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Intensity and timing of defoliation on white cultivar Chasselas under the temperate climate of Switzerland

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Abstract

Aim: The objective of this work is to evaluate the pre-berry-set defoliation on the white cv. Chasselas as a potential vineyard practice in Switzerland, with particular attention to the impact of defoliation intensity on the yield and wine sensory parameters.

Methods and results: Defoliation (removal of 6 basal leaves + 6 lateral shoots per shoot) was completed during three developmental stages of grapevine, i.e., pre-flowering, flowering and bunch closure, and at two intensity levels (50 and 100 % of the fruit zone). The experimentation was performed repeatedly over four years. In addition to vintage effect, pre-flowering defoliation had a consistent impact on vine agronomic behaviour. The yield was highly affected by the technique (more than 30% loss). Earlier and more intense defoliation had more impact on yield, while post-berry-set defoliation had no effect on yield. Pre-floral defoliation affected bud fruitfulness and vigour, i.e., trimming and pruning weights. This result noted a carryover effect that could affect vine sustainability under restrictive conditions. Pre-floral defoliation also increased berry skin thickness and had a positive impact inhibiting Botrytis development. However, white wine composition and sensory analysis were not affected by the practice, provided that a sufficient leaf-to-fruit ratio is maintained to guarantee a proper grape maturation. In terms of methodology, the environmental scanning electron microscopy (ESEM) represented an interesting alternative to conventional transmission electron microscopy (TEM) to observe berry epidermis, as it is less time-consuming.

Conclusion: In the context of this study, pre-flowering defoliation seems to be an interesting practice to reduce vigour and control the high production potential of the cv. Chasselas, without affecting wine sensory parameters. The intensity of early defoliation allows for the modulation of the impact on the yield in order to prevent excessive yield loss.

Significance and impact of the study: Pre-flowering defoliation of white cultivars represents a prophylactic solution to reduce both chemical entrants and bunch-thinning costs.

Key words: defoliation, vigour control, yield limitation, wine sensory parameters, berry ski

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INTRODUCTION

Grapevine defoliation in the cluster zone is usually realized between berry set and bunch closure to create an unfavourable microclimate for cryptogamic diseases, such as *Botrytis cinerea* and powdery mildew (Zoecklein *et al.* 1992; Percival *et al.* 1994; Sternad Lemut *et al.* 2015). When completed after berry set, defoliation does not affect fruit set and yield (Feng *et al*. 2015; Nicolosi *et al*. 2012; Tardaguila *et al.* 2008). However, grape growers are now interested in pre-berry-set defoliation, which presents extra advantages under certain conditions. This practice strongly affects berry set and berry number per bunch (Gómez *et al.* 2012; Kotseridis *et al.* 2012; Poni and Bernizzoni 2010; Sabbatini and Howell 2010). As a consequence, it limits the yield (Poni *et al.* 2006; Palliotti *et al.* 2012; Basile *et al.* 2015) and induces tremendous modifications in berry structure, i.e. skin thickness and skin-to-pulp ratio, and in berry composition (total soluble solids, acidity, and polyphenols) (Palliotti *et al.* 2012; Šuklje *et al.* 2014; Komm et Moyer 2015; Verdenal *et al.* 2017). Inducing strong competition for assimilates between vegetative and reproductive organs, pre-berry-set defoliation also presents some risks: the major part of photosynthetically active foliage is removed at a time of high C and N requirements by the inflorescences, forcing the vine to further dig into its reserves in its wood and roots (Verdenal *et al.* 2017). Consequently, during the year following defoliation, a lower vigour was noted in some situations (Palliotti *et al.* 2012), as well as a lower bud fruitfulness (Risco *et al.* 2014; Uriarte *et al.* 2012). In other situations, no carryover effects could be observed because the vines had sufficient reserves (Acimovic *et al.* 2016).

Pre-flowering defoliation can drastically affect the must composition; the concentration of total soluble solids in the must usually increases in comparison to a non-defoliated control treatment, while acidity is decreased in some situations (Bravetti *et al.* 2012; Diago *et al.* 2010; Palliotti *et al.* 2012; Risco *et al.* 2014). Moreover, the accumulation of phenolic compounds increases (Palliotti *et al.* 2012; Sternad Lemut *et al.* 2013; Talaverano *et al.* 2016), enhancing colour intensity and stability in red wines. Finally, the concentration of volatile compounds increases, possibly enhancing wine aroma quality (Vilanova *et al*. 2012). Verdenal *et al.* (2017) also observed a lower concentration of free glutathione in Pinot noir must as a result of pre-flowering defoliation. This result suggests that glutathione could play a role as an antioxidant protecting berries against external stresses, i.e., UV light and pathogens. However, the quantitative and qualitative parameters of the must and wine are not always affected in a significant manner (Moreno *et al.* 2015; Sivilotti *et al.* 2016; Talaverano *et al.* 2016).

