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Pre-flowering defoliation affects berry structure and enhances wine sensory parameters

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Abstract

Aim: The objective of this work is to investigate the effects of defoliation on cv. Pinot noir under the mild-climate conditions of Switzerland, with particular attention to berry anatomical traits and wine sensory parameters.

Methods and results: Defoliation (removal of 6 basal leaves + 6 lateral shoots per shoot) was completed at three developmental stages of grapevine, i.e., pre-flowering, late flowering and bunch closure. These experimentations were performed repeatedly over six years. In addition to the vintage effect, pre-flowering defoliation had a consistent impact on vine agronomic behaviour. The yield was highly affected by the technique (-30 %). The berry skin thickness doubled, and the polyphenol concentration increased significantly. The free glutathione concentration in the must decreased.

Conclusion: Leaf removal at early pre-flowering stage had tremendous consequences on the vine agronomic performance, mainly to the detriment of berry set, thus having a great impact on yield, berry skin thickness, must composition, and wine composition.

Significance and impact of the study: Hypothesis about the competition for assimilates between the growing canopy and the inflorescences during the early season was developed. Furthermore, the role of glutathione and anthocyanins – as antioxidants against UV stress – was interpreted, demonstrating that grapevine is able to adapt to abiotic stresses and ensure a sustainable development.

Key words: defoliation, skin thickness, anthocyanin, glutathione, millerandage, UV, wine sensory parameters

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Introduction

Grapevine defoliation in the cluster zone is a common practice in most vineyards to improve the microclimate and prevent the development of *Botrytis cinerea*. It is usually realized between the phenological stages berry set and veraison. During the last decade, researchers have shown great interest in pre-flowering defoliation, which is presented as an interesting alternative technique to later-stage defoliation in terms of disease control, yield management and must and wine composition manipulation (Sternad Lemut *et al.,* 2015).

Pre-flowering defoliation strongly affects berry set, berry number per bunch and yield (Kotseridis *et al.,* 2012; Poni and Bernizzoni, 2010; Sabbatini and Howell, 2010). The defoliation impact on berry set mainly depends on vintages (Hed *et al.,* 2015) and grape variety (Kotseridis *et al.,* 2012). Yield reduction can exceed 40 % in extreme cases, which is mainly due to the reduction of the bunch size (Gómez *et al.,* 2012; Uriarte *et al.,* 2012). Bunches showing fewer berries are usually less compact (Kotseridis *et al.,* 2012; Palliotti *et al.,* 2012). Berry size is also reduced and leads to a modification of the skin-to-pulp ratio (Poni *et al.,* 2006). However, fruit set and yield are not affected when defoliation is completed after berry set (Feng *et al.,* 2015; Nicolosi *et al.,* 2012; Tardaguila *et al.,* 2008).

In most cases, grapevine is able to recover from severe pre-flowering defoliation. Higher photosynthetic activity and water use efficiency are recorded in the remaining leaves (Chanishvili *et al.,* 2005; Filippetti *et al.,* 2011; Palliotti *et al.,* 2012). The total leaf area is larger mainly because of more active lateral shoot production (Poni *et al.,* 2006; Tardaguila *et al.,* 2008), resulting in a sufficient leaf-to-fruit ratio for ripening.

Nevertheless, pre-flowering defoliation induces a strong competition for assimilates between vegetative and reproductive organs; the major part of photosynthetically effective foliage is plucked off at a time of high C and N requirements by the inflorescences, forcing the vine to further dig into its reserves, in the wood and roots (Candolfi-Vasconcelos and Koblet 1990). Consequently, during the year following defoliation, a lower vigour was noted (Palliotti *et al.,* 2012), as well as a lower bud fruitfulness and fruit set (Risco *et al.,* 2014; Uriarte *et al.,* 2012), suggesting a strong carry-over effect due to pre-flowering defoliation. In those situations, lower carbohydrate accumulation in the storage organs could limit the development of inflorescence primordia in latent buds, leading to the abortion of mature inflorescences (Noyce *et al.,* 2016). In other situations, no carry-over effects could be observed because the vines have enough reserves (Acimovic *et al.,* 2016).

Bunch area defoliation is known to be efficient against *Botrytis cinerea* development because of better aeration and exposure to sunlight (Hed *et al.,* 2015; Sabbatini and Howell, 2010). Pre-bloom defoliation is even more efficient due to smaller bunch size (Vilanova *et al.,* 2012). Percival *et al.,* (1993) observed a thicker layer of epicuticular waxes and cuticle when berries were exposed to more sunlight and noted the correlation of this thickening with bunch rot resistance. Poni and Bernizzoni (2010) confirmed a higher relative berry skin mass in defoliated vines, which also potentially led to better resistance against berry sunburn.

Pre-flowering defoliation could drastically affect the must composition; the concentration of total soluble solids in the must is usually increased 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), which enhances colour intensity and stability in red wines. Finally, the concentration of volatile compounds is increased, which may enhance wine aroma quality (Vilanova *et al.,* 2012).

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). The impact of pre-flowering defoliation on yield and grape composition is extremely difficult to predict, as it is a function of multiple parameters, such as pedoclimatic conditions, vegetal material, sink-source balance and vine vigour.

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

Year	Total precipitations (mm)	Mean temperatures (°C)
2010	812	10.1
2011	619	11.4
2012	970	10.9
2013	1047	10.2
2014	1005	11.7
2015	686	11.6

Pre-flowering defoliation seems to be promising under the mild-climate conditions of Switzerland. Nevertheless, considering the heterogeneity of the results presented above and the risk of excessive yield loss due to this practice, the present work was required to investigate the effects of pre-flowering defoliation on cv. Pinot noir under the Swiss local conditions, in comparison to alternative defoliation timing, with particular attention to its effects on yield reduction, berry anatomical traits and wine sensory parameters.

