Food Research International 169 (2023) 112826

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Source–Sink manipulations have major implications for grapevine berry and wine flavonoids and aromas that go beyond the changes in berry sugar accumulation

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ARTICLE INFO

Keywords:
Cluster thinning
Defoliation
Grape ripening
Source−sink balance
Aroma compounds
Wine composition

ABSTRACT

Sugar levels in grape berries are necessary for wine production but also, they are the main driver of most ripening processes. Sugar levels are very responsive to canopy and crop load adjustments. The aim of this study is to test the effect of different levels of defoliation and cluster thinning on grape ripening and wine composition. 'Cabernet sauvignon' grapevines (*Vitis vinifera L.*) were subjected to defoliation (keeping 100 %, 66 % and 33 % of the leaves) and fruit thinning treatments (keeping 100 %, 66 % and 33 % of the clusters) combined in a factorial design. The experiment was repeated for 2 consecutive seasons (2017 and 2018) and the plants were left untreated for a third season (2019) to observe the carry-over effects of the treatments. The treatments implied precise adjustments of leaf and cluster numbers. However, the proportion of leaf area to fruit mass tended to compensate each other and interact resulting in smaller differences in leaf area or fruit mass by harvest. Berry mass was strongly reduced by defoliation even in the subsequent season where no defoliation was applied. Berry ripening indicators (soluble solids, acidity and anthocyanin levels) were also more affected by defoliation than fruit thinning. Anthocyanin profile was shifted to a higher proportion of Malvidin-derived anthocyanins for defoliated vines and lower proportion of Malvidin-derived anthocyanins in the case of thinned vines. However, when it came down to wine, the physicochemical parameters as well as the aroma profile were more affected by cluster thinning. There was a clear relationship between sugar levels of the unfermented must and many wine-aroma compounds. Green aromas (2-isobutyl-3-methoxypyrazine, hexanol and *cis*-3-Hexen-1-ol) were among those presenting a negative correlation to must sugar whereas other compounds like Isobutyric acid, Benzyl alcohol, 1-Octen-3-ol and γ-Nonalactone had a positive correlation. This study reveals a higher level of complexity of source sink relations where leaves and clusters do not only act as a source and a sink of carbon, respectively. Therefore, the results of this study should be considered before making comparisons of leaf area to fruit mass ratios across different vine-growing systems.

1. Introduction

Grapes flower late in the season (*i.e.*, May) and grape berry development and ripening are rather long processes, in most cases pushing harvest to the end of the growing season. Such a long ripening stage is in part due to the high levels of sugars accumulated in berries (Orak, 2009), necessary for wine production. In fact, the timing of commercial harvest is often influenced by the monitorization of berry must sugar content (soluble solids), which makes grape berry ripening one of the most studied among fruits. Although sugars are a very important constituent of grapes, their importance is highlighted by the numerous links between sugar accumulation and other physiological processes leading to organoleptic traits. For instance, sucrose by itself may induce the synthesis of anthocyanins in ripening berries (Dai et al., 2014). Furthermore, the transport of sugars into vacuoles during ripening leaves organic acids as one of the main substrates for cell respiration, driving the catabolism of berry acids (Rienth et al., 2016). Therefore, carbohydrate translocation within grapevine is a strong component of berry ripening through direct and indirect mechanisms.

Achieving an adequate speed of ripening and grape composition is determined by several factors such as weather, water status, plant health, and source–sink balance. Warm and dry weather is one of the

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https://doi.org/10.1016/j.foodres.2023.112826
Received 5 December 2022; Received in revised form 27 February 2023; Accepted 11 April 2023
Available online 17 April 2023

strictest requirements to obtain an optimal ripening before the end of the growing season. This is often a requirement for full-bodied red wines, whereas the heat requirement of whites or sparkling wines is lower as lower sugar content is needed to reach readiness for commercial harvest. To ensure an adequate sugar content in the grapes is achieved, cultural practices aim for an adequate canopy development, and a crop load accordingly. Insufficient vegetative growth can be the consequence of severe water deficit, nutrient deficiency or pathogens; and it leads to a reduction in overall carbon fixation and cascading effects such as reduced yields (Lemoine et al., 2013), starch accumulation and root growth (Huck & Hillel, 1983; Martínez-Lüscher & Kurtural, 2021). Studies applying defoliation revealed deeper implications for whole-plant physiology, such as the suppression of ABA signaling (Ren et al., 2006), or transpirational demand, with implications for xylem transport (Hultine et al., 2010). Conversely, a vigorous vegetative growth is usually associated to a good performance and a sign of health. However, the implications of the crop and berry composition, with excessive vigor and canopy congestion may lead to herbaceous aroma/flavor profiles (Torres et al., 2020). Shoot thinning in the first place and cluster thinning ultimately are the two cultural practices that have a greater impact on crop load (Naor et al., 2002). Leaf area (LA) to fruit mass (FM) ratio is well correlated to berry soluble solids (Kliewer & Dokoozlian, 2005; Naor et al., 2002) and thus, the number of clusters can be adjusted to ensure an adequate ripening.

Although historically promoting ripening had a positive connotation, the current progress of global warming and CO_2 concentration have risen the concerns about an excessive advance of ripening, and thus, pushing harvest dates towards the hottest period of the year which exacerbates the problem (Martínez-Lüscher et al., 2016). These concerns are mostly related to the effects of high temperature on grape berry composition, such as a reduction in the content of malic acid, anthocyanins and aroma compounds. However, there is also a reasonable questioning around the intrinsic effects of a fast/slow ripening regardless of environmental factors. In fact, treatments that slow ripening such as manipulations of source– sink ratio have been often proposed as a mean to alleviate the effects of climate change (Gutiérrez-Gamboa et al., 2020; Keller, 2010). However, few studies combine simultaneous manipulations of leaf area and crop mass, apply these more than one season or study carry over effects.

Manipulations of source–sink ratio offer a framework to expand our understanding of carbon allocation, the regulation of ripening and other deep implications of perennial crops management. In addition, studying carry-over effects (i.e., studying untreated plants subjected to treatments on previous seasons) of treatments on perennial crops allows us to understand the incremental effects of treatments in successive seasons. The aim of this experiment was to study the long-term effects of a wide range of source–sink ratio combinations on grape berry composition and wine quality.

2. Materials and methods

2.1. Experimental site and plant material

The experiment was conducted at the University of California Davis, Oakville Experimental Vineyard (38.428°, −122.409°; Oakville, CA) during the 2017, 2018 and 2019 growing seasons. The vineyard block was planted with *Vitis vinifera* L. 'Cabernet Sauvignon' clone FPS08 grafted on 110 Richter (*V. berlandieri* × *V. rupestris*) rootstock in 2008. The soil texture was a clay loam. Plants were trained to bilateral cordons and shoots were vertically shoot-positioned on 30-single bud spurs. Row and vine spacing was 2.4 m × 2.0 m, respectively, and rows were oriented Northwest to Southeast. The plants were drip-irrigated with 2 pressure compensating emitters per plant delivering 2 L/h each. The irrigation schedule from fruit-set to end of harvest delivered 0.5 of crop evapotranspiration applied on a weekly basis as in Yu and Kurtural (2020).

2.2. Experimental design and treatment application

In 2017, all vines were standardized after fruit set to 20 shoots and 30 clusters per vine and laterals were removed prior to defoliation and cluster thinning treatments. To reduce the contribution of fruit exposure to the effects defoliation, 30 % shading factor white nets of 33 cm were placed at bunch closure (E-L number 32) (Coombe, 1995) of season 2017 covering only the southwest side of the fruit zone. In 2018, all vines were thinned to 24 shoots and laterals were removed, leaving 100 % of the clusters. Treatments were applied at pepper-corn size (E-L number 29) (Coombe, 1995). The experimental design was a randomized complete block with 3 (levels of canopy size, keeping either 100 %, 66 % or 33 % of the leaves) by 3 (levels of fruit load, keeping either 100 %, 66 % or 33 % of the leaves) factorial arrangement of treatments. Each treatment combination was replicated 4 times (n = 36) and each replicate consisted of 3 vines. Leaves were removed alternatively on every shoot. For instance, 66 % leaves treatments kept leaves in positions 1st, 2nd, 4th, 5th, 7th, 8th etc. while 33 % of leaves treatments kept leaves in positions 1st, 4th, 7th, etc. in every shoot. In 2017, The 3 levels of fruit load were 100 % of the clusters left (30), 66 % of the clusters left (20) and 33 % of the clusters left (10), whereas in 2018, the final number of clusters were dependent on the cluster bearded by each vine at fruit set (Fig. S1).

In 2019, after 2 seasons of growth under the 9 combinations of defoliation and cluster thinning, the carryover effects were studied by thinning al the vines to 24-shoots and leaving all vines untreated (i.e. no defoliations or cluster thinning applied).