Pre-flowering defoliation seems to be a promising technique under the temperate conditions of Switzerland (Verdenal *et al.* 2017). However, its impact on yield and grape composition seems to be unpredictable as a function of numerous biotic and abiotic factors, e.g., type of cultivar, climatic conditions, and period and intensity of defoliation (Kotseridis *et al.* 2012; Hed *et al.* 2015). Moreover, the results on pre-flowering defoliation were rarely obtained on white cultivars (Komm and Moyer 2015). Considering the heterogeneity of the aforementioned results and the risk of excessive yield loss resulting from this practice, the present work was required to investigate the effects of pre-flowering defoliation on the white cv. Chasselas under local Swiss conditions, in comparison to alternative defoliation timing and intensity, with particular attention paid to its effects on yield reduction and must composition.

MATERIALS AND METHODS

1. Vineyard site and material

The experiment was conducted repeatedly over four years (2013–2016) on a single plot located at the experimental vineyards of Agroscope (Pully, Vaud, Switzerland) using field-grown *Vitis vinifera* L. cv. Chasselas (clone 800) at an altitude of 460 m. The vines were grafted onto rootstock 3309C and then planted in 2007 at a density of 5880 vines/ha (2.00 × 0.85 m).

The average temperature is 11.2°C over the course of the year but 15.7°C during the vine growing season (April–October) and the total annual precipitation is 1150 mm (average 1981–2010, Pully meteorological station, www.meteosuisse.ch). The annual total precipitation and mean temperature in the Leman region for the study period (2013–2016) are shown in Table 1. The vineyard soil is a non-calcareous

Table 1. Total annual precipitations and mean temperatures during the vine growing season in the Leman region during the experiment (Meteo Suisse, Geneva station).

Year	Total precipitations (mm)	Mean temperatures (°C, April-October)
2013	1047	16.0
2014	1005	16.3
2015	686	17.7
2016	886	16.8

colluvial soil containing 15 wt.% clay, 47 wt.% sand and 4 wt.% total $CaCO_3$. The soil organic matter content is 1.7 wt.%, and there is no deficiency/excess of essential elements, such as P, K, and Mg. The water-holding capacity is high (> 250 mm). Annually, 30 kg of N/ha and 30 kg of Mg/ha were applied on the ground early during the season (stage 3–5 leaves); 10 kg of N/ha of foliar urea was also applied (during the veraison stage). The vines were pruned using a single-Guyot training system with seven shoots/plant. The canopy was trimmed to 110 cm in height. The lateral shoots were removed from the fruiting zone during the berry-set stage (BBCH 71, Baggiolini J) as a normal practice in the region.

2. Experimental design

The experiment was structured as a randomized block design, including four blocks with five treatments of 15 vines each (A, B, C, D, and E), consisting of five defoliation treatments as follows (Table 2): A) a non-defoliated control treatment, B) defoliation during the pre-flowering stage (phenological stage BBCH 57, Baggiolini H), C) defoliation during the flowering stage (BBCH 67–69, Baggiolini I) and D) defoliation during the bunch-closure stage (BBCH 77, BaggioliniL). Treatments B, C and D were defoliated intensively, i.e., all six primary leaves and lateral shoots from the base of each shoot were plucked. Treatment E consisted of a pre-flowering defoliation (similar to treatment B) of medium intensity, i.e., only three leaves were removed per shoot.

3. Field measurements and plant sampling

During the flowering stage, phenological differences between the different treatments were estimated by estimating the percentage of fallen flowerhoods on 25 inflorescences. Shoot trimming was conducted two to four times during the season depending on the vintage, and the total trimming fresh weight (g/plant) was determined per replicate at the end of the season. The light-exposed leaf area (m^2/m^2 of ground) was estimated during veraison using Carbonneau's method (1995). The length of the penultimate shoot on the cane was measured on each vine early during the season (when they reached approximately 50 cm) to note an eventual delayed bud burst and a weak return to growth. Pruning weight (g/m) was assessed per replicate during winter from 10 one-metre-long shoots selected on the penultimate cane buds of each vine.

The chlorophyll index was monitored once a month between flowering and harvest using a N-tester (Yara, Paris, France) in the medial zone of the canopy. A leaf diagnosis was completed per treatment every year during veraison on a sample of 25 primary leaves (petiole + blade) collected in the medial zone of the canopy to quantify N, P, K, Mg and Ca (% dry weight, Sol-Conseil laboratory: Gland, VD, Switzerland).

For each replicate, bud fruitfulness was estimated and expressed as the number of bunches per shoot. The potential yield was estimated during July (before bunch closure) from a sample of 50 berries and 10 bunches per replicate, using the method described by Verdenal *et al.* (2017). Bunch thinning was then applied before the bunch-closure stage (BBCH 77), the target being 1.1 kg/m^2. Berry weight was estimated at harvest from a sample of 50 berries per replicate. Bunch weight was estimated at harvest using the ratio of yield-to-bunch number per vine. An attack by *Botrytis cinerea* occurred during 2013. It was quantified per replicate by the percentage of rotten berries per bunch in 25 bunches.

