MECHANIZATION OF PRE-FLOWERING LEAF REMOVAL UNDER THE TEMPERATE-CLIMATE CONDITIONS OF SWITZERLAND

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Introduction

Grapevine leaf removal (LR) in the cluster area is typically done between fruit set and cluster closure to create an unfavorable microclimate for fungal diseases, such as *Botrytis cinerea* and powdery mildew. Grape growers are now turning their attention to pre-flowering LR, which has additional benefits under certain conditions. When applied before flowering, LR strongly affects fruit set and thus the number of berries per cluster. It is therefore a good yield control tool, replacing time-consuming manual cluster thinning (Poni et al. 2006). It also improves berry structure, that is, skin thickness, skin-to-pulp ratio, and berry composition (total soluble solids, titratable acidity, and polyphenols) (Palliotti et al. 2012; Komm and Moyer 2015). By exacerbating competition for assimilates between reproductive and vegetative organs, pre-flowering LR also poses some risks. Excessive yield loss at the same year's harvest due to a too low fruit 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 fruit set rate and make it difficult to predict potential yield at harvest. Repeated and overly intensive preflowering LR can have repercussions over time and induce a decline in bud fruiting and plant vigor (Risco et al. 2014).

The effects of 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 interesting results (Verdenal et al. 2018). An intensive pre-flowering LR (removal of six basal + lateral leaves) confirmed its huge impact on the agronomic performance of the vine, mainly at the expense of fruit set. Yield was therefore strongly affected (about -35% of that of the non-defoliated control treatments). This yield loss was proportional to the initial yield potential, which depends on genetics. The intensity of LR modulated its impact on yield. Preflowering 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. Due to pre-flowering LR, red wines were often preferred for their color 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.

Pre-flowering LR represents a prophylactic solution that reduces both chemical applications and cluster thinning costs. 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 are fragile, the choice of method is essential for optimal results. Mechanical LR by rotary suction was tested at flowering and resulted in the loss of shoots and inflorescences (Intrieri et al. 2016). In comparison, LR by low-pressure dual airflow (Collard, Bouzy, France) seems more suitable for pre-flowering LR. Two trials were conducted in Switzerland for five years on two cultivars to test mechanical pre-flowering LR.

Research Objectives

The objectives of this five-year study were to identify the interest of mechanized pre-flowering LR under the temperate climate conditions of Switzerland, using a double flow of low-pressure air; and to investigate the impact of this practice on the yield parameters, on the grape composition at harvest and on the wine quality. More broadly, this work provides practical insights into the consequences grapevine LR, pointing out the advantages and the limits of intensity, earliness and mechanization of this practice.

Material and methods

Description of the experimental sites

Two experiments were conducted between 2016 and 2020 in the experimental vineyard of Agroscope at Nyon, Switzerland. Two field-grown cultivars of *Vitis vinifera* L. (namely, doral and gamay) were planted in two separate homogeneous plots. The vines were grafted onto rootstock 3309C, planted at a density of 5880 plants/ha and pruned to simple Guyot. Each experiment was structured as a randomized block design, consisting of four homogeneous blocks with four treatments, that is, A) traditional mechanical post-fruit-set LR; B) manual pre-flowering LR; C) mechanical pre-flowering LR; and D) double mechanical LR, pre-flowering and post-fruit-set. Pre-flowering LR was conducted between the phenological stages "separated flower buds" and "flowering" on the same day (BBCH 57-61, May 31, five-year average), as soon as the shoots were tied in the trellis; post-berry-set LR was conducted at "pea size" stage (BBCH 73-75, June 23 on average). Manual LR consisted of hand removal of the first six basal leaves of each shoot, including laterals. Mechanical LR of the equivalent area consisted of using a tractor-mounted compressed air stripper (E 3000 3P, 2003, Collard, Bouzy, France), using a low-pressure dual airflow, with different settings (tractor speed and air flow pressure) for pre-flowering and post-berry-set treatments.

Field measurements

Field measurements were performed per replicate (i.e., four times per treatment), except for leaf mineral composition performed once per treatment. Phenological differences between treatments were assessed on gamay at veraison (average percentage of red berries per cluster at a chosen date). Bud fruiting was estimated (average number of clusters per shoot). Cluster weight was estimated from yield per vine divided by the average number of clusters previously estimated. Pruning weight, which is an indicator of plant vigor, was assessed during winter.