2.3. Berry must soluble solids, pH and titratable acidity

At every sampling point, fifty-five berries were crushed by hand and filtered to obtain must. A digital refractometer (Palette PR-32, Atago, Tokyo, Japan) was then used to measure total soluble solids (TSS) of must. A sample of 5 mL of juice was used to determine must pH and acidity using an auto titrator (Metrohm 862 Compact Titrosampler, Herisau, Switzerland) with NaOH up to pH 8.3 and results are expressed as equivalents of tartaric acid.

2.4. Berry skin and wine anthocyanins and flavonols

At every sampling point, twenty berries per experimental unit were weighed and peeled. Skins where freeze dried and ground into a fine powder. Fifty mg of skin powder were extracted with Methanlol: Water:7M HCl (69:30:1) overnight at 6 °C. Extracts were filtered and transferred into an HPLC vial for analysis following the methods in Martínez-Lüscher et al. (2019). The HPLC system was an Agilent 1260 with a diode array detector (Agilent Technologies, Santa Clara, CA, USA). The column was a C18 Agilent LiChrosphere 100 of 520 × 4 mm and 5 μm particle size. The method had a constant flow of 0.5 mL min^{-1} and two mobile phases consisting in 50 mL/L of formic acid in water (A) and 50 mL/L of formic acid in acetonitrile (B). The gradient had the following proportions of mobile phase A completed by mobile phase B: 91.5 % from 0 to 8 min, 87 % at 25 min, 82 % at 35 min, 62 % at 70 min, 50 % from 70 to 75 min and 91.5 % from 75 to 90 min. Anthocyanins and flavonols where quantified at 520 and 365 nm using Malvidin-3-*O*-glucoside and quecetin-3-*O*-glucoside as quantitative standards, respectively. The 15 anthocyanins detected were different acylations of the 5 basic anthocyanin-*o*-glucosides depending on their substituents in the B-ring (delphinidin, cyanidin, petunidin, peonidin and malvidin derivatives). The 8 flavonols detected were different glycosides of the 3 basic flavonols depending on their substituents in the B-ring (kaempferol, quercetin and myricetin derivatives) (Martínez-Lüscher et al., 2014).

2.5. Yield components

At harvest, clusters were removed, counted, and weighed for each plant in the experiment. Dormant pruning weights were recorded after pruning the vines to one bud spurs in February 2018.

2.6. Wine making

Vinification was conducted in 2018 at a commercial winery using 8 L vessels. The grapes were harvested on September 21st. Grapes were manually destemmed and crushed. Musts were left indoors at 20 °C for 24 h before inoculating them with 0.2 g/L of commercial wine yeast, *Saccharomyces cerevisiae* (Cotes des Blancs: Red Star Yeast Prod. Oakland, CA, USA). Potassium metabisulphite was added aiming for a final concentration of 50 mg/L of free SO_2 to prevent oxidation and glass beads were added when necessary to homogenate head spaces across replicates. Grape solids were left in contact with the must for 20 days and punch downs were done twice a day. Musts were pressed manually, and wines were followed up until the fermentation stopped. Malolactic fermentation was initiated with the addition of *Oenococcus oeni* culture (Viniflora Oenos, Hørsholm, Denmark) at 22 °C. The free SO_2 levels were adjusted to 30 mg/L after malolactic fermentation completed. When finished, wines were bottled, and all analyses were carried out within the following month.

2.7. Wine analyses (technological and phenolic maturity)

Wine alcohol content by volume (%Alc) was determined with an alcohol analyzer (Anton Parr, Ashland, VA, USA), residual sugars (RS) with enzymatic analysis using the Gallery automated analyzer (Thermo Fisher Scientific,Waltham, MA, USA), and pH and titratable acidity with a Mettler-Toledo DL50 titrator (Mettler-Toledo Inc., Columbus, OH, USA). Spectral absorbance of wines was measured using an Agilent Cary 100 spectrophotometer after making the pertinent dilutions to not exceed 0.8 absorbance units (AU). Total anthocyanins were determined as absorbance at 520 nm, Flavonols as absorbance at 365 nm, Color index (CI) as the sum of absorbances at 420 nm, 520 nm and 620 nm, Hue as the ratio of absorbances 520 nm/420 nm and total polyphenol index (TPI) as the absorbance at 280 nm.

2.8. Gas chromatography of IBMP

The herbaceous aroma compound 2-Isobutyl-3-methoxypyrazine (IBMP) was determined using head space (HS)- solid phase micro extraction (SPME)- gas chromatography (GC)-mass spectrometry (MS) in grapes and wines following the procedures in Chapman et al. (2004) and Koch et al. (2010). Samples of 20 berries were combined with an internal standard consisting in 100 μL of deuterated ([$^2H_3$]) IBMP (5 pL L^{-1}) and ground in at 4 °C with a Power Gen 1800D tissue homogenizer (Fisher Scientific, PA-USA). Aliquots of 5 mL of ground berries were combined in an amber GC vial with 5 mL of distilled water. For wines, 100 μL of internal standard were combined with 10 mL. A calibration curve was performed combining different concentrations of IBPM standard (Sigma-Aldrich, St. Louis, MO) with internal standard.

GC–MS instrument was an Agilent 6890 GC with an Agilent 5973 mass selective detector (MSD), a Gerstel MPS2 autosampler (Gerstel Inc., Columbia, MD) and a HP 5MS capillary column (30 m × 0.25 mm and 0.25 film thickness). Extractions were performed exposing a 23 gauge, 2 cm divinylbencene/ carboxen/ polymethylsiloxane (DVB/CARB/PDMS) SPME fiber to the sample HS for 30 min at 40 °C with continuous agitation. SPME fiber was then introduced into the GC–MS inlet with a 0.7 mm straight glass liner. The inlet was kept at 260 °C in splitless mode for 5 min. Inlet flow was then set to 50 mL min^{-1} for another 5 min. Hellium was used as carrier gas at 4.77 psi with an initial flow if 0.8 mL min^{-1}. Oven temperature was held at 40 °C for 5 min, then ramped 2.5 °C min^{-1} up to 80 °C, 5 °C min^{-1} up to 110 °C, 25 °C min^{-1} up to 230 °C and finally kept steady at 230 °C for 5 min. MSD interface was kept at 280 °C. Selected ion monitoring was used at mass channels $m/z = 124$ for IBMP and $m/z = 127$ for [$^2H_3$]IBMP.

Table 1
Effects of defoliation (keeping 100 %, 66 % and 33 % of the leaves) and fruit thinning treatments (keeping 100 %, 66 % and 33 % of the clusters) on per plant basis leaf area, fruit mass and leaf area to fruit mass (LA/FM).

Leaves	100 %		100 %		100 %		66 %		66 %		66 %		33 %		33 %		33 %		100 %		66 %		33 %										
Fruit	100 %		66 %		33 %		100 %		66 %		33 %		100 %		66 %		33 %								100 %		66 %		33 %		$P_{(Leaves)}$	$P_{(Fruit)}$	$P_{(Inter)}$
2017																																	
Leaf area (m^2)	2.63	a	2.67	a	2.60	a	1.84	b	1.98	b	1.82	b	0.96	c	0.94	c	0.88	c	2.63	A	1.88	B	0.93	C	1.81	A	1.86	A	1.76	A	<0.01	0.51	0.95
Fruit mass (kg)	3.21	a	2.51	bc	1.33	fg	2.87	ab	1.84	de	1.47	ef	2.27	cd	1.58	ef	0.88	g	2.35	A	2.06	AB	1.58	B	2.78	A	1.97	B	1.23	C	<0.01	<0.01	0.15
LA/FM (m^2kg^{-1})	0.83	cde	1.08	bc	1.96	a	0.64	def	1.18	bc	1.32	b	0.42	f	0.61	ef	1.00	bcd	1.29	A	1.05	A	0.68	B	0.63	A	0.96	B	1.43	C	<0.01	<0.01	0.06
2018																																	
Leaf area (m^2)	3.61	a	3.50	a	3.18	ab	2.43	c	2.72	bc	2.36	c	1.37	d	1.39	d	1.43	d	3.43	A	2.50	B	1.40	C	2.47	A	2.54	A	2.32	A	<0.01	0.41	0.65
Fruit mass (kg)	5.65	a	5.12	bc	4.23	fg	3.72	ab	3.68	de	2.82	ef	2.11	cd	1.97	ef	1.67	g	4.00	A	3.59	B	2.73	C	4.83	A	3.57	B	1.91	C	<0.01	<0.01	0.15
LA/FM (m^2kg^{-1})	0.64	cd	0.88	b	1.50	a	0.54	d	0.80	bc	1.34	a	0.45	d	0.49	d	0.80	bc	1.00	A	0.90	A	0.58	B	0.54	A	0.72	B	1.21	B	<0.01	<0.01	0.02
2019																																	
Leaf area (m^2)	5.23	a	4.78	ab	4.39	abc	4.18	abc	4.10	abc	3.50	bc	3.62	bc	2.86	c	3.60	bc	4.80	A	3.93	AB	3.36	B	4.34	A	3.92	A	3.83	A	0.01	0.48	0.77
Fruit mass (kg)	6.5	a	5.9	ab	5.5	abc	5.4	abc	4.8	bcd	5.2	a-d	4.0	cd	3.6	d	4.4	bcd	6.0	A	5.1	A	4.0	B	5.3	A	4.8	A	5.0	A	<0.01	0.55	0.76
LA/FM (m^2kg^{-1})	0.79	a	0.82	a	0.80	a	0.80	a	0.86	a	0.71	a	0.89	a	0.81	a	0.82	a	0.81	A	0.79	A	0.84	A	0.83	A	0.83	A	0.78	A	0.81	0.76	0.91