4. Microscopy

In 2013 and 2015, bunch samples were collected before harvest to evaluate berry skin thickness under treatments A, B and D. Three berries from three bunches per treatment were prepared according to Roland and Vian (1991); they were pre-fixed with a solution of 3 % glutaraldehyde-2 % paraformaldehyde in a 0.07 M phosphate buffer at pH 7 and embedded in 2 % agarose and post-fixed with a

Table 2. Description of the four treatments. The lateral shoots were removed in the fruiting zone of all treatments.

Variante	Defoliation timing	Defoliation intensity	Defoliation dates			
			2013	2014	2015	2016
A	Control non defoliated	-	-	-	-	-
B	Pre-flowering (BBCH 57)	High, 6 leaves	11 June	22 May	22 May	31 May
C	Flowering (BBCH 67-69)	High, 6 leaves	8 July	16 June	11 June	27 June
D	Bunch closure (BBCH 77)	High, 6 leaves	5 August	29 July	16 July	25 July
E	Pre-flowering (BBCH 57)	Medium, 3 leaves	11 June	22 May	22 May	31 May

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Table 3. Impact of defoliation timing and intensity on vine phenology, vigour and yield parameters. Six-year averages ± SD. The values followed by different letters in the same row and same table section are significantly different (Newman-Keuls test, P<0.05).

	Defoliation timing				Defoliation intensity		
	Non defoliated	Bunch closure	Flowering	Pre-flowering	Non defoliated	Mid-intensity	High intensity
Number of berries per bunch	210 ± 29 a	201 ± 29 a	160 ± 12 b	124 ± 10 c	210 ± 29 a	182 ± 11 a	124 ± 10 b
Berry weight at harvest (g)	3.0 ± 0.3	2.9 ± 0.3	2.9 ± 0.4	2.9 ± 0.3	3.0 ± 0.3	3.0 ± 0.4	2.9 ± 0.3
Bunch weight at harvest (g)	399 ± 84 a	402 ± 103 a	338 ± 45 ab	265 ± 35 b	399 ± 84 a	374 ± 79 a	265 ± 35 b
Bunch thinning (number removed per vine) (number removed per vine)	8.8 ± 0.9 a	8.3 ± 1.0 a	7.3 ± 0.4 b	5.5 ± 0.3 c	8.8 ± 0.9 a	7.7 ± 0.7 b	5.5 ± 0.3 c
Flowering (%)	53 ± 13 a	48 ± 17 ab	53 ± 14 a	43 ± 18 b	53 ± 13 a	49 ± 17 a	43 ± 18 b
Pruning weight (g/m)	64 ± 7 a	62 ± 4 a	56 ± 5 b	54 ± 7 b	64 ± 7 a	57 ± 6 ab	54 ± 7 b
Shoot length (cm)	57 ± 12	56 ± 11	55 ± 11	54 ± 11	57 ± 12 a	56 ± 11 a	54 ± 11 b
Trimming weight (g/cep)	682 ± 236 a	600 ± 209 b	567 ± 159 b	546 ± 207 b	682 ± 236 a	613 ± 214 ab	546 ± 207 b
Leaf nitrogen (% dry matter)	2.3 ± 0.1	2.5 ± 0.2	2.3 ± 0.2	2.4 ± 0.3	2.3 ± 0.1	2.3 ± 0.2	2.4 ± 0.3
Leaf potassium (% dry matter)	1.6 ±0.3	1.6 ± 0.2	1.7 ± 0.3	1.8 ± 0.2	1.6 ± 0.3 b	1.6 ± 0.2 b	1.8 ± 0.2 a
Bud fruitfulness (bunches/shoot)	1.9 ± 0.2 a	1.9 ± 0.2 ab	1.9 ± 0.1 ab	1.8 ± 0.1 b	1.9 ± 0.2	1.9 ± 0.1	1.8 ± 0.1
Light-exposed leaf area (m^2/m^2 ground)	1.3 ± 0.1 a	1.0 ± 0.1 b	1.0 ± 0.1 b	1.0 ± 0.1 b	1.3 ± 0.1 a	1.2 ± 0.1 b	1.0 ± 0.1 c
Leaf-fruit ratio (m^2/kg)	1.2 ± 0.3	0.9 ± 0.2	0.9 ± 0.2	1.0 ± 0.2	1.2 ± 0.3	1.0 ± 0.1	1.0 ± 0.2

