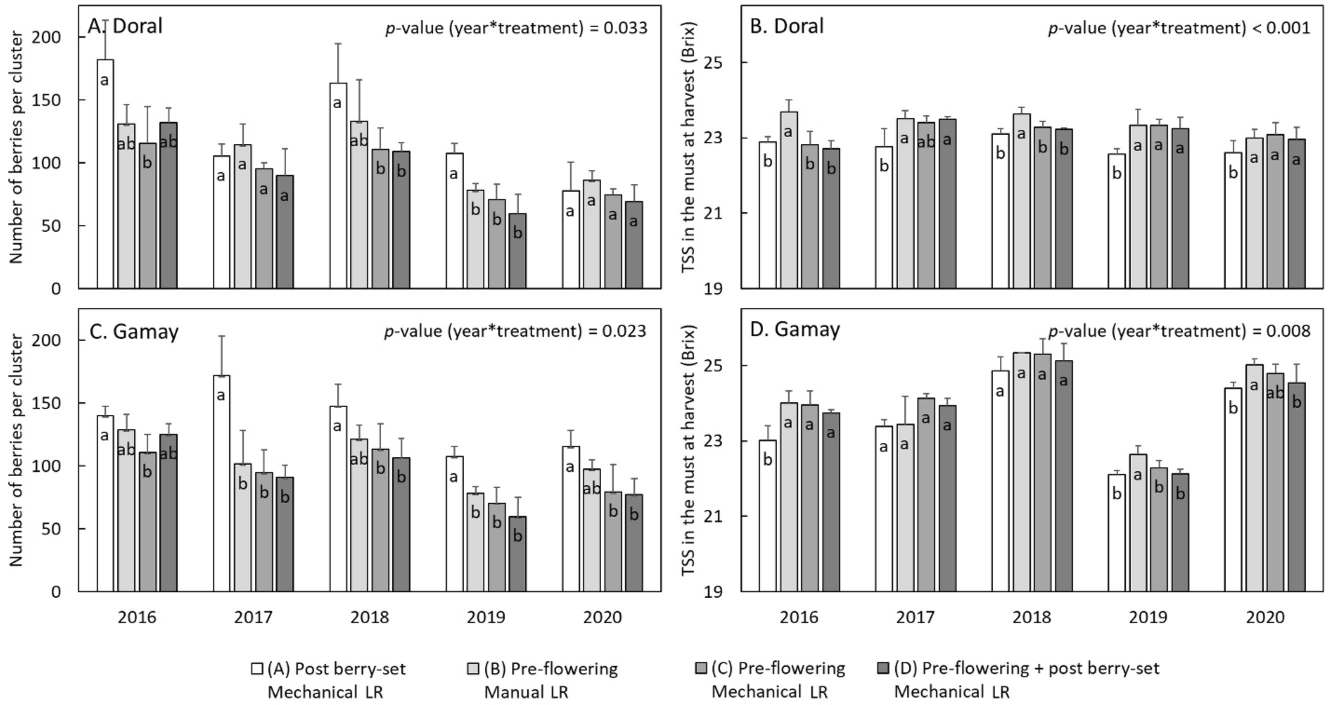

FIGURE 1. Number of berries per cluster and total soluble sugars in the must at harvest (TSS) per year and per treatment for Doral (A, B) and Gamay (C, D). Changins, Switzerland. Treatments with different letters are statistically different in a given year (Tukey's test, $p < 0.05$). Error bars represent standard deviation.