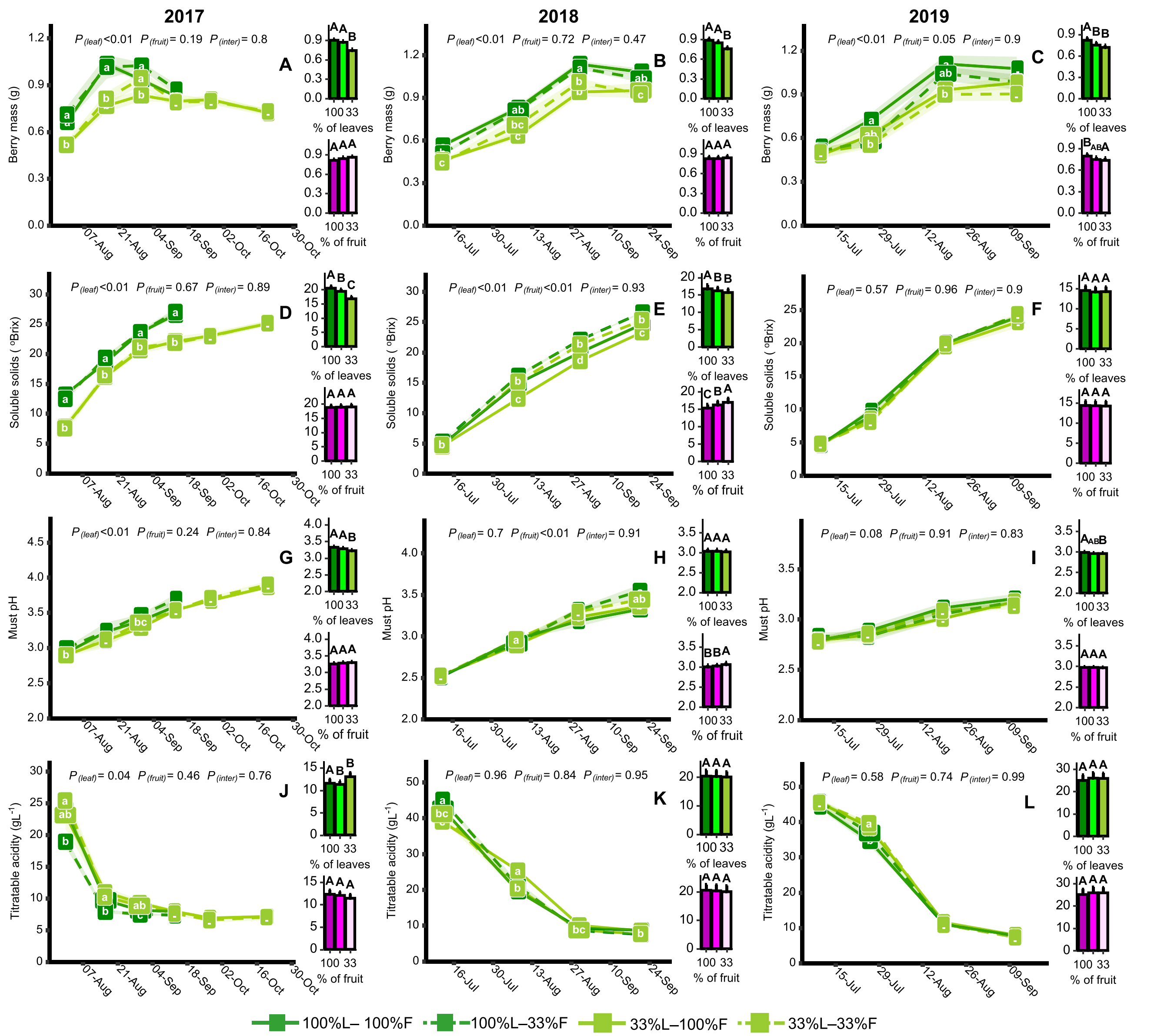

Fig. 1. Effects of defoliation, keeping 100 % (dark green) and 33 % (light green) of the leaves, and fruit thinning, keeping 100 % (solid lines) and 33 % (dashed lines) of the clusters, through seasons 2017 (A, D, G and J), 2018 (B, E, H and K) and 2019 (C, F, I and L)on berry mass (A, B and C), total soluble solids (D, E and F), must pH (G, H and I) and titratable acidity (J, K and L). In 2017, 66 %L and 33 %L treatments were harvested 14 and 37 days later than 100 %L, respectively. These later samplings were not computed in Two-way ANOVA analyses. Groups with no letters in common are statistically different. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.9. Gas chromatography of aroma profile

Forty-two wine aroma compounds were determined in a single run of HS-SPME-GC–MS, following the procedures in Hjelmeland et al. (2013). In brief, 10 mL of wine were transferred to a 20 mL amber GC vial with 3 g of NaCl and an internal standard consisting in 50 μL of 2-undecanone. Vials were sealed and analyzed in triplicate alternating treatments in the sequence. A 1 cm DVB/CARB/PDMS SPME fiber was used for sampling. Samples were warmed at 40 °C and agitated 500 rpm for 5 min before exposing the fiber for 30 min at 40 °C at 250 rpm. The instrument was an Agilent 6890 GC/5975 MSD functioning in electron impact mode at 70 eV with a Gerstel MPS2 autosampler. GC column was a DB-Wax capillary column (30 m × 0.25 mm and 0.25 μm film thickness) (J&W Scientific, Folsom, Ca). Helium at 1 mL min^{-1} flow was used as a carrier gas. During the analysis, the oven temperature was 40 °C for 5 min followed by a ramp of 3 °C min^{-1} up to 180 °C and 30 °C min^{-1} up to 240 °C and finished maintaining 240 °C for 10 min.

2.10. Statistical analysis

Statistical analysis of data was conducted using R v.3.4.1 (R Core Team, 2020). All percentages were log transformed. Data was tested for normality using Shapiro-Wilk's test and were subjected to a one-way and two-way ANOVA and/or LSD post hoc with a significance level set to 0.05. Plots were done with 'ggplot2′ package (Kahle & Wickham, 2013) and heat maps with 'pheatmap' package (Kolde & Kolde, 2018).

3. Results

3.1. Vine balance

Leaf area and yields were highly conditioned by the defoliation and thinning treatments. However, these values of leaf area and crop weight did not correspond exactly to the nominal 100 %, 66 % and 33 % proportions of leaves and clusters remaining (Table 1 and Fig. S1). In 2017,

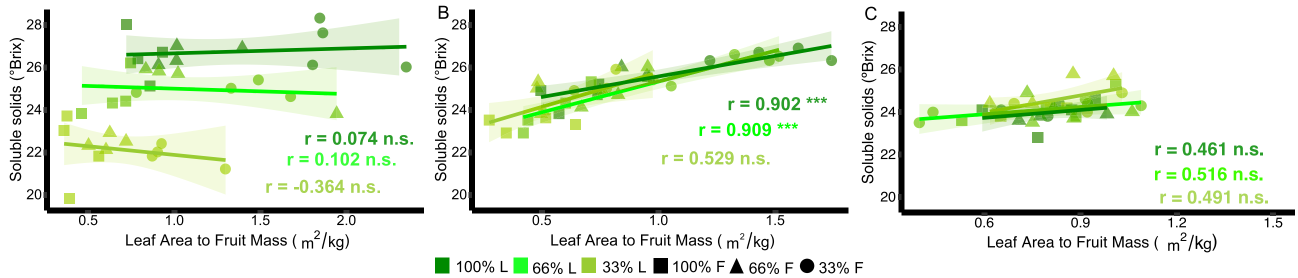

Fig. 2. Effects of defoliation (keeping 100 %, 66 % and 33 % of the leaves) and fruit thinning (keeping 100 %, 66 % and 33 % of the clusters) through seasons 2017 (A), 2018 (B) and 2019 (C) on the relationship between leaf area to fruit mass ratio and total soluble solids (A, B and C). Correlation analysis performed for each level of defoliation.