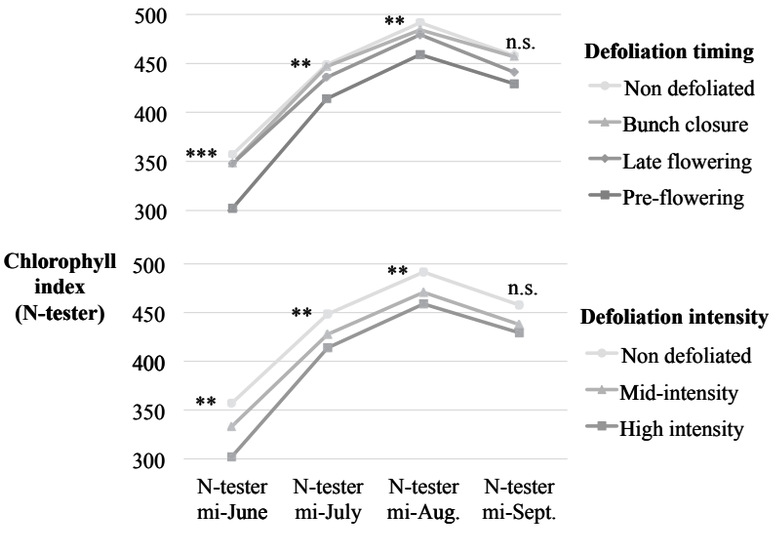

Figure 1. Impact of defoliation timing and intensity on chlorophyll index. 2013-2016 averages. * = highly significant (P value < 0,001); ** = significant (P value < 0,01); n.s. = non significant.**

solution of 1 % OsO4. The samples were then dehydrated in a graded series of ethanol solutions at 30-50-70-95-100 % (v/v) and embedded in LR White resin (14381-UC, London Resin Company, UK). After polymerisation (24 h at 60°C), semi-thin (0.8 µm) sections were cut, stained with a solution of 1 % methylene blue, sodium tetraborate and azure II, and observed using a light microscope (Leica DMLB, Leica Microsystems, Heerbrugg, Switzerland) equipped with a Leica DFC 490 FX camera. Epidermis thickness was measured using the IM50 software provided with the Leica DFC camera. During this process, four sites per berry were randomly measured from the upper epidermis to the limit between the hypodermis (tangential cell layer) and mesocarp (pulp cells).

During 2015, environmental scanning electron microscopy (ESEM) was applied to the samples in parallel to conventional transmission electron microscopy (TEM) observations. During this process, skin fragments sampled from the three berries previously described from the three bunches per treatment were pre-fixed with a solution of 3 % glutaraldehyde-2 % paraformaldehyde in a sodium cacodylate buffer, 0.07 M and pH 7.2, washed with cacodylate buffer and post-fixed with a solution of 1 % OsO4, 0.07 M and pH 7.0, for two hours. The samples were then washed 3 times with cacodylate buffer and stored in cacodylate buffer at 4°C until use. Samples were observed using a Quanta FEG 250 scanning electron microscope (FEI, Hillsboro, Oregon, United States).

5. Grape extract analyses

During three consecutive years (2014, 2015 and 2016) and for each treatment, 100 berries with pedicels were collected twice within a 15-day period, approximately two weeks before the expected harvest date and just before harvest. The berries were pressed with a pneumatic laboratory press using constant pressure (3 bar). The juice was aliquoted in two parts for further analyses.

a. Total polyphenolic content in must

The first aliquot (40 mL) was immediately protected from oxidation with the addition of 40 µl of an aqueous solution of Na_2SO_3 (120 g/L) for analysis of total phenolic content. The total phenolic content was estimated 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) are expressed as the Folin Index.

b. Glutathione determination in must

The second aliquot (40 mL) was mixed immediately with 400 µL of 25 % (m/v) ascorbic acid solution and stored at -25°C for glutathione determination. Glutathione (GSH) concentration was determined using a kinetic enzymatic recycling assay (Oxford Biomedical Research Inc., 2009, Total Glutathione (tGSH) Microplate Assay, Document Control Number: GT20.091001) based on the oxidation of

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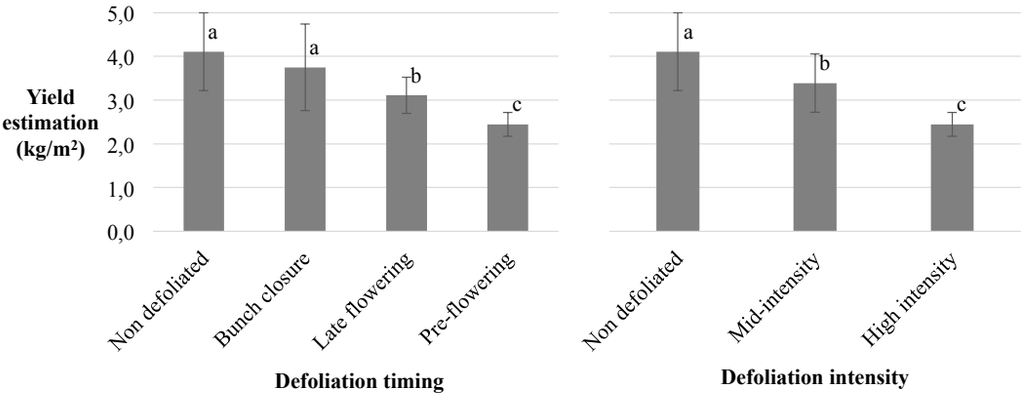

Figure 2. **Impact of defoliation timing and intensity on yield potential, estimated before bunch thinning. 2013-2016 averages ± SD. Treatments with different letters are significantly different (Newman-Keuls test, P<0.05).**