Materials and methods

1. Vineyard site and material

The experiment was conducted over 6 years (2010-2015) in the experimental vineyards of Agroscope (Pully, Vaud, Switzerland) on field-grown *Vitis vinifera* L. cv. Pinot noir (clone FAW-1) at an altitude of 460 m. The vines were grafted onto rootstock 3309C and then planted in 1991 at a density of 5880 vines/ha (2.00 x 0.85 m).

The average temperature during the vine growing season (April-October) is 15.7°C, and the total annual precipitation reaches 1150 mm (average 1981-2010, Pully meteorological station, www.meteosuisse.ch). Annual total precipitations and mean temperatures in the Leman region for the study period (2010-2015) are shown in Table 1. The vineyard soil is a non calcareous colluvial soil containing 15 wt.% clay, 47 wt.% sand and 4 wt.% total $CaCO_3$. The soil organic matter content was 1.7 wt.%, and there were no deficiencies of essential elements, such as P, K, Mg or B. The water-holding capacity is high (> 250 mm). Annually, 30 kg N/ha were applied early in the season (stage 3-5 leaves) on the ground from 2012 to 2015. The vines were pruned using a Cordon training system with 8 shoots/plant. The canopy was trimmed at 110 cm high. The lateral shoots were removed from the fruiting zone at 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 four treatments of 10 vines each (A, B, C, D) consisting of four defoliation timings (Table 2): A) a non-defoliated control treatment, B) defoliation at pre-flowering stage (phenological stage BBCH 57, Baggiolini H), C) defoliation at late flowering stage (BBCH 67-69, Baggiolini I) and D) defoliation at bunch-closure stage (BBCH 77, Baggiolini L). The same intensity of defoliation was applied in all defoliated treatments, i.e., all six primary leaves and lateral shoots from the base of each shoot were plucked off.

3. Field measurements and plant sampling

At flowering stage, phenological differences between the different treatments were estimated by counting the percentage of fallen flowerhoods on 25 inflorescences. The same estimation was conducted at veraison stage, with berries developing colour on 25 bunches. Shoot trimming was conducted two to four times during the season depending on the vintage, and 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 at veraison using Carbonneau's method (1995). The length of the penultimate shoot on the cordon was measured on each vine early in the season (when they reach 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-meter long canes selected in the middle of the cordons. A high millerandage rate – i.e., high proportion of shot berries – occurred in 2010 and 2013 and an average per replicate was hence estimated before bunch thinning on 25 bunches using a percentage scale (0; 10; 25; 50; 75; 90; 100).

The chlorophyll index was monitored once a month between flowering and harvest using an N-tester (Yara, Paris) in the medial zone of the canopy. A leaf diagnosis was completed per treatment every year at

Table 2. Description of the four treatments. The lateral shoots were removed in the fruiting zone of all treatments. Defoliation of treatments B-C-D consisted of removing the first six basal main leaves of each shoot.

Treatment	BBCH scale	Date of defoliation					
		2010	2011	2012	2013	2014	2015
A: Control treatment, no defoliation	-	-	-	-	-	-	-
B: Pre-flowering stage defoliation	57	June 4	May 9	May 24	June 11	May 22	May 22
C: Flowering stage defoliation	67-69	June 25	May 30	June 18	July 2	June 12	June 9
D: Bunch-closure stage defoliation	77	July 28	June 30	July 13	Aug. 5	July 21	July 13

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 in July (before bunch closure) from a sample of 50 berries and 10 bunches per replicate using the following equation:

$$\text{potential yield (kg/m}^2\text{)} = \frac{\frac{\text{bunch wt}_{\text{July}} \times \text{berry wt}_{\text{harvest}}}{\text{berry wt}_{\text{July}}} \times \text{bunch nb}_{\text{vine}}}{\text{plantation density} \times 1000}$$

where berry wt $_{\text{July}}$ and bunch wt $_{\text{July}}$ are the average berry and bunch weights in July (stage BBCH 75-77), respectively; berry wt $_{\text{harvest}}$ is the average berry weight at harvest for Pinot noir in Pully since 2005, i.e., 1.7 g; and bunch nb $_{\text{vine}}$ is the bunch number per vine.

Bunch thinning was applied before bunch-closure stage (BBCH 77) in 2011, 2012, 2014 and 2015, the target being 1.0 kg/m^2. Berry weight was estimated at harvest from a sample of 50 berries per replicate. Bunch weight was estimated at harvest from the following equation:

$$\text{bunch wt}_{\text{harvest}} = \frac{\text{yield}_{\text{vine}}}{\text{bunch nb}_{\text{vine}}}$$

An attack by *Botrytis cinerea* occurred in 2012. It was quantified per replicate by the percentage of rotten berries per bunch on 25 bunches.

4. Microscopy

In 2013 and 2015, bunch samples were collected before harvest to evaluate berry skin thickness in 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 0.07 M phosphate buffer at pH 7 and embedded in 2 % agarose and post-fixed with a 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 IM50 software provided with the Leica DFC camera. For this, four sites per berry were randomly measured from the upper epidermis to the limit between the hypodermis (tangential cell layer) and mesocarp (pulp cells).