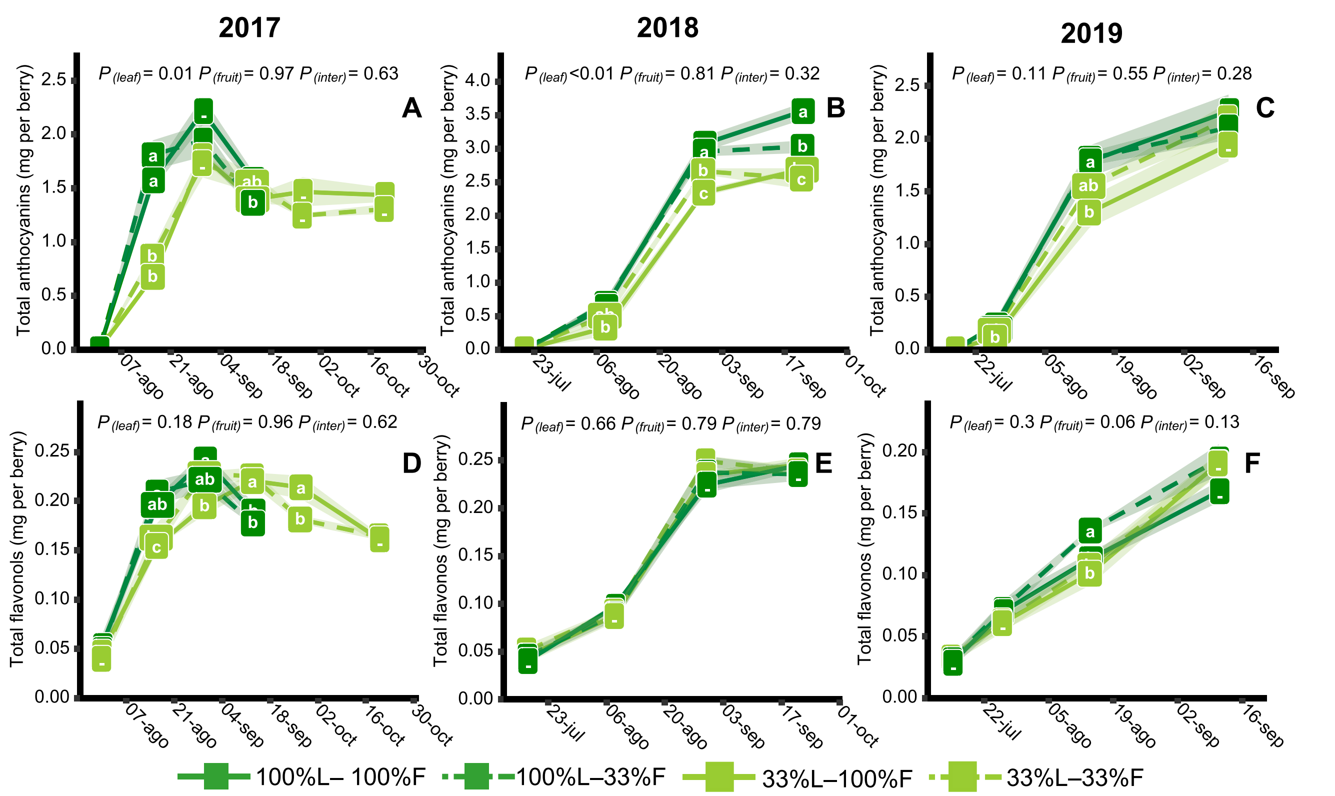

Fig. 3. Effects of defoliation, keeping 100 % (dark green) and 33 % (light green) of the leaves, and cluster thinning treatments, keeping 100 % (solid lines) and 33 % (dashed lines) of the clusters, through seasons 2017 (A and D), 2018 (B and E) and 2019 (C and F) on total anthocyanins per berry (A, B and C), and total flavonols per berry (D, E and F). Groups with no letters in common are statistically different. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

leaf areas were close to the nominal treatment application as 66 %L and 33 %L treatments were 71 % and 35 % of the leaf area that 100 %L treatments had (Table 1). In 2018, leaf areas were on average 73 % and 41 % of the 100 %L treatments. Although cluster counts were very close the prescribed treatment applied (Fig. S1), fruit mass was only reduced to 71 % and 44 % in 2017 and 74 % and 40 % in 2018, for the treatments 66 %F and 33 %F, respectively. Remarkably, defoliation treatments had a great effect on the yield of unthinned vines both years, and in fact, interaction P values were rather low ($P = 0.15$; Table 1). For instance, 100 %L-100 %F had 41 % and 168 % more yield than 33 %L-100 %F treatment in 2017 and 2018, respectively, despite having similar cluster numbers (Table 1 and Fig. S1). These deviations of leaf area and yields from the nominal defoliation and thinning treatments led to compensation in LA/FM values as defoliation, cluster thinning, and their interaction had significant effects (Table 1 and Fig. S2). The nominal treatments were attenuated to the point that differences in LA/FM were just 4.67-fold in 2017 and 3.33-fold in 2018 when comparing between the extreme treatments (i.e. 100 %L-33 %F vs 33 %L-100 %F).

3.2. *Berry mass, must soluble solids, pH and titratable acidity*

Defoliation constantly reduced berry mass in all 3 years of study when measured at most time points (Fig. 1 ABC). Cluster thinning did not have as significant effect overall, but 33 %F plants tended to have higher berry mass and this increase was significant on 31 August 2017 sampling (Fig. 1 A). Interestingly, berry mass was higher in cluster thinned vines in year 2019. Effects on berry must soluble solids were different based on the year (Fig. 1 DEF). In 2017, defoliation reduced total soluble solids by 4°Brix, whereas cluster thinning had no significant effects. Conversely, in 2018, defoliation reduced total soluble solids by 1.2°Brix whereas cluster thinning increased soluble solids by 2.0°Brix. In 2019, no effects on must soluble solids were carried over from previous seasons. These changes in total soluble solids observed in 2017 were not correlated to vine balance as leaf area was the only

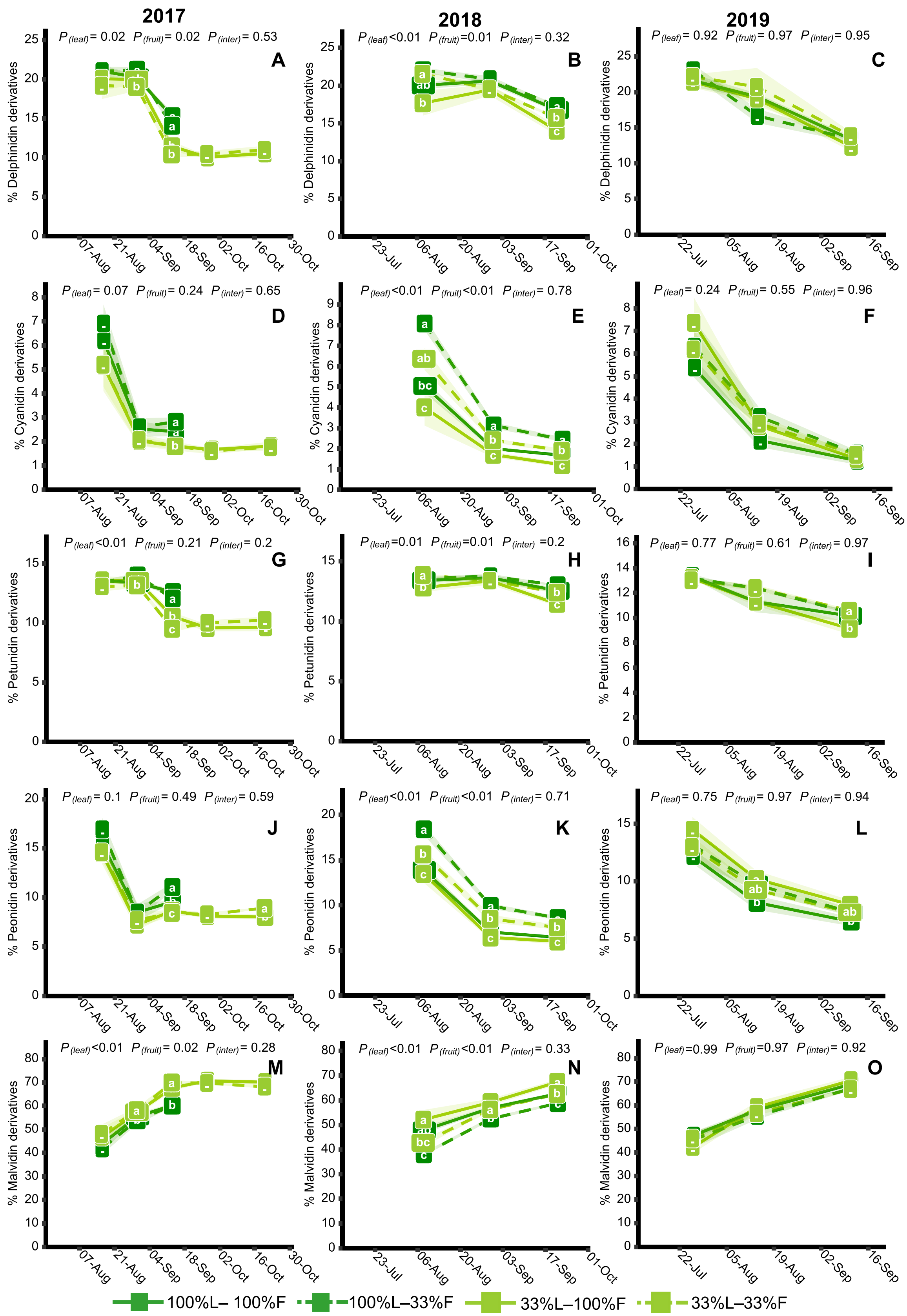

Fig. 4. Effects of defoliation, keeping 100 % (dark green) and 33 % (light green) of the leaves, and cluster thinning treatments, keeping 100 % (solid lines) and 33 % (dashed lines) of the clusters, through seasons 2017 (A, D, G, J and M), 2018 (B, E, H, K and N) and 2019 (C, F, I, L and O) on % of anthocyanins derived from delphinidin (A, B and C), cyanidin (D, E and F), petunidin (G, H and I), peonidin (J, K and L) and malvidin (M, N and O). Groups with no letters in common are statistically different. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