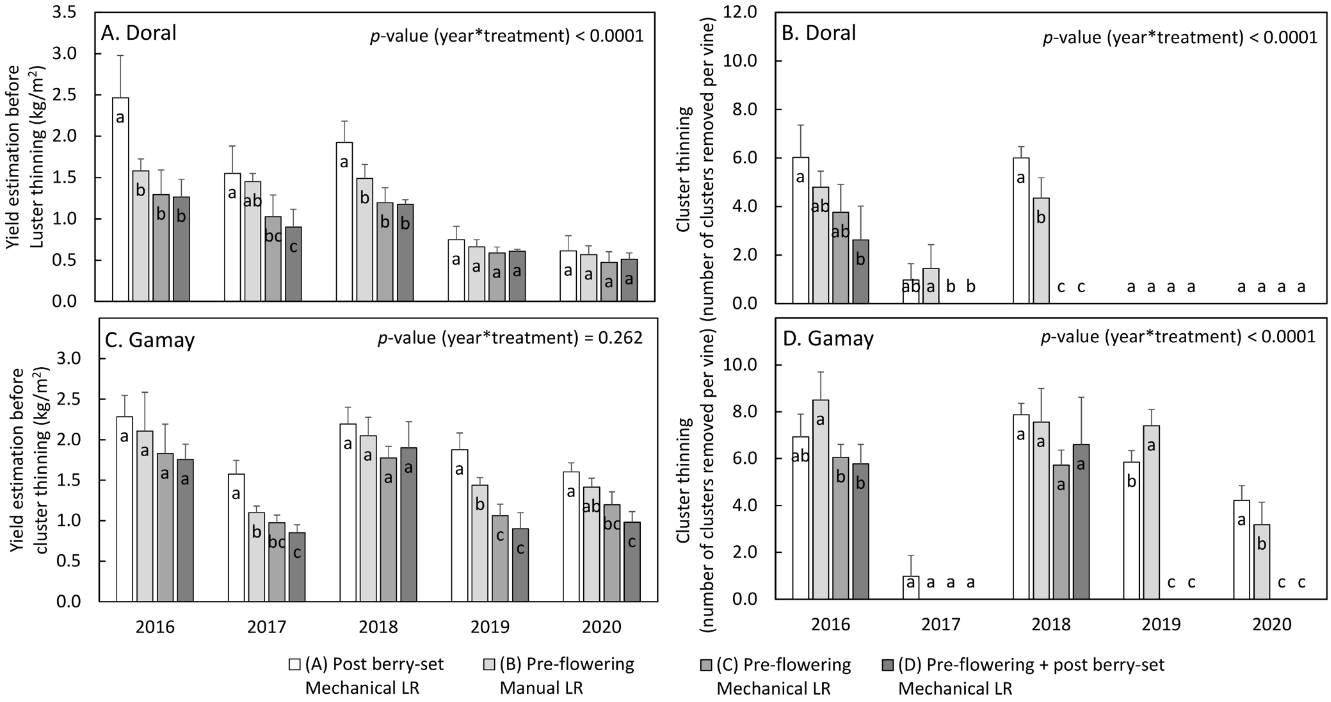

FIGURE 2. Yield estimation and cluster thinning per year and per treatment for Doral (A, B) and Gamay (C, D). Changins, Switzerland. The objective of cluster thinning was to produce 1.0 kg/m^2 (10 T/ha). Treatments with different letters are statistically different in a given year (Tukey's test, $p < 0.05$). Error bars represent standard deviation.

TABLE 2. Vineyard observations and must composition on Doral and Gamay as a function of LR treatment. Average data 2016–2020, Changins, Switzerland (part 1/2).