5. Grape extract analyses

During three consecutive years (2013, 2014 and 2015) and for each treatment, 300 berries with pedicels were collected twice within a 15-day period, approximately two weeks before the expected harvest date and just before harvest. 100 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 was immediately protected from oxidation with the addition of an aqueous solution of Na_2SO_3 (120 g/L) for the 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 dilution factor) are expressed as 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 by 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 GSH by acid 5,5'-dithio-2-nitrobenzoic (DTNB). The method adapted the wine and must samples for an A25 spectrophotometric autoanalyser (BioSystems, Barcelona, Spain). Reactive 1 (R1) was composed of DTNB (60 mg/L) and glutathione reductase (400 µL/L) in KH_2PO_4 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.

c. Total free anthocyanins and anthocyanin profile

Another 100 berries were ground in a mixer (Memory Blender, Switzerland) for 1 min. Approximately 50 g of this mixture was transferred into a glass bottle (250 mL) and weighed precisely before adding 50 mL of Glories solution (aqueous solution of HCl, pH 1). The sample was held for 4 h at 20°C under gentle agitation (200 rpm) to extract most of the anthocyanin content. After 4 h, the supernatant was separated by centrifugation (15 min at 3000 rpm) and used for the determination of total free anthocyanins and for the anthocyanin profile.

Total anthocyanin content was determined using the Puissant-Léon method (Ribéreau-Gayon *et al.,* 1998), which was adapted to an autoanalyser (A25, BioSystems, Barcelona, Spain) by adding 380 µL of 1 % HCl to 20 µL of sample and by measuring the absorbance at 520 nm after 300 s. The results are expressed in mg of malvidin-3-O-glucoside per litre of wine extract.

The determination of the most important free anthocyanins was adapted from the OIV-MA-AS315-11 method (OIV 2016) using an Agilent 1200 HPLC instrument equipped with a DAD detector (Agilent, Germany) and a data acquisition and analysis system (Agilent ChemStation, version B.04.03-SP2). First, 10 µL of sample were injected, and the compounds were separated on a silicon-based reverse phase column Zorbax Eclipse Plus C18 (4.6 mm x 50 mm, 1.8 µm; Agilent, Germany) using water/formic acid/acetonitrile 88:10:2 (v/v/v, solvent A) and then water/formic acid/acetonitrile 2:10:88 (v/v/v, solvent B) at a flow rate of 2 mL/min as mobile phases. Before each analysis, the column was first equilibrated with 100 % solvent A for 2 min at 25°C. The gradient started with 0 % B (0 to 0.2 min) to reach 40 % B at 5.2 min and finally 100 % B at 6 min, which corresponds to the total run time (the entire analysis duration was 8 min). Anthocyanins were detected at 520 nm, and the profile was expressed in percentage of peak area compared to the total peak area. The acetylated forms and then the coumaroylated forms of anthocyanins were not given independently, but as a group.

6. Must 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). The concentration of ammonium and free primary amino acids was determined on an A25 spectrophotometric autoanalyser (BioSystems, Barcelona, Spain) using well described commercial methods; 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, Spain), which used δ-phthalaldehyde/N-acetyl-cysteine as a reagent (also called NOPA method). YAN was calculated as the sum of nitrogen (mg/L) in the form of ammonium and free primary amino acid.

7. Winemaking and analyses

Grapes from each treatment were harvested each year in one day when TSS reached approximately 22 °Brix. Approximately 60 kg of grapes were vinified per treatment following the standard protocol of the Agroscope Institute; the grapes were destemmed, the 6-to-8 day alcoholic fermentation was immediately started at 25°C with yeast addition (Zymaflore FX10, 20 g/hL) and the cap was punched down daily. The wines were then pressurised, centrifuged and lactic bacteria were added (Viniflora CH35, 1 g/hL) to guarantee the completion of malolactic fermentation. The wines were then stabilized (50 ppm SO_2), kept one month at 0°C, filtrated with 0.65 µm filters 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 total phenolic content was estimated by measuring the absorbance of the sample at 280 nm (Ribéreau-Gayon *et al.,* 1998). The results (absorbance at 280 nm corrected by dilution factor) are expressed as Total Polyphenol Index.

The total anthocyanin content and the determination of the most important free anthocyanins were evaluated in the wine using the same methods described above for the grape analysis.

The «chromatic characteristics» of the wines were described according to the CIELab procedure using the OIV-MA-AS2-11 method as a reference (OIV, 2016). The absorption spectra were obtained on a classic spectrophotometer (Cary 60 UV-VIS, Agilent), and the chromaticity coordinates and derived magnitudes (Chroma and Tone) were calculated with a dedicated algorithm software (Agilent Cary WinUV Color, Agilent).

A sensory analysis was completed every year; the trained Agroscope panel described the wines according to pre-defined criteria using a 1-to-7 scale.

In 2016, the 2010-2014 wines were tasted again to evaluate and compare the ageing potential.

8. Statistical analyses

ANOVAs, Newman-Keuls multiple comparisons and principal component analysis were completed using statistical software ©XLSTAT 2016.01.26633 (Addinsoft, Paris). Differences were considered significant when p-value < 0.05.

Results

1. Phenology and plant behaviour

The six-year results on phenology, vigour and yield parameters are presented in Table 3. The pre-flowering treatment (B) consistently showed earliness: at flowering stage, 72 ± 8 % of flowering was completed against an average of 57 ± 13 % in the three other treatments (A, C, D). This tendency was confirmed at veraison stage; the two latest leaf defoliation treatments (C) and (D) showed a delay (-9 % on average) in comparison to the pre-flowering and control treatments (B) and (A). No nitrogen deficiency was noticed in the leaf diagnosis; nevertheless, the control treatment (A) had a lower concentration (2.19 % dry weight) compared to defoliated treatments. Concerning vigour, no differences were observed in neither shoot length in the early season nor in trimming and pruning weights in the winter. The light-exposed leaf area was larger in the pre-flowering defoliated treatment (B), which presented more developed lateral shoots when compared to the two other defoliated treatments (C and D).