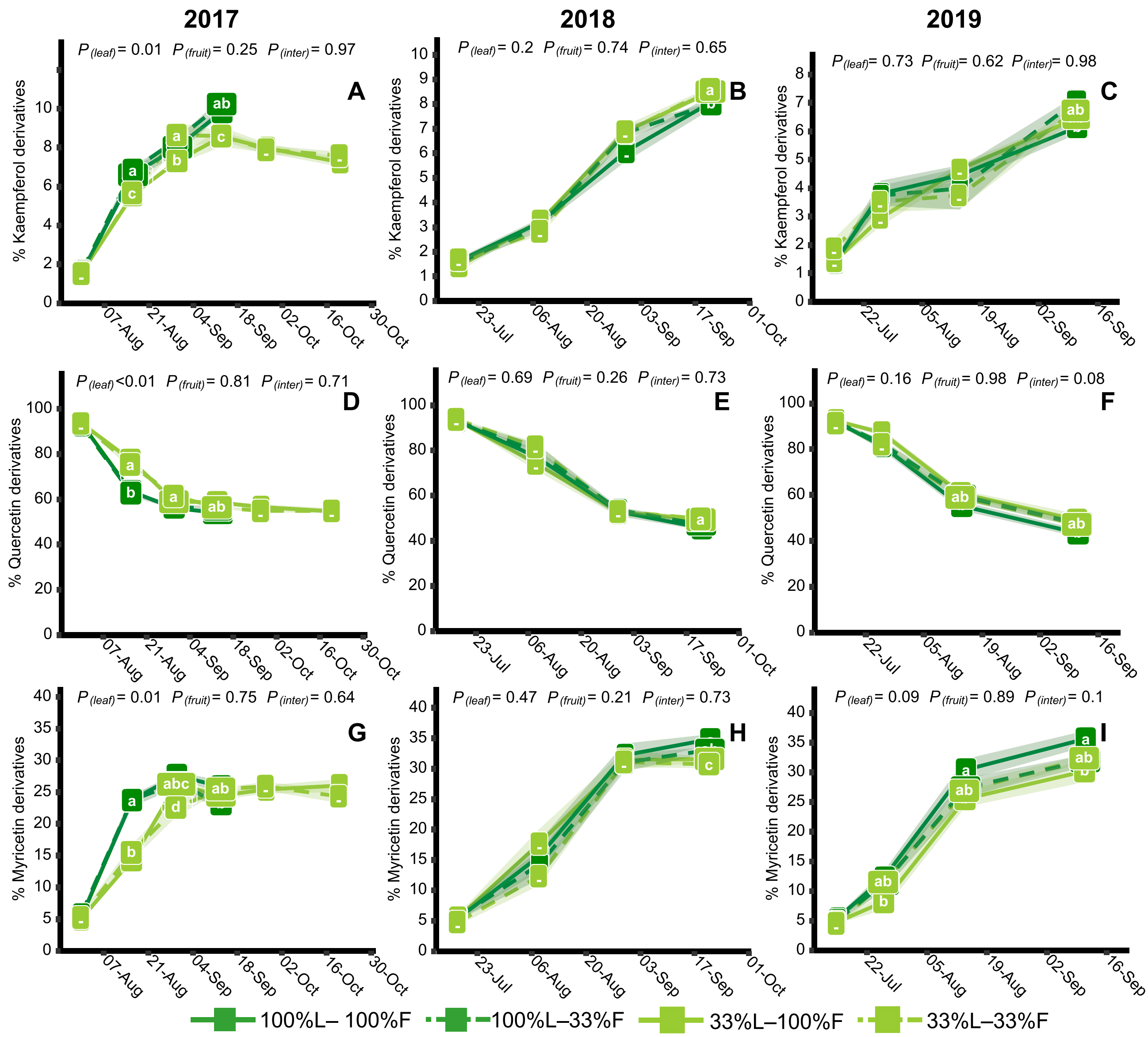

Fig. 5. Effects of defoliation, keeping 100 % (dark green) and 33 % (light green) of the leaves, and cluster thinning treatments, keeping 100 % (solid lines) and 33 % (dashed lines) of the clusters, through seasons 2017 (A, D and G), 2018 (B, E and H) and 2019 (C, F and I) on % of flavonols derived from kaempferol (A, B and C), quercetin (D, E and F) and myricetin (C, F and I). Groups with no letters in common are statistically different. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

significant main factor (Fig. 2A), whereas in 2018, total soluble solids were tightly correlated to LA/FM (Fig. 2B). Likewise, must pH displayed contrasting results between seasons (Fig. 1 GHI). It was lower for 33 %L treatments in 2017 and higher for 33 %F vines in 2018. Similarly, TA was higher in 33 %L vines in 2017 (Fig. 1 J), whereas, in 2018, the effects were more focused on 33 %L-100 %F treatment that had lower acidity in June but higher acidity than other treatments during ripening (Fig. 1 K).

3.3. Grape skin flavonoids

In 2017, total anthocyanins started higher with the 100 %L treatments compared to other treatments. However, these differences were reduced through ripening and by the end of the season all treatments had similar anthocyanin content (Fig. 3 A). These results were not fully reproduced in 2018 where 100 %L treatments and specially 100 %F-100 %L had a higher anthocyanin content and maintained the highest content even though all treatments reached technological ripeness. Anthocyanin profile was similar between the two years of treatments in 2017 and 2018 where the 100 %L had a lower proportion of malvidins (Fig. 4 MN) in favor of compounds with a lower degree of 3́4́5́hydroxylation and/or methylation (Fig. 4 ABDEGHJK). This pattern was not observed in 2019 and no carry over effects were observed on anthocyanin profile (Fig. 4 CFILO).

Total flavonols responded in the same manner as anthocyanins in 2017 with greater content in 100 %L for initial stages but similar to all other treatments by the end of the study (Fig. 3 D). Flavonol profile was affected only transiently in 2017 where the proportion of kaempferol- and quercetin-derived flavonols (Fig. 5 AD) were higher in detriment of myricetin-derived flavonols in ripening berries (Fig. 5 G).

3.4. Grape and wine IBMP

The IBMP content monitored in 2018 grapes followed a pronounced decline from June (pea size) until harvest (Fig. 6). Both defoliation and cluster thinning had significant effects on IBMP levels, and these effects

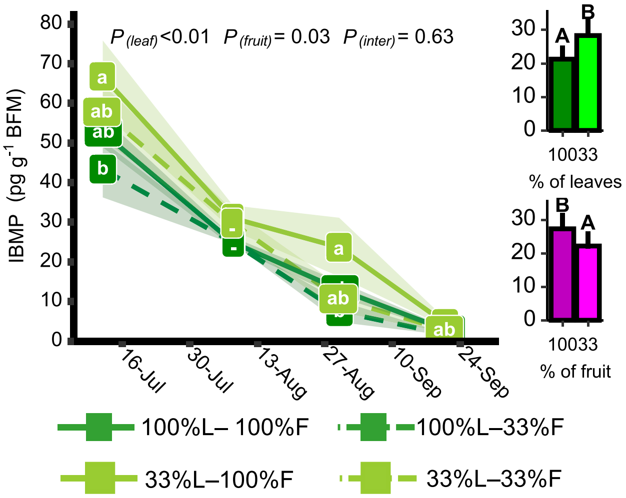

Fig. 6. Effects of defoliation, keeping 100 % (dark green) and 33 % (light green) of the leaves, and fruit thinning treatments, keeping 100 % (solid lines) and 33 % (dashed lines) of the clusters, on IBMP. Groups with no letters in common are statistically different. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

were consistent through berry development and in the wine. In fact, IBMP levels of grapes and wines were strongly correlated to must soluble solids (Table 2).