	Doral					
	(A) Post berry-set	(B) Pre-flowering	(C) Pre-flowering	(D) Pre-flowering + post berry-set	*p*-value	Interaction
	Mechanical	Manual	Mechanical	Mechanical		Year*Treatment
Vineyard observations						
Pruning weight (g/m)	54	52	52	53	n.s.	n.s.
Bud fruitfulness (clusters per shoot)	1.9 a	2.0 a	1.8 b	1.8 b	***	•
Veraison (% red berries at a chosen date)	-	-	-	-	-	-
Chlorophyll index (N-tester at veraison)	546	543	531	544	n.s.	n.s.
Leaf nitrogen (% dry mass)	2.26	2.36	2.24	2.37	•	–
Leaf phosphorus (% dry mass)	0.21	0.21	0.22	0.21	n.s.	–
Leaf potassium (% dry mass)	1.4	1.3	1.3	1.3	n.s.	–
Leaf calcium (% dry mass)	2.7	2.8	2.6	2.7	n.s.	–
Leaf magnesium (% dry mass)	0.3	0.3	0.3	0.3	n.s.	–
Light-exposed leaf area (m^2/m^2 of ground)	1.01	1.01	1.05	0.98	*	**
Leaf-to-fruit ratio (m^2/kg)	1.2	1.2	1.4	1.3	***	**
Early estimated yield (kg/m^2)	1.5	1.1	0.9	0.9	***	***
Cluster thinning (clusters removed per vine)	2.6	2.1	0.8	0.5	***	***
Number of berries per cluster	127	109	93	92	***	**
Berry weight at harvest (g)	1.6 ab	1.6 b	1.7 a	1.7 a	***	n.s.
Cluster weight at harvest (g)	165	144	123	125	***	***
Yield at harvest (kg/m^2)	1.0	0.9	0.8	0.8	***	***
Must composition at harvest						
TSS (Brix)	22.8	23.4	23.2	23.1	***	***
pH	3.09	3.10	3.09	3.10	n.s.	n.s.
TA (g tartrate/L)	8.3	8.1	8.2	8.3	***	**
Tartaric acid (g/L)	8.3	8.0	8.1	8.2	**	**
Malic acid (g/L)	2.2 ab	2.1 b	2.3 a	2.3 a	**	•
Ammonium (mg/L)	74 a	63 b	63 b	80 a	***	–
Alpha amino N (mg N/L)	115	122	117	131	n.s.	–
YAN (mg N/L)	176 ab	174 b	169 b	197 a	*	–
Folin Index	13.4	12.3	12.8	13.9	•	–
Total glutathione (mg/L)	46.2 b	50.3 a	47.5 ab	49.7 ab	*	–
Total anthocyanins (mg/L)	–	–	–	–	–	–
Delphinidol-3-glucoside (% total anthocyanins)	–	–	–	–	–	–
Cyanidol-3-glucoside (% total anthocyanins)	–	–	–	–	–	–
Petunidol-3-glucoside (% total anthocyanins)	–	–	–	–	–	–
Peonidol-3-glucoside (% total anthocyanins)	–	–	–	–	–	–
Malvidol-3-glucoside (% total anthocyanins)	–	–	–	–	–	–
Acetylated anthocyanins (% total anthocyanins)	–	–	–	–	–	–
Coumaroylated anthocyanins (% total anthocyanins)	–	–	–	–	–	–

Note: Numbers 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.

TABLE 2. Vineyard observations and must composition on Doral and Gamay as a function of LR treatment. Average data 2016–2020, Changins, Switzerland (part 2/2).

	Gamay					
	(A) Post berry-set	(B) Pre-flowering	(C) Pre-flowering	(D) Pre-flowering + post berry-set	*p*-value	Interaction
	Mechanical	Manual	Mechanical	Mechanical		Year×Treatment
Vineyard observations						
Pruning weight (g/m)	45	42	44	42	•	n.s.
Bud fruitfulness (clusters per shoot)	2.3 a	2.3 a	2.2 b	2.1 b	***	n.s.
Veraison (% red berries at a chosen date)	51 b	61 a	64 a	55 b	***	**
Chlorophyll index (N-tester at veraison)	565	558	557	554	n.s.	n.s.
Leaf nitrogen (% dry mass)	2.06	2.07	2.06	2.03	n.s.	–
Leaf phosphorus (% dry mass)	0.19	0.19	0.19	0.19	n.s.	–
Leaf potassium (% dry mass)	1.1	1.2	1.2	1.2	n.s.	–
Leaf calcium (% dry mass)	3.0 ab	3.1 a	2.9 b	3.1 ab	*	–
Leaf magnesium (% dry mass)	0.4	0.4	0.4	0.4	n.s.	–
Light-exposed leaf area (m^2/m^2 of ground)	1.02	0.96	1.05	0.99	***	**
Leaf-to-fruit ratio (m^2/kg)	1.2	1.4	1.4	1.3	**	***
Early estimated yield (kg/m^2)	1.9 a	1.6 b	1.4 c	1.3 c	***	n.s.
Cluster thinning (clusters removed per vine)	5.7	6.4	3.6	3.6	***	***
Number of berries per cluster	137	105	94	92	***	*
Berry weight at harvest (g)	2.1 a	2.0 b	2.1 a	2.1 a	***	n.s.
Cluster weight at harvest (g)	149 a	129 b	116 c	122 bc	***	n.s.
Yield at harvest (kg/m^2)	0.9	0.8	0.8	0.8	***	***
Must composition at harvest						
TSS (Brix)	23.5	24.1	24.1	23.9	***	**
pH	3.11 b	3.16 a	3.15 a	3.14 a	***	n.s.
TA (g tartrate/L)	10.2	9.6	10.1	10.1	***	*
Tartaric acid (g/L)	8.9 a	8.5 c	8.5 c	8.7 b	***	n.s.
Malic acid (g/L)	3.9	3.6	4.1	3.8	***	*
Ammonium (mg/L)	94 a	87 a	97 a	93 a	n.s.	–
Alpha amino N (mg N/L)	114 b	125 ab	133 a	116 b	**	–
YAN (mg N/L)	192	196	213	193	•	–
Folin Index	16.9	19.6	14.5	20.3	•	–
Total glutathione (mg/L)	19.9 ab	25.7 a	15.6 b	22.7 ab	*	–
Total anthocyanins (mg/L)	602	647	608	659	n.s.	–
Delphinidol-3-glucoside (% total anthocyanins)	5.7 b	6.5 ab	5.6 b	6.7 a	*	–
Cyanidol-3-glucoside (% total anthocyanins)	1.0 b	1.1 ab	1.0 b	1.2 a	*	–
Petunidol-3-glucoside (% total anthocyanins)	7.0 b	7.8 a	7.1 b	7.9 a	***	–
Peonidol-3-glucoside (% total anthocyanins)	12.8	12.5	12.4	13.3	•	–
Malvidol-3-glucoside (% total anthocyanins)	63.8 a	62.5 ab	64.0 a	61.8 b	*	–
Acetylated anthocyanins (% total anthocyanins)	3.1	3.9	4.0	3.0	n.s.	–
Coumaroylated anthocyanins (% total anthocyanins)	6.7 a	6.5 a	6.8 a	6.1 a	•	–