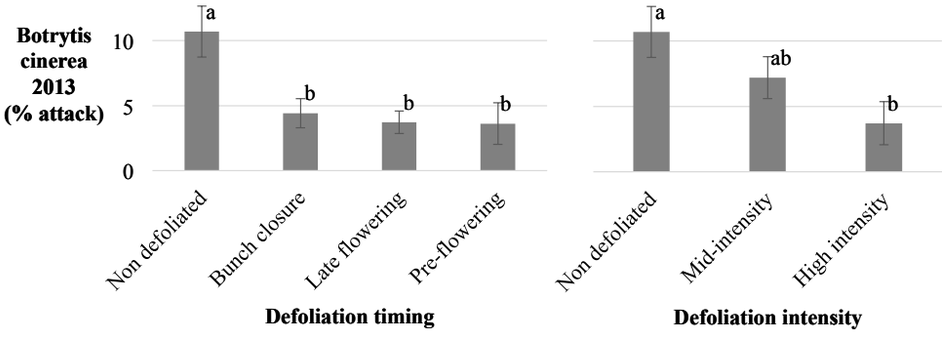

Figure 3. **Impact of defoliation timing and intensity on *Botrytis* attack on the bunches. 2013 averages ± SD. Treatments with different letters are significantly different (Newman-Keuls test, P<0.05).**

GSH by acid 5,5' dithio 2 nitrobenzoic (DTNB). The method was adapted to wine and must samples using an A25 spectrophotometric autoanalyser (BioSystems, Barcelona, Spain). Reactive 1 (R1) was composed of DTNB (60 mg/L) and glutathione reductase (400 µL/L) in a KH2PO4 buffer (125 mM) at pH 7. First, 250 µL of R1 and 5 µL of the sample were mixed in a micro-vial; then, 80 µL of NADPH at a concentration of 200 mg/L were added after 120 s, and the absorbance was measured at 405 nm after 150 and 210 s. The concentration of total GSH was calculated from the standard curve and expressed in mg/L.

6. Must and wine analyses

At harvest, must samples were collected per replicate during crushing. The general must parameters were determined using an infrared spectrophotometer (FOSS WineScan™), i.e., total soluble solids (TSS, °Brix), titratable acidity (TA, g/L as tartaric acid), tartaric and malic acids (g/L), pH, and yeast assimilable nitrogen (YAN, mg/L). Grapes from each treatment were harvested each year during one day when TSS reached approximately 18.5 °Brix. Approximately 150 kg of grapes were vinified per treatment following the standard protocol of the Agroscope Institute; the grapes were crushed, cold settled overnight at 12°C and then racked the next day. The 6-to-8-day alcoholic fermentation started at 25°C with yeast addition (Zymaflore FX10, 20 g/hL). The wines were centrifuged, and lactic bacteria were added (Viniflora CH35, 1 g/hL) to guarantee the completion of the malolactic fermentation. The wines were then stabilized (50 ppm SO_2), stored for one month at 0°C, filtrated with a 0.65-µm filter and bottled. Finished wines were analysed using an infrared spectrophotometer (FOSS WineScan™) for the following parameters: alcohol; dry weight; pH; volatile acid; titratable acidity; tartaric, malic and lactic acids; glycerol; and free and combined SO_2. The «chromatic characteristics» of the wines were described according to the CIELab procedure using 1-to-7 scale.

7. Statistical analyses

ANOVAs and Newman-Keuls multiple comparisons were completed using the statistical software ©XLSTAT 2016.01.26633 (Addinsoft, Paris, France).

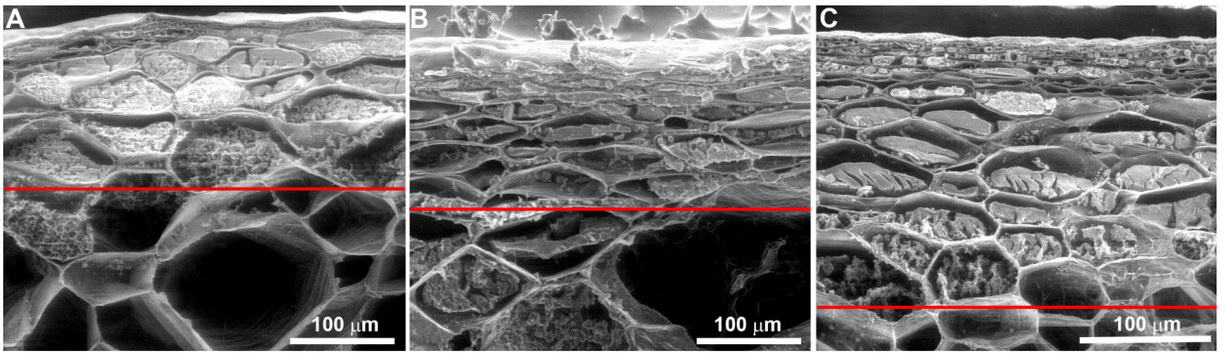

Figure 4. Environmental scanning electron microscopy (ESEM) of berry epidermal cells showing the effects of two defoliation stages on berry skin thickness at harvest 2015. A: non-defoliated control (treatment A); B: bunch-closure defoliation (treatment D); C: pre-flowering defoliation (treatment B). Berry skin thickness was measured from the upper epidermis to the limit (red line) between the hypodermis (tangential cell layer) and mesocarp (pulp cells below the line). Scale bars represent 100 µm.