2. Yield parameters

The average bud fruitfulness was 1.7 ± 0.2 bunches per shoot for all treatments. No carry-over effect due to early defoliation was noticed during the entire trial. However, the early defoliation treatments (B and C) presented very different bunch structures in comparison to the bunch-closure defoliation and control treatments (D and A). Their clusters were globally smaller (-28 % wt.); they had fewer berries per bunch (-33 %), and their berries were smaller (-0.1 to -0.2 g). As a consequence, the average 2011-2015 yield potential estimation showed a 35 % loss in the pre-flowering treatment (B) in comparison to the control treatment (A), a 25 % loss in the flowering treatment (C) and no significant loss in the bunch-closure treatment (D) (Figure 1). Bunch thinning was completed only once in six years in the pre-flowering treatment (B) and every year in the control and bunch-closure treatments (A and D) (Table 3).

Even if it was not significant, the leaf-to-fruit ratio at harvest tended to be higher in the pre-flowering treatment (B) in comparison to the later defoliated treatments (C and D), which was due to both a lower yield and a larger light-exposed leaf area (Table 3).

Table 3. Impact of defoliation timing on vine phenology, vigour and yield. Six-year averages ± SD.

Treatment	A	B	C	D	P value
Defoliation stage	Control No defoliation	Pre-flowering stage	Flowering stage	Bunch-closure stage	
Flowering (%)	58 ± 13 b	72 ± 8 a	56 ± 14 b	58 ± 12 b	**<0.0001**
Veraison (%)	51 ± 9 a	52 ± 14 a	44 ± 6 ab	40 ± 9 b	**0.008**
Leaf nitrogen (% dry matter)	2.19 ± 0.08 b	2.34 ± 0.06 a	2.25 ± 0.08 ab	2.32 ± 0.17 a	**0.006**
Shoot length (cm)	43 ± 5	44 ± 6	42 ± 6	40 ± 5	1.000
Trimming weight (g/vine)	470 ± 176	518 ± 181	475 ± 144	488 ± 126	0.331
Pruning weight (g/m)	62 ± 6	61 ± 3	61 ± 4	63 ± 6	0.406
Light-exposed leaf area (m^2/m^2 ground)	1.4 ± 0.1 a	1.2 ± 0.1 b	1.1 ± 0.1 c	1.1 ± 0.1 c	**<0.0001**
Bud fruitfulness (bunches/shoot)	1.7 ± 0.1	1.7 ± 0.2	1.7 ± 0.1	1.7 ± 0.2	0.427
Bunch weight at harvest (g)	176 ± 52 a	119 ± 40 b	124 ± 34 b	165 ± 46 a	**<0.0001**
Number of berries per bunch	151 ± 31 a	98 ± 13 b	106 ± 23 b	148 ± 23 a	**<0.0001**
Berry weight at harvest (g)	1.6 ± 0.3 a	1.4 ± 0.3 b	1.4 ± 0.3 b	1.5 ± 0.3 a	**<0.0001**
Bunch thinning (removed per vine)	3 ± 3 a	0 ± 1 ab	2 ± 2 b	3 ± 2 a	**0.004**
Leaf-to-fruit ratio (m^2/kg)	1.6 ± 0.3	1.8 ± 0.8	1.4 ± 0.3	1.3 ± 0.2	0.051
Yield (kg/m^2)	0.9 ± 0.1 a	0.8 ± 0.2 b	0.8 ± 0.1 b	0.9 ± 0.1 ab	**0.009**
Botrytis cinerea attack in 2012 (%)	8.3 ± 3.0 a	0.3 ± 0.2 b	0.2 ± 0.2 b	1.2 ± 0.9 b	**0.000**

The values followed by different letters in the same row are significantly different (Newman-Keuls test, P<0.05).

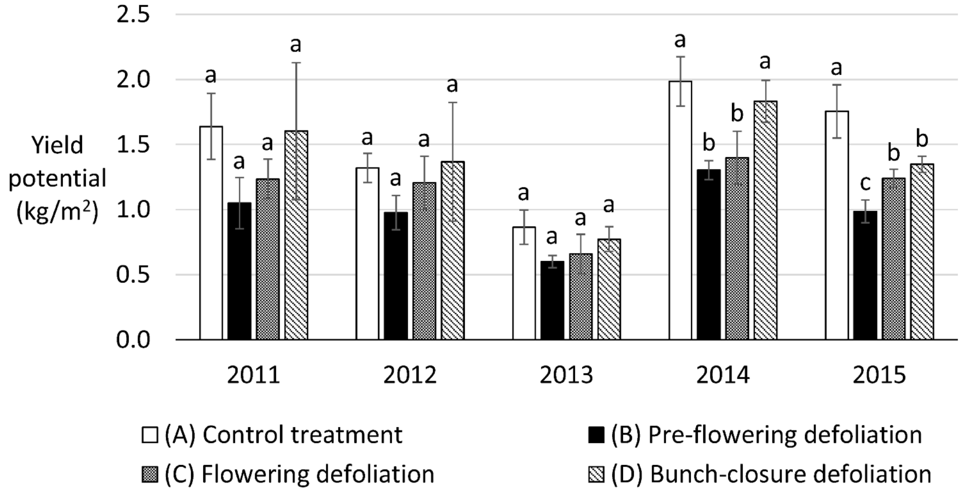

Figure 1. Impact of defoliation timing on yield potential, estimated before bunch thinning. 2011-2015 averages ± SD. Treatments with different letters are significantly different (Newman-Keuls test, P<0.05).