Note: Numbers 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.

TABLE 3. Wine analysis and tasting data on Doral and Gamay as a function of LR treatment. Average data 2016–2020, Changins, Switzerland (part 1/2).

	Doral				
	(A) Post berry-set Mechanical	(B) Pre-flowering Manual	(C) Pre-flowering Mechanical	(D) Pre-flowering + post berry-set Mechanical	*p*-value
Wine composition					
Alcohol (%vol.)	12.9	13.7	13.3	13.3	n.s.
pH	3.4	3.4	3.4	3.4	n.s.
TA (g tartrate/L)	5.5	5.3	5.3	5.2	n.s.
Tartaric acid (g/L)	2.2	2.0	2.0	2.0	n.s.
Lactic acid (g/L)	1.2	1.3	1.3	1.3	n.s.
Glycerol (g/L)	8.6	8.6	8.6	8.8	n.s.
Succinic acid (g/L)	1.0	1.0	0.9	1.0	n.s.
Proline (mg N/L)	81 b	85 ab	90 ab	93 a	*
Total glutathione (mg/L)	1.5	2.1	1.7	1.6	•
Folin Index	6.6	6.6	6.3	6.2	n.s.
Total anthocyanins (mg/L)	–	–	–	–	–
Delphinidol-3-glucoside (% total anthocyanins)	–	–	–	–	–
Cyanidol-3-glucoside (% total anthocyanins)	–	–	–	–	–
Petunidol-3-glucoside (% total anthocyanins)	–	–	–	–	–
Peonidol-3-glucoside (% total anthocyanins)	–	–	–	–	–
Malvidol-3-glucoside (% total anthocyanins)	–	–	–	–	–
Acetylated anthocyanins (% total anthocyanins)	–	–	–	–	–
Coumaroylated anthocyanins (% total anthocyanins)	–	–	–	–	–
Lightness L	99	98	98	98	n.s.
Colour a (red/green)	–1.6	–1.4	–1.5	–1.4	n.s.
Colour b (yellow/blue)	8.8	8.9	9.0	8.7	n.s.
Wine tasting (scores 1 to 7)					
Colour intensity	4.6	4.6	4.6	4.7	•
Fruitiness	4.4	4.5	4.3	4.5	n.s.
Floral	2.7	2.8	2.7	2.8	n.s.
Herbaceous	1.7	1.6	1.6	1.6	n.s.
Lactic	1.4	1.5	1.4	1.5	n.s.
Empyreumatic	1.1	1.2	1.1	1.1	n.s.
Global nose impression	4.2	4.3	4.2	4.3	n.s.
Volume	4.6	4.7	4.6	4.7	n.s.
Acidity	4.4	4.3	4.4	4.4	n.s.
Tannin intensity	–	–	–	–	–
Tannin quality	–	–	–	–	–
Bitterness	2.6	2.5	2.4	2.5	n.s.
General impression	4.1	4.2	4.1	4.2	n.s.