Differences were considered significant when the p-value < 0.05.

RESULTS

1. Phenology and plant behaviour

The four-year results of phenology, vigour and yield parameters are presented in Table 3. The pre-flowering treatment (B) consistently showed a delay in the completion of flowering: during the flowering stage, 43 ± 18 % of the flowering was completed against an average of 51 ± 14 % for the four other treatments (A, C, D, and E). No mineral deficiency was noticed in the leaf diagnosis, and no difference could be observed except in K: defoliation intensity increased the K concentration in the leaves of the pre-floral treatment (B). In spite of the good nitrogen concentration in the leaves, the monitoring of the chlorophyll index during the season noted a consistent lower chlorophyll concentration under the pre-floral treatment, which becomes non-significant by the time of harvest (Figure 1). The intensity also played a role in the chlorophyll index variability: the mid-intensity treatment (E) presented intermediate results between the high-intensity and non-defoliated treatments.

Concerning vegetative development and vigour, the light-exposed leaf area was larger under the control treatment (A, 1.3 m^2/m^2 of soil), followed by treatment E (1.2 m^2/m^2 of soil), and then the other treatments (B, C, and D, 1.0 m^2/m^2 of soil) according to defoliation intensity. The timing of defoliation had no impact on the development of lateral shoots. However, high-intensity and pre-floral defoliation (B) reduced the shoot length during the early season by 3 cm, when compared to that under the non-defoliated treatment (A) (Table 3). Despite the variability between vintages, defoliated treatments (B, C and D) had a lower trimming weight (an average of 571 ± 205 g versus 682 ± 236 g under the non-defoliated treatment A). Mid-intensity defoliation (E) modulated the impact on the trimming weight (613 ± 214 g). Moreover, both the high-intensity and earliness of defoliation (B) induced lighter pruning weights during the winter (54 ± 7 g/m under treatment B versus 64 ± 7 g/m under treatment A) (Table 3).

Table 3. Impact of defoliation timing on vine phenology, vigour and yield parameters. Four-year averages ± SD. The values followed by different letters in the same row and same table section are significantly different (Newman-Keuls test, P<0.05).

2. Yield parameters

Intensive pre-flowering defoliation (B) induced a slightly lower bud fruitfulness (-0.1 bunch/shoot in comparison to that of the other treatments) (Table 3). Treatment B also presented different bunch structures in comparison to those of the other treatments (A, C, D, and E): bunches were globally smaller (-30 % wt.) and had fewer berries per bunch (-36 %), although their berries were not smaller (Table 3). As a consequence, the average 2013–2016 yield potential estimation showed a 40 % loss under the pre-flowering treatment (B) in comparison to that under the control treatment (A), a 24 % loss under the late-flowering treatment (C) and no significant loss under the bunch-closure treatment (D) (Figure 2). Once again, the mid-intensity treatment (E) modulated the impact of pre-floral defoliation with only an 18 % loss. Bunch thinning was still completed every year

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Table 4. Impact of defoliation timing and intensity on yield and must composition at harvest. Four-year averages ± SD. The values followed by different letters in the same row and same table section are significantly different (Newman-Keuls test, P<0.05).

	Defoliation timing				Defoliation intensity		
	Non defoliated	Bunch closure	Flowering	Pre-flowering	Non defoliated	Mid-intensity	High intensity
Yield (kg/m^2)	1.2 ± 0.2	1.2 ± 0.2	1.2 ± 0.3	1.1 ± 0.2	1.2 ± 0.2	1.2 ± 0.2	1.1 ± 0.2
Total soluble solids (°Brix)	18.2 ± 2 a	17.9 ± 2 ab	17.8 ± 2 b	18 ± 2 ab	18.2 ± 2	18.2 ± 2	18 ± 2
Total acidity (g/L tart. ac.)	7.0 ± 1.1	7 ± 1.3	7.2 ± 1.4	7.0 ± 1.4	7.0 ± 1.1	6.9 ± 1.1	7.0 ± 1.4
Tartaric acidity (g/L)	5.8 ± 0.8	5.9 ± 0.7	5.9 ± 0.9	5.8 ± 0.8	5.8 ± 0.8	5.8 ± 0.6	5.8 ± 0.8
Malic adidity (g/L)	3.5 ± 1.0	3.5 ± 1.1	3.5 ± 1	3.5 ± 1.1	3.5 ± 1	3.3 ± 0.9	3.5 ± 1.1
pH	3.3 ± 0.1 ab	3.3 ± 0.1 ab	3.27 ± 0.1 b	3.31 ± 0.1 a	3.30 ± 0.1	3.31 ± 0.1	3.31 ± 0.1
YAN (mg N/L)	181 ± 32	188 ± 32	174 ± 28	189 ± 32	181 ± 32	171 ± 28	189 ± 32
Glutathione (mg/L)	70 ± 9	70 ± 22	64 ± 16	58 ± 15	70 ± 9 a	63 ± 11 ab	58 ± 15 b

under all treatments, due to the high yield potential of Chasselas.