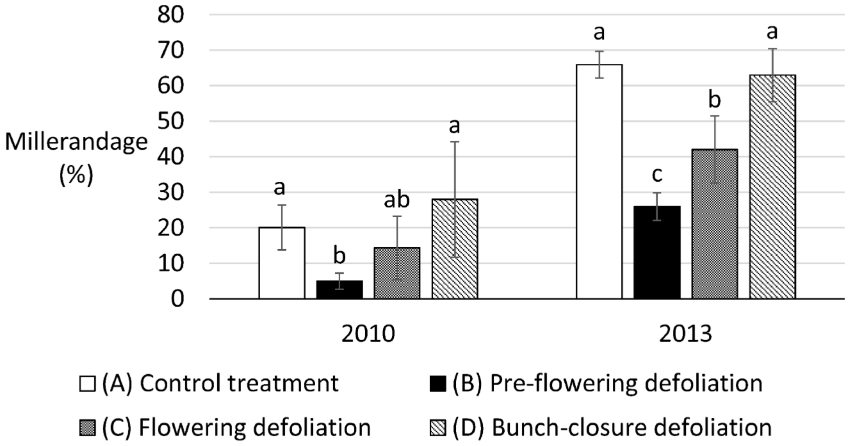

Figure 2. Impact of defoliation timing on the development of millerandage symptoms. Treatments with different letters are significantly different (Newman-Keuls test, P<0.05).

Nevertheless, the leaf-to-fruit ratio was considered as high enough in all treatments to ensure complete grape maturation, according to Murisier and Zufferey (1997). High millerandage rates were recorded in 2010 and 2013: both years, the earlier the defoliation, the lower the millerandage rate, while no differences were noticed between the control and the bunch-closure treatments (A and D) (Figure 2).

A *Botrytis cinerea* attack occurred in 2012. The control treatment (A) had an 8 % loss due to grey mould, while the three defoliated treatments had less than a 2 % loss (Table 3).

3. Berry structure

Defoliation treatments significantly affected berry skin thickness (P value < 0.0001), while the vintage effect was negligible. Berries in the control treatment (A) presented thinner skins (two-year average, 110 ± 8 µm), followed by the bunch-closure treatment (D) (149 ± 13 µm) and then the pre-flowering treatment (B) (219 ± 17 µm) (Figure 3). These results had consequences on the grape composition as presented below.

4. Grape extract analysis

The free glutathione, Folin index and anthocyanin concentrations and profiles are presented in Table 4. The free glutathione concentration is not significantly influenced by the period of defoliation (P value = 0.063); however, the defoliated treatments (B and C) tended to have a lower glutathione concentration (average 37 mg/L) than the control and bunch-closure treatments (A and D; average 45 mg/L). The Folin index measured from the grape extract did not show any significant difference between treatments.

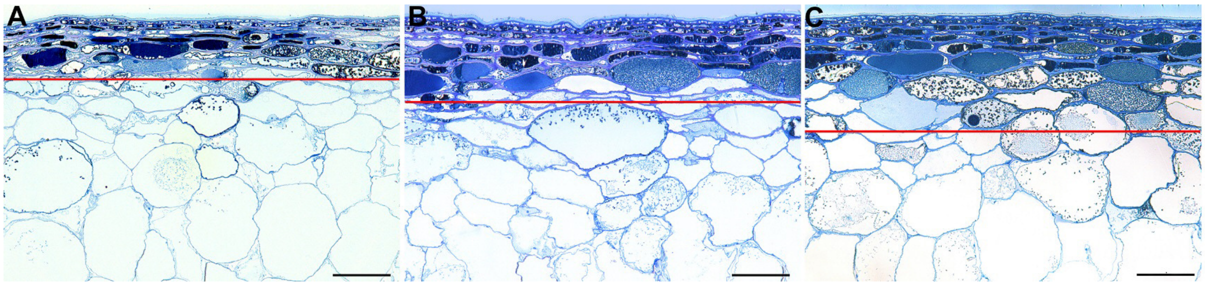

Figure 3. Semi-thin sections of berry epidermal cells showing the effects of two defoliation stages on berry skin thickness at harvest 2013. A: non-defoliated control (treatment A); B: bunch-closure defoliation (treatment D); C: pre-flowering defoliation (treatment B). Scale bars represent 100 µm.

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

Treatment	A	B	C	D	
Defoliation stage	Control No defoliation	Pre-flowering stage	Flowering stage	Bunch-closure stage	P value
Free glutathione (mg/L)	46 ± 8	35 ± 10	39 ± 11	44 ± 5	0.063
Folin index	25.6 ± 0.9	28.3 ± 1.8	27.5 ± 1.6	25.9 ± 1.2	0.553
Total anthocyanins (mg/L)	386 ± 40 b	512 ± 52 a	483 ± 60 a	475 ± 118 a	**0.027**
Delphinidin (%)	7.4 ± 1.2 b	8.9 ± 1.5 a	8.7 ± 1.6 a	8.6 ± 1.6 a	**0.008**
Cyanidin (%)	2.5 ± 0.3	2.7 ± 0.3	2.5 ± 0.2	2.7 ± 0.4	0.065
Petunidin (%)	8.5 ± 0.8 b	9.5 ± 1.0 a	9.5 ± 1.2 a	9.3 ± 0.9 a	**0.004**
Peonidin (%)	19.8 ± 1.2	19.6 ± 0.9	18.6 ± 1.4	19.1 ± 1.4	0.058
Malvidin (%)	61.1 ± 2.7	59.0 ± 2.2	60.2 ± 1.6	59.8 ± 2.0	0.202
Acetyl anthocyanins (%)	0.7 ± 0.8	0.3 ± 0.3	0.4 ± 0.3	0.4 ± 0.4	0.396
Coumaroyl anthocyanins (%)	0.1 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.1	0.455