Numbers with different letters are statistically different (Tukey's test, $p < 0.05$). The wine tasting data are scores based on a predefined 1–7 scale. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; • $p < 0.10$; n.s., non-significant.

TABLE 3. Wine analysis and tasting data on Doral and Gamay as a function of LR treatment. Average data 2016–2020, Changins, Switzerland (part 2/2).

	Gamay				
	(A) Post berry-set	(B) Pre-flowering	(C) Pre-flowering	(D) Pre-flowering + post berry-set	*p*-value
	Mechanical	Manual	Mechanical	Mechanical	
Wine composition					
Alcohol (%vol.)	13.4 b	13.8 a	13.8 a	13.6 ab	**
pH	3.5	3.5	3.5	3.5	n.s.
TA (g tartrate/L)	5.9	5.8	5.9	5.8	n.s.
Tartaric acid (g/L)	2.7	2.5	2.6	2.6	n.s.
Lactic acid (g/L)	1.7	1.7	1.8	1.7	n.s.
Glycerol (g/L)	9.6	9.8	9.9	9.7	•
Succinic acid (g/L)	1.3	1.2	1.3	1.2	n.s.
Proline (mg N/L)	94 b	94 b	105 a	95 b	**
Total glutathione (mg/L)	2.6	2.8	3.3	3.0	n.s.
Folin Index	36.7 b	37.6 ab	36.6 b	39.4 a	*
Total anthocyanins (mg/L)	574.0	572	587	582	n.s.
Delphinidol-3-glucoside (% total anthocyanins)	3.7	3.6	3.4	4.3	n.s.
Cyanidol-3-glucoside (% total anthocyanins)	0.4	0.4	0.4	0.5	n.s.
Petunidol-3-glucoside (% total anthocyanins)	7.2	6.0	5.7	6.3	n.s.
Peonidol-3-glucoside (% total anthocyanins)	8.8	8.5	9.2	9.5	n.s.
Malvidol-3-glucoside (% total anthocyanins)	72.9	74.2	73.4	73.3	n.s.
Acetylated anthocyanins (% total anthocyanins)	1.3	1.4	1.6	1.4	n.s.
Coumaroylated anthocyanins (% total anthocyanins)	5.6	5.8	6.3	4.8	n.s.
Lightness L	27	24	25	24	n.s.
Colour a (red/green)	60.3	57.8	58.3	57.2	•
Colour b (yellow/blue)	37.7	37.7	35.3	33.3	•
Wine tasting (scores 1 to 7)					
Colour intensity	5.2	5.3	5.2	5.3	n.s.
Fruitiness	4.4	4.4	4.5	4.4	n.s.
Floral	1.6	1.6	1.6	1.6	n.s.
Herbaceous	1.6	1.6	1.6	1.6	n.s.
Lactic	1.2	1.2	1.1	1.2	n.s.
Empyreumatic	1.1	1.1	1.1	1.1	n.s.
Global nose impression	4.5	4.3	4.5	4.4	n.s.
Volume	4.6	4.6	4.6	4.6	n.s.
Acidity	4.3	4.3	4.2	4.3	n.s.
Tannin intensity	4.6	4.6	4.5	4.6	n.s.
Tannin quality	4.4	4.4	4.4	4.4	n.s.
Bitterness	1.8	1.9	1.8	1.8	•
General impression	4.3	4.3	4.4	4.3	n.s.

Numbers with different letters are statistically different (Tukey's test, $p < 0.05$). The wine tasting data are scores based on a predefined 1–7 scale. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; • $p < 0.10$; n.s., non-significant.

3. Wine composition and tasting

LR treatments had a negligible impact on wine composition. For Doral, only the proline concentration increased from 81 mg N/L in the post-berry-set mechanical LR to 93 mg N/L in the double mechanical LR (Table 3). Glutathione concentration tended to be higher in the manual pre-flowering LR (2.1 mg/L; $p < 0.10$). In the tasting of the Doral wines, colour intensity was the only parameter that tended to vary with LR, with a slightly higher value for the double mechanical treatment ($p < 0.10$).

Gamay wines subjected to post-berry-set LR had slightly lower alcohol concentration (-0.3 %vol.), lower proline concentration (94 mg N/L) and among the lowest Folin Index values (36.7) (Table 3). No difference among treatments was found in terms of anthocyanins, either in total concentration or in proportion. The colour intensity of Gamay wines from the mechanical post-berry-set LR was identical to that of the other LR treatments (i.e., same L) but tended to be redder and yellower (higher a and b; both $p < 0.10$). However, other than a minor variation in bitterness ($p < 0.10$), no differences were observed when tasting the Gamay wine as a function of LR treatments in terms of colour or any other organoleptic parameters (Table 3).