3.5. Wine composition (technological and phenolic maturity)

Several significant interactions were found between defoliation and cluster thinning where 33 %L-33 %F was separated from the rest of the treatments for its lower color index, flavonols (360 nm AU), anthocyanins (520 nm AU) and total polyphenol index (280 nm AU) (Fig. 7 and Table S1). There was also an interaction between defoliation and cluster thinning making extreme treatments (i.e. 100 %L-33 %F vs 33 %L-100 % F) have a larger difference in alcohol content that the decrease or the increase corresponding defoliation and cluster thinning affected, respectively. Defoliation caused a significant increase in anthocyanin polymerization. Contrarily, cluster thinning had effects where must pH, potassium, wine hue and the percentage of polymeric pigments and reducing titratable acidity increased. The PCA constructed with two principal components that aggregated 47.2 % (PC1) and 32.5 % (PC2) of the variability (Fig. 8 A). Treatments were well separated from each other, although there was clustering between 100 %L-100 %F and 33 % L-100 %F. Technological maturity variables (Alcohol, pH, potassium and titratable acidity) and wine hue were clustered together with 33 %F treatments (Fig. 8 B). Phenolic maturity variables Anthocyanins, Flavonols, TPI and color index and % polymeric anthocyanins in opposition clustered together and contributed to the separation of 100 %L-33 %F and 33 %L-33 %F wines.

3.6. Wine aroma composition and profile

Out of the 42 aroma compounds identified in the single run as well as the targeted analyses of IBMP, 18 of these compounds were affected by the interaction between defoliation and cluster thinning (Fig. 7, Table 2 and Table S2). Out of the eighteen, 33 %L33 %F treatment was singled out by 8 compounds for having higher (farnesol, b-linalool and b-myrcene) or lower (*cis*-3-Hexen-1-ol, ethyl acetate, ethyl butanoate, ethyl lactate and isoamyl alcohol) levels, and 100 %L100 %F wines were singled out by 3 compounds (nerolidol, phenetyl acetate and phenetyl alcohol) for having a higher content. Four compounds with significant interactions exacerbated the results in the extreme treatments, either increasing 100 %L-33 %F (*cis*-2-Hexen-1-ol, hexanoic acid and isobutiric acid) or 33 %-L100 %F (IBMP) levels. In regards to main effects, defoliation was the main factor for which 11 compounds were significantly affected. Out of the 11 compounds 1 increased and 10 decreased by the level of defoliation. Cluster thinning had significant effects on 17 compounds, increasing 2and decreasing 15 in the 33 %Fruit wines. Many (15) compounds were correlated to TSS (Table 2). Among these, 4 (1-octen-3-ol, isobutyric acid, benzyl alcohol, g-Nonalactone) had a very strong positive correlation to TSS whereas 5 (ethyl 3-methylbutyrate, ethyl dihydrocinnamate, *cis*-3-hexen-1-ol, Hexanol, and IBMP) had a strong negative relationship.

The two most important components revealed by PCA aggregated 43.7 % and 22.8 % of the variability in the aroma profile (Fig. 8 C and D). The wine samples were clustered together with some overlap between 100 %L-100 %F and 33 %L-100 %F but distinctly separated from 100 %L-33 %F and 33 %L-33 %F (Fig. 8 C), just as happened with wine physicochemical parameters (Fig. 8 A). Four terpenoids (2,5,6 and 8) and one aliphatic alcohol (4) were clustered together around 33 %F33 % L (Fig. 8 D). However, the rest of the treatments did not cluster any aroma compound class in any way.

4. Discussion

4.1. Source and sink manipulations interact to compensate vine balance

Nominal treatments of defoliation and cluster thinning did not correspond to equivalent reductions of leaf area and yields. The most evident example was that the thinning of 66 % of the clusters of non-defoliated plants in 2018 which resulted in 25 % less yield. Thus, changes in berry size, and berries per cluster to a lesser extent, were the main mechanism of compensation of over or under cropping. This adjustment led an attenuation of the differences in LA/FM among treatments; only a x4.7-fold and 3.3-fold change from one extreme treatment to another in 2017 and 2018, respectively. This homeostasis of yields has been reported before, where a higher cluster number led to smaller clusters and/or berries (Brillante et al., 2018; Terry & Kurtural, 2011). However, this homeostasis of berry size has been far less reported with defoliation, which was the strongest effect and interactive effects with thinning in the present study. For instance, Bennett et al. (2005) reported small reductions in yield with no change in berry diameter, whereas Parker et al. (2015) reported no changes in yield or berry mass after 50 % defoliations either from fruit set or veraison. Changes in berry size have been long time associated to changes in water status (Chaves et al., 2010). This may be due to higher hydraulic conductivity of well-watered plants enabling expansion of berry cells through maturity and later in the season compensating for water losses associated to shriveling (Zhang & Keller, 2015). The presence of more leaves could be associated to shading, and thus, lower berry water loss leading to bigger berries (Martínez-Lüscher et al., 2020). However, a severe defoliation could also reduce the plant's water consumption making defoliated vines have more water available and better water status (Balducci et al., 2020). After water, sugars are the largest constituent of berry mass. In fact, increased carbon assimilation rates have been related to increased fruit size regardless of leaf area (Ito et al., 1999; Martínez-Lüscher et al., 2015). Sucrose is probably the most universal signaling molecule in plants; therefore, it would not be surprising that fruits respond to surplus of sucrose increasing their growth rates just as buds do (Mason et al., 2014). Interestingly, defoliated and unthinned vines had smaller berries even in the following season, in 2019, when no treatments where applied. This result suggested that the carbohydrate starvation experienced by the defoliated vines in the two years of treatments was not recovered in a single season. One factor that could have mediated this response was the reduced root development of defoliated vines (Fig. S3) (Martínez-Lüscher & Kurtural, 2021). Other authors have pointed out the strong relationship between the number of seeds and berry mass which could explain why carry over effects where so strong in this study (Walker et al., 2005). Given the smaller berry size in defoliated vines,

Table 2
Two-way ANOVA analysis of the effect of defoliation and cluster thinning on wine aroma compounds in season 2018. Correlation analysis of wine aroma compounds with must total soluble solids prior to the fermentation (Table S1) showing spearman's ρ (rho) and signification levels * ($P < 0.05$) and ** ($P < 0.01$).

Code	Class	Compound	$P_{(Leaf)}$	$P_{(Fruit)}$	$P_{(Inter.)}$	ρ	Odor type/Flavor type[a]
A_2	Aliphatic acid	Isobutyric acid	0.82	<0.01	0.01	0.88**	Acidic
B_2	Benzenoids	Benzyl alcohol	0.02	<0.01	0.16	0.88**	Floral/Fruity
O_1	Aliphatic alcohol	1-Octen-3-ol	0.35	<0.01	0.47	0.85**	Earthy/Mushroom
L_1	Other	γ-Nonalactone	0.07	<0.01	0.83	0.73**	Coconut/Coconut
P_1	Volatile phenols	Phenethyl alcohol	<0.01	0.03	0.01	0.62*	Floral
A_1	Aliphatic acid	Hexanoic acid	0.02	0.38	0.01	0.56	Fatty/Cheesy
O_2	Aliphatic alcohol	*cis*-2-Hexen-1-ol	0.29	0.08	0.04	0.53	Fruity
T_7	Monoterpene	*cis*-Rose oxide	0.2	0.5	0.2	0.45	Floral/Green
O_4	Aliphatic alcohol	Farnesol	<0.01	<0.01	<0.01	0.29	Floral
E_13	Aliphatic ester	Isoamyl alcohol	<0.01	0.05	<0.01	0.26	Fermented/Fusel
O_6	Aliphatic alcohol	Isobutanol	<0.01	0.1	0.05	0.19	Ethereal
E_5	Aliphatic ester	Ethyl cinnamate	0.01	0.59	0.88	0.12	Balsamic
T_8	Monoterpene	Geraniol	0.97	0.39	0.15	0.10	Floral
T_6	Monoterpene	β-Myrcene	0.2	0.7	0.02	0.09	Spicy/Woody
T_2	Monoterpene	β-Citronellol	0.12	0.81	<0.01	0.08	Floral
E_3	Aliphatic ester	Ethyl acetate	<0.01	<0.01	<0.01	−0.02	Ethereal
O_7	Aliphatic alcohol	*trans*-3-Hexen-1-ol	<0.01	0.27	0.11	−0.08	Green
K_2	Aliphatic ketone	Diacetyl	0.44	0.17	0.01	−0.15	Buttery
E_12	Aliphatic ester	Isoamyl acetate	<0.01	<0.01	0.08	−0.16	Fruity
B_1	Benzenoids	Benzaldehyde	0.42	0.34	0.01	−0.20	Fruity
T_4	Monoterpene	β-Ionone	0.02	0.65	0.63	−0.20	Floral
E_4	Aliphatic ester	Ethyl butanoate	<0.01	<0.01	<0.01	−0.30	Fruity
T_5	Monoterpene	β-Linalool	<0.01	<0.01	<0.01	−0.32	Floral/Citrus
A_3	Aliphatic acid	Octanoic acid	<0.01	0.02	0.25	−0.32	Farry/Soapy
E_8	Aliphatic ester	Ethyl hexanoate	0.03	0.01	0.58	−0.33	Fruity
E_6	Aliphatic ester	Ethyl decanoate	0.04	0.03	0.17	−0.34	Waxy
N_1	Norisoprenoid	β-Damascenone	0.13	0.34	0.09	−0.34	Fruity
E_10	Aliphatic ester	Ethyl lactate	0.05	<0.01	0.04	−0.43	Fruity
T_3	Monoterpene	β-Cyclocitral	0.54	0.02	0.17	−0.44	Tropical
E_11	Aliphatic ester	Ethyl octanoate	0.04	0.01	0.26	−0.48	Waxy
T_10	Sesquiterpene	Nerolidol	0.01	0.02	0.01	−0.48	Floral/Green
E_14	Aliphatic ester	Phenethyl acetate	0.01	0.03	0.02	−0.47	Floral/Honey
T_9	Monoterpene	Limonene	0.75	0.01	0.9	−0.56	Citrus
K_1	Aliphatic ketone	Acetoin	<0.01	<0.01	0.48	−0.61*	Buttery/Creamy
T_1	Monoterpene	α-Terpinene	0.42	<0.01	0.33	−0.67*	Woody/Terpenic
E_9	Aliphatic ester	Ethyl isobutyrate	0.01	<0.01	0.07	−0.68*	Fruity/Ethereal
B_3	Benzenoids	p-Cymene	0.63	<0.01	0.55	−0.68*	Terpenic
E_2	Aliphatic ester	Ethyl 3-methylbutyrate	0.45	<0.01	0.09	−0.72*	Fruity
E_1	Aliphatic ester	Ethyl 2-methylbutyrate	0.1	<0.01	0.53	−0.74**	Fruity
E_7	Aliphatic ester	Ethyl Dihydrocinnamate	0.19	<0.01	0.53	−0.75**	Floral
O_3	Aliphatic alcohol	*cis*-3-Hexen-1-ol	0.88	<0.01	0.03	−0.76**	Green
O_5	Aliphatic alcohol	Hexanol	0.39	<0.01	0.38	−0.78**	Herbal/Green
M_1	MethoxyPyrazin	2-isobutyl-3-methoxypyrazine	0.01	<0.01	0.01	−0.79**	Green