However, the anthocyanin concentration was highly increased by defoliation, irrespectively of the period of leaf removal; the control treatment (A) had significantly less anthocyanins (average 386 mg/L) than the three other defoliated treatments. The anthocyanin profile was also affected by leaf removal; the control treatment (A) had lower delphinidin and petunidin proportions. Even if not significant, treatment (A) tended to have less cyanidin and more peonidin, while malvidin and the acetyl/coumaroyl anthocyanins were not influenced by the defoliation treatments.

5. Must composition at harvest

The results on the composition of must at harvest over six years of experiments are summarized in Table 5. No differences could be noted in either TSS or pH measurements. TA ranged between 10.4 and 11.2 g/L, with the control treatment (A) consistently being the most acidic. The flowering treatment (C) was less acidic in terms of tartaric (7.1 g/L) and malic acid (4.8 g/L). YAN was the lowest (average 132 mg/L) in the flowering treatment (C) and the highest (average 166 mg/L) in the bunch-closure treatment (D).

6. Wine composition

Table 6 presents the results regarding wine composition, which globally confirms the results obtained in the musts. No differences between treatments could be observed in terms of alcohol, volatile acidity, tartaric and malic acids, and SO_2 in the wines (results not shown). Considering the six-year averages, the control treatment (A) showed a higher titratable acidity (5.0 g/L) and a lower pH (3.57) than the defoliated treatments. The control and pre-flowering treatments (A and B) led to a slightly higher concentration of glycerol (8.7 g/L).

Table 5. Impact of defoliation timing on yield and must composition at harvest. Six-year averages ± SD. The values followed by different letters in the same row are significantly different (Newman-Keuls test, P<0.05).

Treatment	A	B	C	D	P value
Defoliation stage	Control No defoliation	Pre-flowering stage	Flowering stage	Bunch-closure stage	
TSS (°Brix)	22.1 ± 1.1	22.4 ± 1.2	22.4 ± 1.2	22.1 ± 1.1	0.069
TA (g/L tart. ac.)	11.2 ± 2.3 a	10.8 ± 2.5 b	10.4 ± 2.4 c	10.8 ± 2.6 b	**<0.0001**
Tartaric acidity (g/L)	7.6 ± 1.0 a	7.2 ± 1.0 b	7.1 ± 1.0 b	7.5 ± 1.2 a	**0.002**
Malic acidity (g/L)	5.4 ± 2.0 a	5.2 ± 2.2 a	4.8 ± 2.0 b	5.1 ± 2.2 a	**0.001**
pH	3.01 ± 0.12	3.03 ± 0.12	3.02 ± 0.12	3.02 ± 0.12	0.126
YAN (mg N/L)	150 ± 30 b	147 ± 34 b	132 ± 28 c	166 ± 34 a	**0.000**

Table 6. Impact of defoliation timing on wine composition. Six-year averages ± SD. The values followed by different letters in the same row are significantly different (Newman-Keuls test, P<0.05).

Treatment	A	B	C	D	P value
Defoliation stage	Control No defoliation	Pre-flowering stage	Flowering stage	Bunch-closure stage	
Titratable acidity (g/L)	5.0 ± 0.5 a	4.9 ± 0.5 b	4.9 ± 0.5 b	4.9 ± 0.5 b	**0.013**
pH	3.57 ± 0.1 b	3.61 ± 0.1 a	3.61 ± 0.1 a	3.58 ± 0.1 ab	**0.019**
Glycerol (g/L)	8.7 ± 0.2 a	8.7 ± 0.4 a	8.6 ± 0.3 ab	8.5 ± 0.3 b	**0.040**
Dry extract (g/L)	22.1 ± 0.5 b	22.7 ± 0.8 a	22.4 ± 0.5 b	22.1 ± 0.8 b	**0.000**
Total polyphenolic index	29 ± 3 b	33 ± 4 a	31 ± 4 ab	29 ± 2 b	**0.002**
Total anthocyanins (mg/L)	293 ± 36 b	331 ± 42 a	303 ± 58 ab	311 ± 54 ab	**0.022**
Lightness L	47 ± 5 a	41 ± 8 c	43 ± 7 bc	45 ± 7 ab	**0.001**
Colour (red/green) a	50 ± 4	52 ± 5	50 ± 5	50 ± 6	0.175
Colour (yellow/blue) b	25 ± 3 c	30 ± 4 a	29 ± 4 ab	27± 4 bc	**0.001**
Chroma C	56 ± 4 b	60 ± 5 a	58 ± 6 ab	57 ± 6 b	**0.009**
Hue H	27 ± 4 c	30 ± 3 a	29 ± 4 ab	28 ± 4 bc	**0.003**

In addition to a higher proportion of malvidin (average 78 %), the wine anthocyanin profiles were similar to the must profiles with less constancy, which is probably due to the winemaking process; only delphinidin was significantly lower in the control treatment (A) (results not shown). The pre-flowering treatment (B) regularly presented more dry extract, along with a higher polyphenol index and a higher concentration of anthocyanins (331 mg/L). As a consequence, the wines from treatment (B) regularly had a more intense colour and a more purple shade, as evidenced by their lower lightness, higher chroma, higher hue and higher colour b.