Analysis of leaves and grapes

The leaf mineral composition (N, P, K, Ca and Mg) was assessed at veraison and analyzed by an external laboratory (Sol-Conseil, Gland, Switzerland). General must parameters were determined at harvest using an infrared spectrophotometer (FOSS WineScan^{TM}) (i.e., total soluble solids, titratable acidity, tartaric and malic acids, and pH). Other analyses were performed on the grape extracts: from 2017 to 2020, the total phenolic content was determined and expressed as Folin index; the concentration of ammonium was determined by enzymatic method; and the concentration of free alpha-amino acids by a spectrophotometric method, as described in Verdenal et al. (2018). Yeast assimilable nitrogen (YAN) was calculated as the sum of nitrogen (mg N/L) as ammonium and free alpha-amino acids. Total glutathione concentration was determined using a liquid chromatography mass spectrometer (LC-MS/MS). A final aliquot was used to evaluate total free anthocyanins and anthocyanin profile for gamay only.

Analysis and tasting of the wines

For each cultivar, the harvest date was determined according to the sugar content. Grapes were harvested per replicate, each year in one day, and yield was assessed. The four replicates of each treatment were then assembled and approximately 50 kg of grapes were vinified per treatment according to the standard Agroscope protocol. The finished wines were analyzed with an infrared spectrophotometer (FOSS WineScan^{TM}) for the following parameters: alcohol, dry extract, pH, volatile acid, titratable acidity, tartaric, malic and lactic acids, glycerol, proline and succinic acid. Folin index, total glutathione, total free anthocyanins and anthocyanin profile were evaluated in gamay wines, as previously described for grape extracts. The chromatic characteristics of the wines were described according to the CIELab procedure. Sensory analysis was performed annually in a dedicated tasting room; the trained Agroscope panel (12 permanent members) described the wines according to predefined criteria using scores from 1 to 7.

Statistical analysis

Data analysis was performed using XLSTAT software (Addinsoft©, Paris, France). Analyses were conducted for each cultivar separately, as two distinct trials. The description and significance of differences between treatments were assessed using analysis of variance (ANOVA, p values < 0.05). Tukey's post hoc test was used for multiple comparisons. Significance of the year*treatment interaction was calculated for parameters that were evaluated by replicate (i.e., field parameters and some must analysis), considering treatment as a fixed factor, and year and replicate as random factors (mixed model). Sensory data were analyzed with the FIZZ program (Biosystems©, Courtenon, France).

Results

Vegetative development and yield parameters (Table 1)

The five-year average of bud fruiting was slightly lower for the two pre-flowering mechanical treatments for both cultivars (1.8 and 2.1 clusters per shoot, respectively for doral and gamay). Doral bud fruiting decreased in the last year of the trial (1.6 clusters per shoot), while it remained stable for gamay. Leaf mineral composition did not vary among LR treatments, except for calcium in gamay, which was slightly lower in the prebloom mechanical LR. Variations in exposed leaf area between treatments were small, approximately 1.0 m^2/m^2 soil. Early estimated yield showed a decrease in 2019 and 2020 for doral (i.e., 0.6 kg/m^2, compared to an average of 1.4 kg/m^2 in 2016-17-18), but not for gamay. Estimated yield was strongly influenced by LR treatments for both cultivars, with the lowest estimates in mechanical LR before flowering (average of 0.9 and 1.3 kg/m^2 for doral and gamay, respectively); 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). This variation between treatments was due to the number of berries per cluster (i.e., -27% and -21% for the pre-bloom mechanical LR of doral and gamay, respectively, compared to the post-set LR), and consequently to cluster weight (-25% and -18%, respectively). In both cultivars, the average yield at harvest varied only between 0.8 and 1.0 kg/m^2, due to homogenization by cluster thinning. A year*treatment interaction was observed for most observations related to leaf/fruit ratio (i.e., leaf area, berry number, cluster thinning, yield) and grape maturity at harvest (i.e., TSS and TA).