DISCUSSION

The trial was designed to confirm the effectiveness of pre-flowering LR and to test the sustainability of its mechanisation; moreover, we tested the interest of a second LR after berry-set, as a complement to limit the growth of lateral shoots.

1. The interest in pre-flowering LR

When compared with mechanical post-berry-set LR (A), mechanical pre-flowering LR (C) affected the vineyard observations and the must composition at harvest, although the gain in grape maturity at harvest was small and primarily related to year and cultivar. In the end, its impact on the wine composition and tasting was overall negligible for both cultivars. Pre-flowering LR induced a lower bud fruitfulness (i.e., -8 % and -6 % on average for Doral and Gamay, respectively). Shoot photosynthesis and carbon allocation to cluster sinks are reduced, which reduces bud fruitfulness and increases flower abscission (VanderWeide *et al.*, 2020). As mentioned in previously published literature (Frioni *et al.*, 2019; Nicolosi *et al.*, 2021; Tardaguila *et al.*, 2012; VanderWeide *et al.*, 2021), pre-flowering LR induced a lower berry set resulting in fewer berries per cluster (-26 % and -31 %, respectively) and a proportionally lower yield estimation before cluster thinning (Figure 2). Despite climate unpredictability (year*treatment interaction), pre-flowering LR had a consistent effect on vine physiology. The yield loss was usually bigger in the years with higher yield estimation (Figure 1). In fact, the intensive mechanical pre-flowering LR led consistently to approximately 30 % yield loss (i.e., 33 ± 11 and 29 ± 11 kg/m^2, respectively, for Doral and Gamay) in comparison to the post-berry-set mechanical LR, as confirmed by the literature (Palliotti *et al.*, 2012). Regardless of each cultivar's natural potential yield (i.e., around 1.1 kg/m^2 for Doral and 1.6 kg/m^2 for Gamay in the post-berry-set mechanical treatments), the yield loss due to pre-flowering LR remained proportional to the initial yield potential. The year*treatment interaction on the leaf area can be justified by the development of lateral shoots (depending on year conditions) in the cluster area after mechanical pre-flowering LR, while the laterals were removed in the manual pre-flowering LR treatment. However, the differences remained negligible at the physiological level (average difference of only 0.08 m^2 of leaves per m^2 of ground) and the leaf-to-fruit ratio, mostly affected by the yield, remained above 1.0 m^2/kg of fruit after LR treatment, thus, not limiting grape maturation. In terms of must composition, pre-flowering LR enhanced grape ripening, by inducing a better accumulation of TSS (+3 % and +2 % for Doral and Gamay, respectively) along with a lower concentration of tartaric acid (-3 % and -4 %, respectively). This improvement in grape maturity in both cultivars has already been demonstrated by other researchers (Poni *et al.*, 2005; VanderWeide *et al.*, 2020). It could be explained by the lower yield and the earlier exposure to the sun, although in our situation the gain in grape maturity was not significant in each year. The concentration of glutathione remained unchanged for Doral and was lower for Gamay. No changes were observed in the concentrations of anthocyanins in Gamay grapes, contradicting the results from other studies (Bubola *et al.*, 2017; Sivilotti *et al.*, 2016). No significant difference was observed in the wines of both cultivars as a function of LR timing. Most of the studies cited previously did not regulate the yield according to the local allowable quotas, but the yield highly influences the must composition in terms of maturity. In this study, cluster thinning has probably minimized the impact of pre-flowering LR on grape and wine composition. Yet, Gamay has already shown its great plasticity in a previous study about pre-flowering LR, while Pinot noir or Merlot have shown greater anthocyanin concentration after pre-flowering LR and, consequently, a deeper red colour in wines under similar conditions (Verdenal *et al.*, 2019).