The leaf-to-fruit ratio at harvest did not show any difference between treatments due to high variability between the vintages (Table 3). Nevertheless, the leaf-to-fruit ratio, 1.0 ± 0.2 m^2/kg, was considered sufficiently high under all treatments to ensure complete grape maturation, according to Murisier and Zufferey (1997).

A *Botrytis cinerea* attack occurred during 2013. The control treatment (A) was the most affected treatment with 11 ± 2 % loss due to grey mould, while the treatments B, C and D (intensive defoliation) have had only 4 ± 1 % loss (Figure 3). The period of defoliation had no impact on the *Botrytis cinerea* attack. However, the intensity of defoliation played an important role against *Botrytis* development, as it affects directly the bunch microclimate in term of humidity and sun exposure.

3. Berry structure

The epidermis thickness of berries was measured using TEM as well as on ESEM images. The results did not show any differences between both microscopy techniques.

Both vintage conditions and defoliation treatments strongly affected berry skin thickness (P values < 0.0001). Berries under the control treatment (A) presented thinner epidermis (two-year average, 119 ± 21 µm), followed by those under the bunch-closure treatment (D, 138 ± 11 µm, +16 % in comparison to A) and then those under pre-flowering treatment (B, 179 ± 21 µm, +50 %) (Figure 4).

4. Must and wine compositions, sensory analysis

The results of the composition of must at harvest over four years of experiments are summarized in Table 4. The yield was constant amongst the treatments at 1.2 ± 0.2 kg/m^2. The only differences in must composition were in terms of TSS and pH measurements: TSS ranged between 17.8 and 18.2 °Brix, with the late-flowering treatment (C) consistently being less concentrated in TSS than the non-defoliated treatment (A), even if the differences were small. The late-flowering treatment (C) also presented a slightly lower pH, i.e., 3.27, versus an average of 3.30 for the other treatments (A, B, D, and E). The defoliation treatments had no impact on titratable acidity or YAN concentration.

In terms of grape extract composition, no difference was observed among Folin index values (average 8.5 ± 1.0). However, the free glutathione concentration

Table 5. Impact of defoliation timing and intensity on wine sensory profile. Four-year averages ± SD.

Defoliation timing	No defoliation	Pre-flowering	Flowering	Bunch closure	Pre-flowering	P value
Defoliation intensity	-	High	High	High	Medium	
Fruitiness	4.2 ± 0.1	4.2 ± 0.2	4.2 ± 0.2	4.1 ± 0.3	4.3 ± 0.2	0.466
Global noze appreciation	4.1 ± 0.1	4.2 ± 0.2	4.1 ± 0.2	4.1 ± 0.2	4.2 ± 0.2	0.775
Volume	4.2 ± 0.3	4.2 ± 0.3	4.1 ± 0.4	4.2 ± 0.3	4.2 ± 0.3	0.216
Overall hedonistic impression	4.1 ± 0.2	4.0 ± 0.2	4.0 ± 0.3	4.0 ± 0.3	4.1 ± 0.2	0.248

under the intensive and pre-floral treatment (B) was lower when compared to that under the non-defoliated treatment (A) (58 ± 15 against 70 ± 9 mg/L) (Table 4). This tendency was noted systematically each year during the study.As a confirmation of must composition, no differences were observed in wines between treatments in terms of tartaric and lactic acids (respectively 1.4 ± 0.2 and 2.2 ± 0.4 g/L), glycerol (6.3 ± 0.7 g/L), SO_2 total (72 ± 8 mg/L) and the Folin index (4.4 ± 0.5). The wines were visually identical according to their CIELab coordinates (results not shown). The results from the sensory analysis did not permit one to distinguish the wines from the different defoliation treatments (Table 5).

DISCUSSION

The results of the present work showed the strong impact of pre-flowering defoliation on vine physiology and yield. The following points allow us to consider the possible implementation of pre-floral defoliation in the common practices. The differences among the treatments were consistent within each vintage and can be summarized as follows.

1. Impact on yield parameters

Pre-floral defoliation significantly reduced berry-set rate and, thus, induced a decrease of approximately 30 % in the potential yield before bunch thinning, as also reported by Palliotti *et al.* (2012). One can suppose a major trophic competition between the growing canopy and inflorescences, as suggested by Verdenal *et al.* (2107). A good recovery was observed in lateral shoot development, as mentioned by Poni *et al.* (2006): the light-exposed leaf area was maintained, and the balanced leaf-to-fruit ratio at harvest allowed for the proper maturation of the grapes under all defoliated treatments (B, C, D and E), as advised by Murisier and Zufferey (1997).