Must composition at harvest (Table 1)

The LR treatment after fruit set generally contained less TSS, as well as the highest titratable acidity among the treatments (i.e., 8.3 and 10.2 g tartrate/L, for doral and gamay, respectively), generally related to more tartaric acid. The pH in the LR treatment after fruit set was lower only in gamay. Grape nitrogen content was influenced by cultivar: for doral, YAN concentration was lower in the pre-flowering (i.e., manual and mechanical) treatments compared to the double mechanical treatment in LR. Conversely, YAN of gamay tended (p-value < 0.10) to be higher in the mechanical pre-flowering treatment in LR, due to a higher concentration of amino nitrogen. Folin index tended (p value < 0.10) to be higher in the dual mechanical LR treatment for both cultivars. Anthocyanin concentration as a function of LR treatments was unchanged in gamay grapes (mean 629 mg/L). 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 value < 0.10).

Wine Composition and Tasting (Table 2)

LR treatments had a minor impact on wine composition. For doral, only proline concentration increased from 81 mg N/L in the post berry-fixation mechanical LR to 93 mg N/L in the double mechanical LR. Glutathione content tended to be higher in the prefloral manual LR (2.1 mg/L; p value < 0.10). When tasting the doral wines, color intensity was the only parameter that tended to vary with LR, with a slightly higher value for the double mechanical treatment (p-value < 0.10). Gamay wines subjected to the LR berry post-fixation mechanical treatment had slightly lower alcohol content (-0.3%vol.), lower proline content (94 mg N/L), and some of the lowest Folin index values (36.7). No differences between treatments were found in terms of anthocyanins, either in total concentration or in proportion. The color intensity of gamay wines from the post-fixation mechanical LR berry treatment 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 values <0.10). However, other than a minor variation in bitterness (p-value <0.10), no differences were observed when tasting the gamay wine as a function of the LR treatments in terms of color or any other organoleptic parameters.

Conclusion

The pre-flowering LR treatments affected vegetative parameters and must composition at harvest, while their impact on wine composition was negligible overall. The intensive LR treatment applied in these trials had an effect on doral bud fruiting and could potentially affect long-term production. Moderate LR just prior to flowering appears to be a sustainable and prophylactic practice under temperate climate conditions to reduce chemical applications and cluster thinning costs.

The low-pressure dual airflow provided an effective pre-flowering LR and gave better results than the rotary suction system, without damaging any fragile shoots at this early phenological stage. Adapted settings were required compared to post-flowering LR (lower speed and higher airflow).

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Tables and Figures

Table 1. Vineyard observations and must composition on doral and gamay as a function of leaf removal treatment. Numbers with different letters are statistically different (Tukey test, $p < 0.05$). ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; •, $p < 0.10$.

Leaf removal treatment	Doral						Gamay					
	Post berry-set Mechanical	Pre-flowering Manual	Pre-flowering Mechanical	Pre-flowering + post berry-set Mechanical	P-value	Interaction Year*Treatment	Post berry-set Mechanical	Pre-flowering Manual	Pre-flowering Mechanical	Pre-flowering + post berry-set Mechanical	P-value	Interaction Year*Treatment
Vineyard observations												
Pruning weight (g/m)	54	52	52	53	n.s.	n.s.	45	42	44	42	•	n.s.
Bud fruitfulness (clusters per shoot)	1.9 a	2.0 a	1.8 b	1.8 b	***	•	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)	546	543	531	544	n.s.	n.s.	565	558	557	554	n.s.	n.s.
Leaf nitrogen (% dry mass)	2.26	2.36	2.24	2.37	•	–	2.06	2.07	2.06	2.03	n.s.	–
Leaf phosphorus (% dry mass)	0.21	0.21	0.22	0.21	n.s.	–	0.19	0.19	0.19	0.19	n.s.	–
Leaf potassium (% dry mass)	1.4	1.3	1.3	1.3	n.s.	–	1.1	1.2	1.2	1.2	n.s.	–
Leaf calcium (% dry mass)	2.7	2.8	2.6	2.7	n.s.	–	3.0 ab	3.1 a	2.9 b	3.1 ab	*	–
Leaf magnesium (% dry mass)	0.3	0.3	0.3	0.3	n.s.	–	0.4	0.4	0.4	0.4	n.s.	–
Light-exposed leaf area (m^2/m^2 of ground)	1.01 ab	1.01 ab	1.05 a	0.98 b	*	**	1.02 ab	0.96 c	1.05 a	0.99 bc	***	**
Leaf-to-fruit ratio (m^2/kg)	1.2 b	1.2 b	1.4 a	1.3 ab	***	**	1.2 b	1.4 a	1.4 a	1.3 ab	**	***
Early estimated yield (kg/m^2)	1.5 a	1.1 b	0.9 c	0.9 c	***	***	1.9 a	1.6 b	1.4 c	1.3 c	***	n.s.
Cluster thinning (number removed per vine)	2.6 a	2.1 a	0.8 b	0.5 b	***	***	5.7 a	6.4 a	3.6 b	3.6 b	***	***
Number of berries par cluster	127 a	109 b	93 bc	92 c	***	**	137 a	105 b	94 bc	92 c	***	*
Berry weight at harvest (g)	1.6 ab	1.6 b	1.7 a	1.7 a	***	n.s.	2.1 a	2.0 b	2.1 a	2.1 a	***	n.s.
Cluster weight at harvest (g)	165 a	144 b	123 c	125 c	***	***	149 a	129 b	116 c	122 bc	***	n.s.
Yield at harvest (kg/m^2)	1.0 a	0.9 b	0.8 b	0.8 b	***	***	0.9 a	0.8 b	0.8 b	0.8 b	***	***