7. Wine tasting

The results from the sensory analysis are presented in Table 7. On a six-year average, the wines from the pre-flowering treatment (B) had a visually higher colour intensity. The wines of the control treatment (A) were described as less fruity and more herbaceous in comparison to those of the other treatments. In terms of mouth feel, the wines of the two early defoliation treatments (B and C) tended to have more volume and had more intensity; they presented more pleasant and structured tannins. On the whole, over the six years of experiment, the pre-flowering treatment (B) was significantly preferred to the other wines, and the control treatment (A) was classified as the least pleasant.

All the wines vinified from 2010 to 2014 were tasted a second time in 2016; for each year, the wine differences were similar to the initial tasting or no longer significant; the control treatment (A) still showed less colour intensity and gave a lower overall hedonistic impression (results not shown). Moreover, for three years out of five, the control treatment (A) had oxidized flavours, suggesting an early evolution of the wine. These results confirmed the long-term impact of defoliation on wine parameters, but they

Table 7. Impact of defoliation timing on wine sensory profile. Six-year averages ± SD. The values followed by different letters in the same row are significantly different (Newman-Keuls test, P<0.05).

Treatment Defoliation stage	A Control No defoliation	B Pre-flowering stage	C Flowering stage	D Bunch-closure stage	P value
Colour intensity	4.1 ± 0.2 c	4.4 ± 0.4 a	4.3 ± 0.3 ab	4.2 ± 0.3 bc	**0.004**
Fruitiness	4.2 ± 0.2 b	4.4 ± 0.2 a	4.4 ± 0.3 a	4.4 ± 0.2 a	**0.006**
Floral	1.9 ± 0.2	1.9 ± 0.2	2.0 ± 0.2	2.0 ± 0.1	0.369
Herbaceous	2.0 ± 0.2 a	1.8 ± 0.2 b	1.8 ± 0.2 b	1.8 ± 0.2 b	**0.030**
Spicy	2.5 ± 0.3	2.7 ± 0.5	2.6 ± 0.4	2.6 ± 0.4	0.242
Global nose appreciation	4.2 ± 0.2 b	4.4 ± 0.2 a	4.4 ± 0.2 a	4.4 ± 0.2 a	**0.015**
Volume	4.2 ± 0.2	4.4 ± 0.2	4.3 ± 0.3	4.2 ± 0.2	0.062
Acidity	4.2 ± 0.2	4.1 ± 0.2	4.2 ± 0.2	4.1 ± 0.2	0.306
Tannin intensity	4.3 ± 0.2	4.5 ± 0.2	4.4 ± 0.2	4.3 ± 0.1	**0.051**
Dry tannins	2.9 ± 0.3	2.7 ± 0.3	2.6 ± 0.2	2.6 ± 0.3	0.132
Soft tannins	3.3 ± 0.4	3.4 ± 0.4	3.5 ± 0.3	3.5 ± 0.4	0.138
Structured tannins	3.1 ± 0.3 b	3.4 ± 0.4 a	3.4 ± 0.4 a	3.1 ± 0.3 b	**0.005**
Bitterness	1.9 ± 0.2	1.8 ± 0.2	1.8 ± 0.1	1.8 ± 0.3	0.352
Overall hedonistic impression	4.0 ± 0.3 b	4.3 ± 0.3 a	4.2 ± 0.3 ab	4.2 ± 0.2 ab	**0.033**

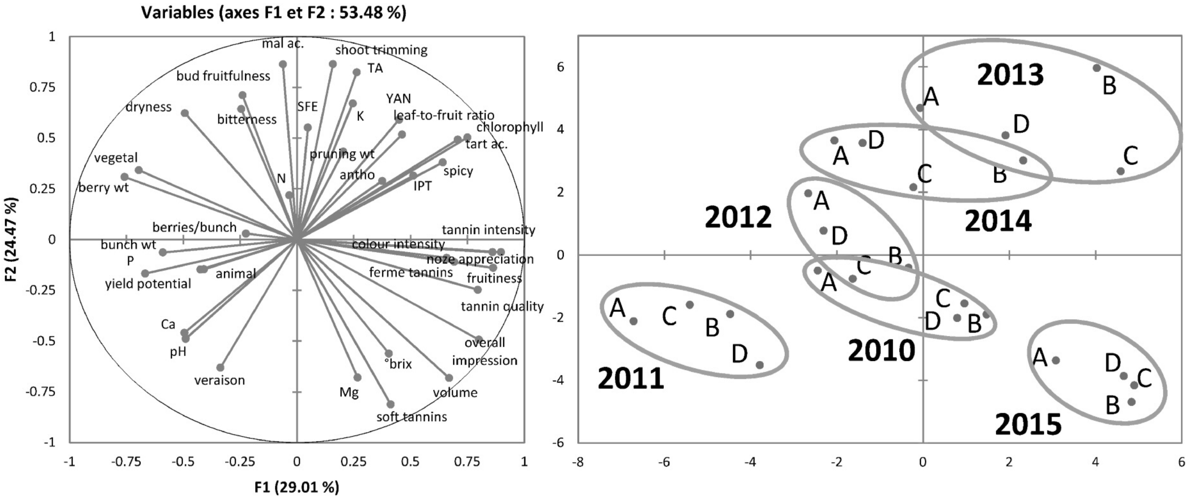

Figure 4. Principal componant analysis considering the physiological and yield parameters, must analysis and sensory analysis, from 2011 to 2015.

were not sufficient enough to allow a safe conclusion about its impact on wine ageing capacity.