2. Reduction of vigour

However, the bud fruitfulness slightly decreased under the pre-floral treatment (B) in comparison to that under the non-defoliated treatment (A). The vigour, i.e., shoot length and trimming and pruning weights, was also affected by intense pre-flowering defoliation (B). Moreover, the leaf potassium concentration slightly increased and the chlorophyll index decreased under the pre-floral treatment (B). These results prove the presence of a carryover effect during the year following pre-floral defoliation, which confirms the warning results from other studies (Risco *et al.* 2014; Uriarte *et al.* 2012). Indeed, intensive defoliation can induce lower carbohydrate reserves in the vine by dormancy, affecting bud fruitfulness the following year (Bennet *et al.* 2005; Noyce *et al.* 2016). In the context of this study, carryover symptoms – i.e., smaller bud fruitfulness, lower chlorophyll index, phenological delay, lighter trimming and pruning weights – were not pronounced after four years of defoliation, and vine sustainability was not affected (in terms of longevity, health, vine balance and grape composition), but it could definitely have become an issue under more restrictive conditions. However, under the exact same pedo-climatic conditions (the same site, experimental treatments and methods), cv. Pinot noir did not show any carry overover effects after six years of pre-floral defoliation (Verdenal *et al.* 2017). This comparison demonstrates the importance of cultivar genetics in their physiological responses to pre-floral defoliation.

3. Berry skin thickness and Botrytis cinerea development

Berry skin thickness significantly increased under the pre-flowering treatment (B) in comparison to that under the control treatment (A), as observed for Pinot noir by Verdenal *et al.* (2017). This effect would offer not only physical and but also chemical resistance to infection: higher concentration of active anti-*Botrytis* compounds in the berry skin would help in the resistance to biotic stresses (Fournioux and Adrian 2011; Pezet *et al.* 2003; Spring *et al.* 2013). As an example, polymeric proanthocyanidins inhibit macerating enzyme activities crucial to *B. cinerea* development (Perret *et al.*, 2003; Deytieux-Belleau *et*

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al. 2009). In the present trial, the 2013 bunch rot attack confirmed the defoliation efficiency against *Botrytis cinerea*. However, due to local climatic conditions, a *Botrytis* attack occurred only once, which was not sufficient to confirm the correlation between berry skin composition and grape resistance. On the other hand, this resistance was clearly related to defoliation intensity, which reduces humidity and creates an unfavourable microclimate for fungus inoculation (Zoecklein *et al.* 1992; Percival *et al.* 1994; Sternad Lemut *et al.* 2015). In addition to this result, Kunz *et al.* (2006) suggested that early UV light exposure may also be beneficial to plants by increasing cellular immunity to pathogens. In terms of methodology, one interesting result is the reliability of both microscopy techniques used in this work. The ESEM technique is less time-consuming compared to TEM, especially when numerous samples have to be embedded and cut.

4. Physiological response to abiotic stress

A lower free-glutathione concentration in grape extracts under the pre-floral treatment (B) suggests that glutathione could have played a role as an antioxidant in responding to pre-floral defoliation and early grape exposure to sunlight. Verdenal *et al.* (2017) obtained the same results on Pinot noir and developed the following hypothesis: for the same total-glutathione concentration, the free-glutathione concentration might be lower as a result of the higher bounded-glutathione concentration, due to the higher level of UV stress (Chanishvili *et al.* 2005; Pastore *et al.* 2013).

5. Impact of defoliation on wine composition and overall appreciation

Only negligible differences, i.e., TSS and pH, could be observed in must composition, but these had no consequence on the wine composition in the end. Pre-floral defoliation usually increases polyphenolic concentration in red cultivar berry skins (Šuklje *et al.* 2014; Osrečak *et al.* 2016; Sivilotti *et al.* 2016). However, Chasselas grapes, as a white cultivar, contain no anthocyanins, and there is usually no skin maceration in the winemaking. These two points greatly reduce the role of pre-floral defoliation on wine quality, as there is no enological interest in terms of polyphenol accumulation and colour intensity in white wine, in contrast to red wine. As a confirmation in the present trial, no difference was observed between the wines from the five treatments. No difference was observed in terms of polyphenolic concentration in the musts (Folin index).

CONCLUSION

Pre-flowering defoliation resulted in major effects on vine physiology – i.e., reduction of berry set rate and yield, modifications in berry structure, reduction of vigour – and represents an interesting sustainable practice to control yield and enhance resistance to pathogens under the temperate climate of Switzerland. These results are possibly related to the competition between the growing canopy and the inflorescences for assimilates during the early season. Therefore, the intensity of pre-flowering defoliation allows for the modulation of its impact. Hypotheses regarding the role of glutathione, as an antioxidant against UV stress, were based on the results and confirmed in earlier publications. However, this practice also presents risks, as it can affect vine vigour and thus can potentially reduce vine sustainability under restrictive conditions. Thus, the implementation of pre-floral defoliation should be considered depending on the local conditions – i.e., regional climatic condition, vegetal material health, compatibility with other practices. Regarding white cultivars such as Chasselas, pre-flowering defoliation has only insignificant consequences on wine composition and sensory parameters, as colour intensity and tannin composition are not considered in white winemaking.

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