Must composition at harvest												
Total soluble sugars (Brix)	22.8 c	23.4 a	23.2 b	23.1 b	***	***	23.5 b	24.1 a	24.1 a	23.9 a	***	**
pH	3.09	3.10	3.09	3.10	n.s.	n.s.	3.11 b	3.16 a	3.15 a	3.14 a	***	n.s.
Titratable acidity (g tartrate/L)	8.3 a	8.1 b	8.2 ab	8.3 a	***	**	10.2 a	9.6 b	10.1 a	10.1 a	***	*
Tartaric acid (g/L)	8.3 a	8.0 b	8.1 b	8.2 ab	**	**	8.9 a	8.5 c	8.5 c	8.7 b	***	n.s.
Malic acid (g/L)	2.2 ab	2.1 b	2.3 a	2.3 a	**	•	3.9 b	3.6 c	4.1 a	3.8 b	***	*
Ammonium (mg/L)	74 a	63 b	63 b	80 a	***	–	94 a	87 a	97 a	93 a	n.s.	–
Alpha amino N (mg N/L)	115	122	117	131	n.s.	–	114 b	125 ab	133 a	116 b	**	–
Yeast assimilable nitrogen (mg N/L)	176 ab	174 b	169 b	197 a	*	–	192	196	213	193	•	–
Folin index	13.4	12.3	12.8	13.9	•	–	16.9	19.6	14.5	20.3	•	–
Total glutathions (mg/L)	46.2 b	50.3 a	47.5 ab	49.7 ab	*	–	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 anthocanins)	–	–	–	–	–	–	5.7 b	6.5 ab	5.6 b	6.7 a	*	–
Cyanidol-3-glucoside (% total anthocanins)	–	–	–	–	–	–	1.0 b	1.1 ab	1.0 b	1.2 a	*	–
Petunidol-3-glucoside (% total anthocanins)	–	–	–	–	–	–	7.0 b	7.8 a	7.1 b	7.9 a	***	–
Peonidol-3-glucoside (% total anthocanins)	–	–	–	–	–	–	12.8	12.5	12.4	13.3	•	–
Malvidol-3-glucoside (% total anthocanins)	–	–	–	–	–	–	63.8 a	62.5 ab	64.0 a	61.8 b	*	–
Acetylated anthocyanins (% total anthocanins)	–	–	–	–	–	–	3.1	3.9	4.0	3.0	n.s.	–
Coumaroylated anthocyanins (% total anthocanins)	–	–	–	–	–	–	6.7 a	6.5 a	6.8 a	6.1 a	•	–

Table 2. Wine analysis and tasting data on doral and gamay as a function of leaf removal treatment. Numbers with different letters are statistically different (Tukey test, $p < 0.05$). The wine tasting data are scores based on a predefined 1-to-7 scale. ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; •, $p < 0.10$.