[a] Odor and flavor type descriptors were obtained from PubChem database (2023).

the concentration of some constituents may have been reconcentrated in berries or wines, especially those constituents that were in the skin, as smaller berries tend to have a higher skin to pulp ratio. For instance, this is the reason why in some instances anthocyanin contents per berry were lower per gram of berry but not per berry.

Although some authors have pointed out the relationship between small berries and higher wine quality, and berry size sorting is a commercial practice, others have shown no intrinsic relationship between berry size and composition (Roby et al., 2004; Walker et al., 2005). Walker et al. (2005) suggested that cultural practices that typically reduce berry size may be behind the increased quality of smaller berries. Hence, a regulatory mechanism involving a change in the synthesis or degradation, and not allometry itself. In the case of the present study, few effects could be explained through allometry, suggesting a more complex regulation being involved.

4.2. Source–sink balance affected grape composition through shifts in ripening as well as more complex ripening regulation mechanisms

The effect of source–sink balance manipulations on grape sugar accumulation have been known for some time (Bravdo et al., 1985), and the great majority of studies support that must sugar levels increase with a higher proposition LA/FM ratio up to a point where there is no further effect (Kliewer & Dokoozlian, 2005; Naor et al., 2002). However, when it comes to secondary metabolites responses are often not so unanimous across the literature. Anthocyanins levels have a natural trend to increase up to a ca. 21°Brix and a subsequent decrease (Martínez-Lüscher et al., 2019). Therefore, the strategy of sampling and how results are presented can influence their interpretation. This was evident in 2017 where the faster accumulation of sugars with 100 % of the leaves initially led to a higher concentration of Anthocyanins, then the rest of the treatments transfixed by the September sampling, the most advanced treatment (i.e. 100 %L33 %F) had lower anthocyanin levels than the 33 %Leaves treatments. This was not the case of the second year of treatments where 100 % leaves maintained their higher anthocyanin content until the harvest of all treatments.

Flavonoid profiles are strongly influenced by genetic factors (Mattivi et al., 2006). Nevertheless, they are greatly modulated by the progress of ripening and environmental factors such as water deficit or solar radiation (Martínez-Lüscher et al., 2019). In our results, anthocyanin profiles in general were more 3́45 hydroxylated and methoxylated (higher %Malvidin-derived anthocyanins) in defoliated vines. These effects were present when comparing berries sampled on the same date or when comparing grapes at the same Brix. Furthermore, these trends were not observed in flavonol profiles as clearly. This clear anthocyanin-specific effect could not be due entirely to environmental factors such as water

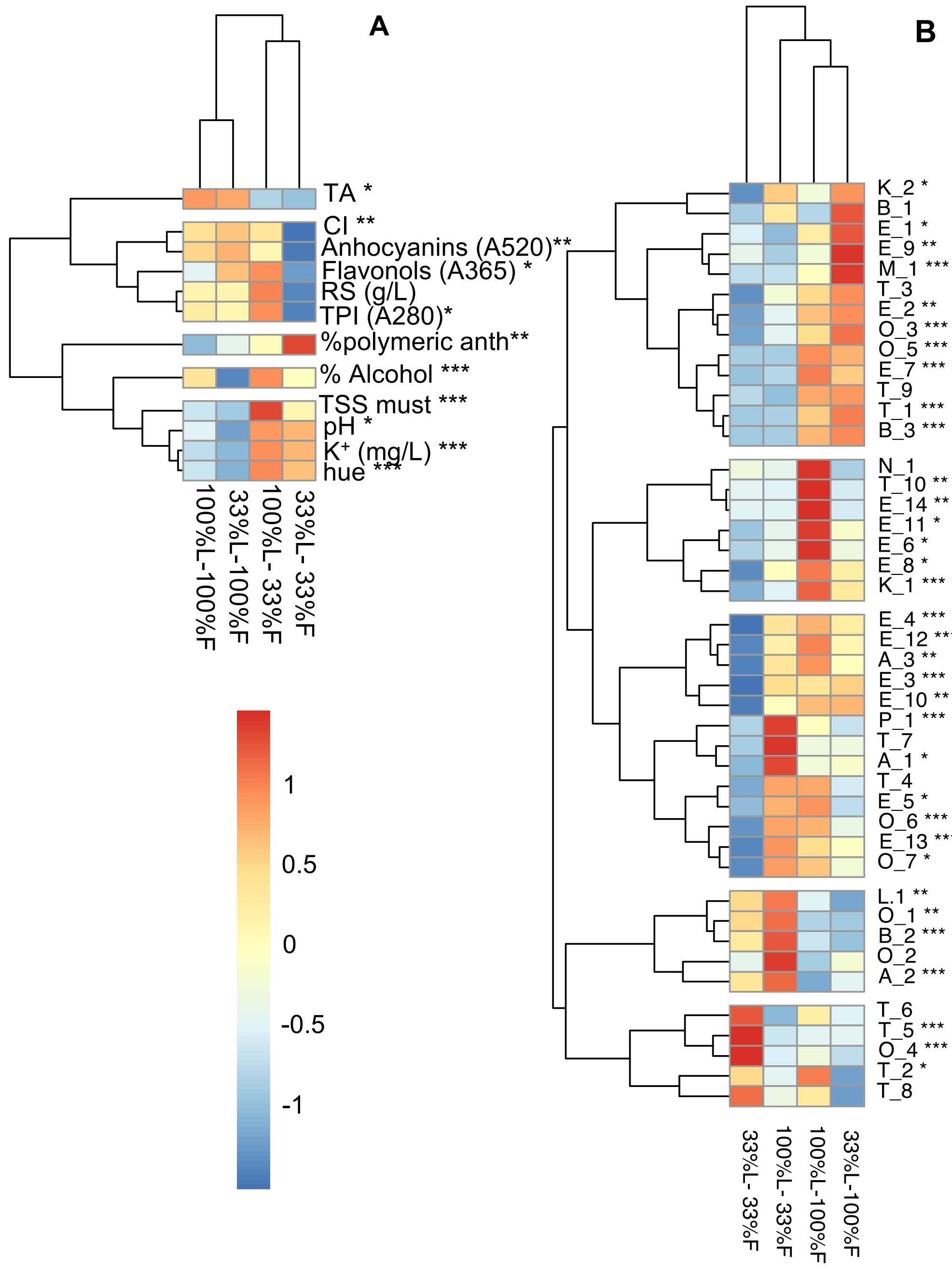

Fig. 7. Heretical analyses and heat map of 2018 vintage wines from vines subjected to defoliation (keeping 100 % and 33 % of the leaves) and fruit thinning treatments (keeping 100 % and 33 % of the clusters). Wine analyses separated by technologic and phenolic maturity (A) and aroma profile (B). ANOVA significance levels of P < 0.05 (*), P < 0.01 (**) and P < 0.001 (***). Full dataset in Tables S1 and S2. Full names of the aroma compounds in Table 2.

deficit or solar radiation as water status and berry flavonol profile was similar in defoliated vines. Berli et al. (2011) reported an increase in total anthocyanin levels while the proportion of malvidin-derived anthocyanins decreased in response to the application of exogenous ABA. Considering that ABA synthesis takes place in leaves (Zhang et al., 2018), and it is suppressed by defoliation (Ren et al., 2006), it is plausible that the increase in the proportion of malvidin-derived anthocyanins observed would be mediated by lower levels of ABA.