2. The interest in mechanising pre-flowering LR

When compared with manual LR on the same date (B), mechanical pre-flowering LR (C) was more brutal to the plant and had a stronger impact on yield formation and delayed grape ripening. The number of berries per cluster was lower (-14 % and -11 %, respectively) along with the estimated yield (-20 % and -16 %, respectively). In terms of must composition, mechanical pre-flowering LR limited the accumulation of TSS for Doral (i.e., -1 %), while it remained unchanged for Gamay. It also induced a higher TA (+2 % and +6 % for Doral and Gamay, respectively), mainly due to more malic acid (+8 % and +13 %, respectively). The YAN concentration remained unchanged in Doral musts, while it was higher in Gamay musts after mechanical LR in comparison with manual LR. When compared two-by-two, no difference was observed among Doral wines, while Gamay wines from mechanical LR tended to be slightly less bitter

(-7 %) with smoother tannins (+6 %) than wines from manual LR (both $p < 0.10$, results not shown). In the context of this experiment, and despite the positive impact on Gamay wines, mechanical LR seemed too intense and negatively affected bud fruitfulness and grape maturity. Mechanical treatment induced a greater loss in bud fruitfulness (i.e., -10 % and -8 % on average for Doral and Gamay, respectively). This could potentially affect the yield at harvest over the years (Figure 1). The risk of the long-term impact of intensive pre-flowering LR has already been pointed out by other researchers, for example, reduction of the grapevine reserves, vigour, fruitfulness and, potentially, the lifespan (Palliotti *et al*., 2012; Risco *et al*., 2014; Uriarte *et al*., 2012). Even if no shoots were broken during mechanical LR, some damage was observed on the inflorescences due to the intensity of the treatment, which resulted in a lower yield than with manual LR and probably induced a lower bud fruitfulness. Thus, a moderate mechanical pre-flowing LR could be an effective practice, while an intensive pre-flowering LR should be manually done. Further experiments on the intensity of mechanical pre-flowering LR are required.

3. The lack of interest in a second LR after berry-set

When compared with mechanical pre-flowering LR (C), double LR (D, i.e., pre-flowering + post-berry-set) had a minor impact on the vine development and must composition. The purpose of the second LR was to limit the growth of laterals and clean the clusters from the remaining flower caps to prevent the development of fungal diseases. Double LR resulted in an even smaller leaf area (-7 % for both Doral and Gamay), without significantly affecting the leaf-to-fruit ratio. Pruning weight and bud fruitfulness were not affected. Grape ripening was not affected for Doral, while it was slightly altered for Gamay (-1 % TSS and +2 % tartaric acid). Doral wines were not affected at all, while only the Folin Index was higher in Gamay wines from double LR (+9 %). In the context of this study, the presence of flower caps in the fruits after cluster closure did not promote fungal diseases due to the absence of fungal diseases. Moreover, the growth of lateral shoots was not excessive after pre-flowering LR, which could have justified a second LR after the berry set. Thus, double LR was not a useful method in the context of this trial. As previously discussed, the impact of single intensive pre-flowering LR was already strong, nullifying the benefits of double LR.

4. The sustainability of mechanical pre-flowering LR

The low-pressure dual airflow provided an effective pre-flowering LR without damaging any fragile shoots, although the loss of a few flower buds was observed on the inflorescence. Adapted settings were required compared with post-berry-set LR (lower speed, Table 1) to maintain an LR efficiency equivalent to manual LR, due to the smaller leaf area at that early stage of the season. After mechanical pre-flowering LR, cluster thinning work has been reduced by 69 % and 27 %, respectively for Doral and Gamay, in comparison with post-berry-set LR (Table 2).

The year conditions strongly affected plant physiology (Supplementary Tables S1 and S2), particularly the yield parameters, that is, bud fruitfulness, berry number and cluster weight which determine the initial yield potential. Despite the variability in year effects, primarily due to climate unpredictability and cultivar, yield loss was generally proportional to yield potential and, therefore, greater in years with higher yield estimates (Figure 1).

However, the carryover impact of pre-flowering LR could potentially affect long-term production. The intensive pre-flowering LR applied in this trial affected bud fruitfulness. Double LR was excessive and did not evidence any benefit over a single pre-flowering LR. As a consequence, pre-flowering LR should not be recommended for too young or too weak vines.

As a confirmation of our previous trials conducted under similar conditions (Verdenal *et al.*, 2019), we concluded that a single moderate pre-flowering LR appears to be a sustainable and prophylactic practice under temperate climatic conditions, to effectively limit the yield while improving grape ripening in some years. In addition, the present trial validates the sustainability of mechanical pre-flowering LR using a low-pressure double airflow, to reduce the cost of laborious cluster thinning.

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