Leaf removal treatment	Doral						Gamay					
	Post berry-set Mechanical	Pre-flowering Manual	Pre-flowering Mechanical	Pre-flowering + post berry-set Mechanical	P-value	Interaction Year* Treatment	Post berry-set Mechanical	Pre-flowering Manual	Pre-flowering Mechanical	Pre-flowering + post berry-set Mechanical	P-value	Interaction Year* Treatment
Wine composition												
Alcohol (%vol.)	12.9	13.7	13.3	13.3	n.s.	–	13.4 b	13.8 a	13.8 a	13.6 ab	**	–
pH	3.4	3.4	3.4	3.4	n.s.	–	3.5	3.5	3.5	3.5	n.s.	–
Titratable acidity (g tartrate/L)	5.5	5.3	5.3	5.2	n.s.	–	5.9	5.8	5.9	5.8	n.s.	–
Tartaric acid (g/L)	2.2	2.0	2.0	2.0	n.s.	–	2.7	2.5	2.6	2.6	n.s.	–
Lactic acid (g/L)	1.2	1.3	1.3	1.3	n.s.	–	1.7	1.7	1.8	1.7	n.s.	–
Glycerol (g/L)	8.6	8.6	8.6	8.8	n.s.	–	9.6	9.8	9.9	9.7	•	–
Succinic acid (g/L)	1.0	1.0	0.9	1.0	n.s.	–	1.3	1.2	1.3	1.2	n.s.	–
Proline (mg N/L)	81 b	85 ab	90 ab	93 a	*	–	94 b	94 b	105 a	95 b	**	–
Total glutathions (mg/L)	1.5	2.1	1.7	1.6	•	–	2.6	2.8	3.3	3.0	n.s.	–
Folin index	6.6	6.6	6.3	6.2	n.s.	–	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 anthocanins)	–	–	–	–	–	–	3.7	3.6	3.4	4.3	n.s.	–

Cyanidol-3-glucoside (% total anthocanins)	–	–	–	–	–	–	0.4	0.4	0.4	0.5	n.s.	–
Petunidol-3-glucoside (% total anthocanins)	–	–	–	–	–	–	7.2	6.0	5.7	6.3	n.s.	–
Peonidol-3-glucoside (% total anthocanins)	–	–	–	–	–	–	8.8	8.5	9.2	9.5	n.s.	–
Malvidol-3-glucoside (% total anthocanins)	–	–	–	–	–	–	72.9	74.2	73.4	73.3	n.s.	–
Acetylated anthocyanins (% total anthocanins)	–	–	–	–	–	–	1.3	1.4	1.6	1.4	n.s.	–
Coumaroylated anthocyanins (% total anthocanins)	–	–	–	–	–	–	5.6	5.8	6.3	4.8	n.s.	–
Lighness L	99	98	98	98	n.s.	–	27	24	25	24	n.s.	–
Color a (red/green)	-1.6	-1.4	-1.5	-1.4	n.s.	–	60.3	57.8	58.3	57.2	•	–
Color b (yellow/blue)	8.8	8.9	9.0	8.7	n.s.	–	37.7	37.7	35.3	33.3	•	–
Wine tasting (scores 1 to 7)												
Color intensity	4.6	4.6	4.6	4.7	•	–	5.2	5.3	5.2	5.3	n.s.	–
Fruitiness	4.4	4.5	4.3	4.5	n.s.	–	4.4	4.4	4.5	4.4	n.s.	–
Floral	2.7	2.8	2.7	2.8	n.s.	–	1.6	1.6	1.6	1.6	n.s.	–
Herbaceous	1.7	1.6	1.6	1.6	n.s.	–	1.6	1.6	1.6	1.6	n.s.	–
Lactic	1.4	1.5	1.4	1.5	n.s.	–	1.2	1.2	1.1	1.2	n.s.	–
Empyreumatic	1.1	1.2	1.1	1.1	n.s.	–	1.1	1.1	1.1	1.1	n.s.	–
Global nose impression	4.2	4.3	4.2	4.3	n.s.	–	4.5	4.3	4.5	4.4	n.s.	–
Volume	4.6	4.7	4.6	4.7	n.s.	–	4.6	4.6	4.6	4.6	n.s.	–
Acidity	4.4	4.3	4.4	4.4	n.s.	–	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	2.6	2.5	2.4	2.5	n.s.	–	1.8	1.9	1.8	1.8	•	–
General impression	4.1	4.2	4.1	4.2	n.s.	–	4.3	4.3	4.4	4.3	n.s.	–