Smaller berry sizes have been associated to a re-concentration of berry seed and skin contents during the wine making (Singleton, 1972). In 2018, grapes were harvested with lower berry mass and lower anthocyanin levels per grape in the defoliated vines. This was only transferred to lower anthocyanin levels in the 33 %L33F wine. Contrarily to berry parameters, wine parameter results were dominated by effects of cluster thinning and interactions as through higher pH, lower titratable acidity, higher potassium, higher hue, higher anthocyanin polymerization. Cluster thinning typically increases the sugar content of grapes and this results in lower acidity, higher anthocyanin levels, and color properties (Condurso et al., 2016; Mawdsley et al., 2019). This was clearly observed when cluster thinning enhances ripening towards its optimum (typically 21-23°Brix), but results can be different when the most advanced berries enter in the shriveling stage (Yu et al., 2020) as was the case in the present study. Total soluble solids of wine musts were affected by both defoliation and cluster thinning and average values ranged from 25.7 to 28.0°Brix which is a range in which total soluble solids is clearly related with the progress of shriveling. This tended to increase the complexity of the results from wine aroma compounds, as compounds may have a different optimal stage for their

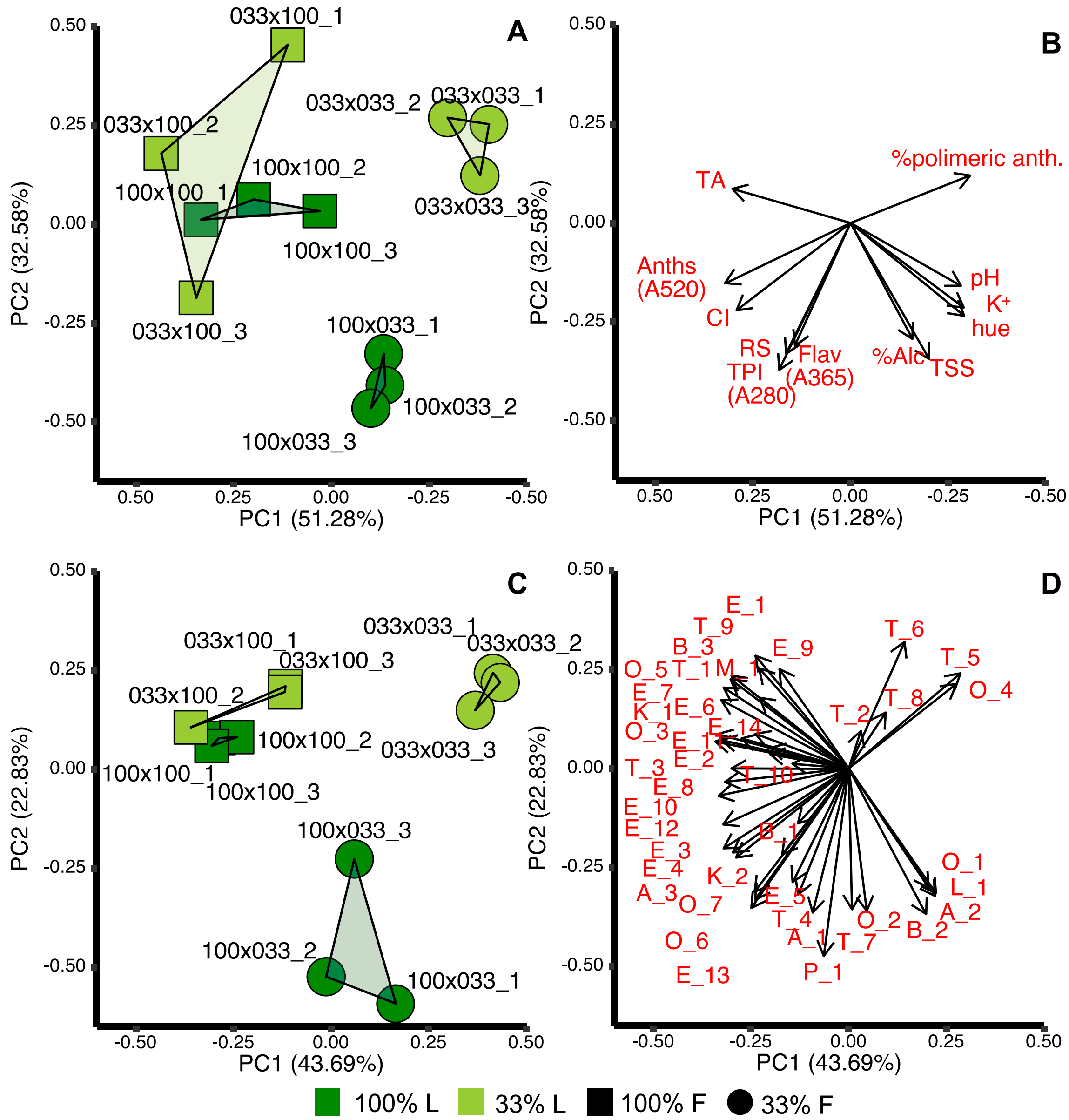

Fig. 8. Principal components analysis of 2018 vintage wines from vines subjected to defoliation (keeping 100 % and 33 % of the leaves) and fruit thinning treatments (keeping 100 % and 33 % of the clusters). Wine analyses separated by technologic and phenolic maturity (A and B) and aroma profile (C and D). Full dataset in Tables S1 and S2. Full names of the aroma compounds in Table 2.

accumulation (Kalua & Boss, 2009). Still, a third of the aroma compounds had a significant relationship with must total soluble solids, and most of these were negatively correlated indeed.

Herbaceous aromas such as IBMP, Hexanol, and *cis*-3-Hexen-1-ol were strongly and negatively correlated to total soluble solids. However, these reductions were stronger in cluster thinned treatments. Alba et al. (2022) compared cluster thinning treatments with the same brix also reported clear reductions in Hexanol, and *cis*-3-Hexen-1-ol levels, which suggests that thinning may have effects beyond independent from the shift in technological ripening typically observed after cluster thinning. In that study, thinning also resulted in much higher Benzyl alcohol levels which also increased with thinning in our results. The treatments with a lower LA to FM ratio tended to an aroma profile with higher levels of compounds with fruity and floral character and lower levels of compounds with green character. The treatments with cluster thinning were very well separated from the unthinned treatments. Interestingly, the wines from 33 %L-100 %F vines were not separated from 100 %L-100F % and both retained herbaceous aroma profiles. This finding suggests that more leaf area may be able to compensate for the higher sugar requirements of high yields but not reducing a green aroma. Green aroma compounds such as IBMP may be more sensitive to grape berry exposure to solar radiation, and thus, a full canopy could contribute to a more occluded fruit zone in this study (Koch et al., 2012; Torres et al., 2020).

5. Conclusion

Berry total soluble solids measurement is the simplest and most indicative parameter of berry ripening, and it is very responsive to canopy and crop load adjustments. This study revealed a higher level of complexity to the concept of LA to FM balance. For instance, berry mass was strongly reduced by defoliation even in the subsequent season where no defoliation was applied. Berry total soluble solids, acidity and anthocyanin levels were also more affected by defoliation than fruit thinning. However, when it came down to wine composition, the two cluster thinned treatments were clearly separated from the unthinned treatments and from each other whereas the unthinned were clustered toguether. The results of this experiment revealed a great level of complexity of source–sink relations in which leaves may play an additional role than carbon translocation (e.g. ABA synthesis), and likewise, for clusters (e.g. sink of potassium and fate of hormones). This was clearly visible by the great differences between treatments 100 %L-100 %F and 33 %L-33 %F. Therefore, when considering LA to FM ratio one must also consider allometric relationships and not expect same results when downsizing source–sink balances.

CRediT authorship contribution statement

Johann Martínez-Lüscher: Conceptualization, Funding acquisition, Project administration. **Sahap Kaan Kurtural:** Conceptualization, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgement

American Vineyard Foundation funded this study (AVF Proj.ID: 2185). The authors also acknowledge the valuable assistance of Marshall Pierce, Runze Yu, Luca Brillante, and Christopher Chen during field work. We thank Groth Vineyards and Winery for their help in the winemaking process.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2023.112826.

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