Discussion

The principal component analysis (Figure 4) noted the dominant impact of the vintage on the overall grape maturation. According to Figure 1, 2013 was a vintage with a lower average berry-set rate (higher flower abortion) for all treatments, which was due to climatic conditions before and during flowering.

However, the results of the present work showed the strong impact of pre-flowering defoliation on yield and grape composition, and the differences between treatments were consistent within each vintage and can be summarized as follows:

1. Impact on agronomic and ripening parameters

A good recovery was observed in lateral leaf development, as mentioned by Poni *et al.* (2006), and there were no carry-over effects in terms of vigour and bud fruitfulness, as stated by Acimovic *et al.* (2016). Early defoliation significantly reduced berry-set rate and the millerandage rate; the potential yield before cluster thinning consequently dropped by approximately 30 % due to a lower berry-set rate and smaller clusters and berries, as also reported by Palliotti *et al.* (2012). One can suppose a major trophic competition between the growing canopy and inflorescences. The shot berries (berries without seeds) seemed to be more sensitive to the conditions of flower abortion, inducing a lower millerandage rate in the pre-flowering treatment (B). This result differs from Nicolosi *et al.* (2012), who completed defoliation only after flowering (BBCH 71).

2. Impact on berry development and morphology

In the present trial, skin thickness was doubled by the pre-flowering defoliation treatment (B; 219 µm), and anthocyanin concentration increased by 33 % in comparison to the control treatment (A), without any modification in the Pinot noir anthocyanin profile, which confirmed previous results (Feng *et al.,* 2015; Osrečak *et al.,* 2016; Pastore *et al.,* 2013; Sternad Lemut *et al.,* 2013).

3. Impact on *Botrytis cinerea* development

The 2012 bunch rot attack confirmed the defoliation efficiency against *Botrytis cinerea*. However, this resistance was not related to defoliation timing as it was observed for all defoliation treatments. Defoliation seemed to have a dual impact against *Botrytis cinerea*. First, it exposes the clusters and decreases their size, which reduces humidity and creates an unfavourable microclimate for fungus inoculation; and second, it increases the concentration of active anti-*Botrytis* compounds in the berry skin, such as polymeric proanthocyanidins, which inhibit macerating enzyme activities crucial to *Botrytis cinerea* development (Deytieux-Belleau *et al.,* 2009; Perret *et al.,* 2003). In addition, skin thickness could be closely related to the higher resistance level of berries against *Botrytis cinerea* (Fournioux and Adrian, 2011; Pezet *et al.,* 2003; Spring *et al.,* 2013). Unfortunately, in the present trial, *Botrytis* attack occurred only once in 2012, which was not sufficient to confirm the correlation between berry thickness and *Botrytis* resistance.

4. Physiological response to abiotic stress

The higher concentration of total anthocyanins in the defoliated treatments (B, C and D) confirms that total anthocyanin content can vary considerably, being affected by both biotic and abiotic stresses (i.e., genes, light, temperature, and agronomic factors) (Bueno *et al.,* 2012). In the present trial, leaf removal exposed the grapes to direct sunlight and higher temperatures, as demonstrated by Pastore *et al.,* (2013). Higher UV doses promoted the accumulation of reactive oxygen species (ROS) (Bueno *et al.,* 2012). The anthocyanins may have acted as antioxidants: their accumulation helped neutralizing ROS produced by UV stress, thus preventing cellular damage due to prolonged exposure to sunlight (Kunz *et al.,* 2006). Another trial was carried out in the same conditions on the cultivar Gamay, which is more sensitive to sunburn than Pinot noir: the pre-flowering defoliation reduced significantly the sunburn symptoms (results not published yet).

Glutathione is another crucial antioxidant element in plant cellular defence and protection (Carvalho *et al.,* 2015). In the present work, the early-defoliation treatments (B and C) tended to have a lower concentration of free glutathione. This result suggests that glutathione could have played a role in the detoxification of ROS in earlier defoliated treatments: the early-defoliation treatments may not have decreased the concentration of total glutathione, but only increased the proportion of bound glutathione (not included in the analysis) which reacted with ROS (Chanishvili *et al.,* 2005; Pastore *et al.,* 2013).

5. Impact of defoliation on wine composition and overall appreciation

Ultimately, pre-flowering defoliation had a long-term positive impact on wine composition, mainly due to a higher pH and a higher polyphenolic index, as demonstrated by other studies (Sternad Lemut *et al.,* 2013; Talaverano *et al.,* 2016). The wine tasting also confirmed the enhancement of wine colour and aromas through significant changes in the concentration of volatile compounds, as demonstrated by Vilanova *et al.* (2012) and Feng *et al.* (2017).

Conclusion

The removal of leaves at the early pre-flowering stage induced tremendous consequences on vine yield potential, berry skin thickness, resistance against *Botrytis cinerea*, grape and wine composition, and wine organoleptic properties. These results are possibly related to the competition between the growing canopy and the inflorescences for assimilates during early season. Pre-flowering defoliation was proved to be an interesting sustainable practice to control yield and enhance wine quality and resistance to pathogens in cv. Pinot noir under the temperate climate of Switzerland. Hypotheses about the role of glutathione and anthocyanins – as antioxidants against UV stress – were based on the results and confirmed earlier publications.

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