Effect of cluster thinning within the grapevine variety 'Welschriesling' on yield, grape juice and wine parameters

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The effect of cluster thinning (CT) within the grapevine variety 'Welschriesling' (*Vitis vinifera* L.) on yield parameters, grape juice parameters and wine parameters was monitored over three consecutive vintages. Cluster thinning was carried out at veraison (BBCH 83 to 85), removing 33 to 53 % of clusters, resulting in yield reductions of 41 %, 30 % and 45 % in 2017, 2018 and 2019, respectively. A significant effect of cluster thinning on wine quality was observed in cooler vintages, i.e. 2017 and 2019, with lower GDD (Growing Degree Days) summation and yield reduction of more than 40 % compared to the control. No effect of grape thinning was observed in a warmer vintage (2018) and with a yield reduction of 30 %. Grape thinning significantly increased concentrations of 3-mercaptohexyl acetate in wines, with trends toward higher 3-mercaptohexan-1-ol also noted in wines from CT treatments compared to the control. Number of clusters/vine, yield/vine, titratable acidity in grape juice and total dry matter in wine were also significantly affected by CT. In years with lower GDD accumulation and with sufficient yield reduction, cluster thinning can be an efficient tool to adjust wine styles already in the vineyard.
Keywords: 'Welschriesling', thiols, terpenes, yield, grape juice

Einfluss der Traubenausdünnung bei der Rebsorte 'Welschriesling' auf Ertrags-, Most- und Weinparameter. Der Einfluss der Traubenausdünnung bei der Rebsorte 'Welschriesling' (*Vitis vinifera* L.) auf Ertrags-, Most- und Weinparameter wurde in drei aufeinanderfolgenden Jahrgängen verfolgt. Die Ausdünnung wurde zum Reifebeginn (BBCH 83 bis 85) durchgeführt, wobei 33 bis 53 % der Trauben entfernt wurden, was zu Ertragsminderungen von 41 % (2017), 30 % (2018) bzw. 45 % (2019) führte. In kühleren Jahrgängen (2017 und 2019) mit weniger Wachstumsgradtagen und einer Ertragsreduzierung von mehr als 40 % im Vergleich zur Kontrolle wurde ein signifikanter Effekt der Traubenausdünnung auf die Weinqualität beobachtet. In einem wärmeren Jahrgang (2018) mit einer Ertragsminderung von 30 % war kein Einfluss der Ausdünnung zu beobachten. Sie erhöhte die Konzentrationen von 3-Mercaptohexylacetat in Weinen signifikant, wobei auch Tendenzen zu einem höheren 3-Mercaptohexan-1-ol-Gehalt festgestellt wurden. Die Traubenanzahl pro Rebe, die Höhe des Stockertrags, die titrierbare Säure im Traubensaft und die Gesamttrockenmasse im Wein wurden ebenfalls signifikant von der Traubenausdünnung beeinflusst. In Jahren mit weniger Wachstumsgradtagen und ausreichender Ertragsreduzierung kann die Traubenausdünnung ein effizientes Instrument sein, um Weinstile bereits im Weinberg anzupassen.
Schlagwörter: 'Welschriesling', Thiole, Terpene, Ertrag, Traubenmost

The ability of a vine to adequately ripen fruit is influenced by total leaf area and its exposure to sunlight in relation to fruit load, in addition to environmental conditions and the physiological state of the vine (Kliewer and Ough, 1970). Cluster thinning, shoot hedging and leaf removal are the most commonly used practices in vineyards to modify the leaf area/fruit yield ratio (Cola et al., 2014; Frioni et al., 2017; Kliewer and Dokoozlian, 2005; Poni et al., 2013; Šuklje et al., 2013). The suggested required ratio of leaf area/fruit yield to allow fruit to ripen adequately varies widely (from 7 to 14 cm^2/g) depending on variety, climate and trellising system (Kliewer and Dokoozlian, 2005; Kliewer and Ough, 1970).

Cluster thinning is commonly used by grape growers to achieve required crop load, especially in cooler climates with a short ripening period (Frioni et al., 2017; Kliewer and Dokoozlian, 2005). Therefore, cluster thinning is often performed to prevent overcropping and to improve fruit and wine composition. The effects of cluster thinning are inconsistent among studies for the majority of measured yield parameters and grape and wine metabolites (Bubola et al., 2011; Preszler et al., 2013; Reščič et al., 2015). The poor reproducibility of results related to cluster thinning may be influenced by environmental factors, variety, vine physiological status, initial yield potential, and the timing and intensity of cluster thinning. Although the effects of cluster thinning on yield parameters, such as bunch and berry weight, grape juice, and wine composition are not consistent, the benefit of cluster thinning in the season of exceptional yield potential coinciding with a cool growing season was found to be beneficial in obtaining better grape quality (Keller et al., 2005). Similarly, Frioni et al. (2017); observed higher fruit uniformity and improved grape composition as a result of cluster thinning and leaf removal only in a cooler vintage among two studied.

'Welschriesling' (*Vitis vinifera* L.) is a variety grown in Central and Eastern Europe (Austria, Croatia, Czech Republic, Hungary, Slovakia, and Slovenia), and it is the commonly most planted grape variety in Slovenia (Simončič et al., 2017). Despite its importance for the production of varietal wines or as a blending variety, very little is known about yield potential, grape and wine composition of 'Welschriesling'. Recently, we reported the presence of varietal thiols in 'Welschriesling' wines at concentrations that can importantly influence the sensory perception of the wine (Čuš et al., 2017; Šuklje and Čuš, 2021). The average measured concentration of 3-mercaptohexan-1-ol (3MH) in commercial Slovene 'Welschriesling' wines was 820.1 ng/l, while the average concentration of 3-mercaptohexyl acetate (3MHA) was 277.5 ng/l and of 4-mercapto-4-methyl-2-pentanone (4MMP) it was 5.5 ng/l (Šuklje and Čuš, 2021). The thiol concentrations of the 'Welschriesling' wines are comparable to the reported average concentrations of 923 ng/l and 110 ng/l of 3MH and 3MHA in Slovene 'Sauvignon blanc' wines (Lisjak et al., 2011). The aromatic expression of 'Welschriesling' is considered to be highly dependent on planting material, geographical location, yield per vine, grape maturity, and winemaking practices (Flak et al., 2006; Flak et al., 2003). The selection of commercial yeast starters and also lactic acid bacteria can significantly alter the volatile composition of 'Welschriesling' wines, especially the varietal thiols (Šuklje and Čuš, 2021). Although 'Welschriesling' is a variety with high yield potential, i.e. 4.3 to 5.9 kg/vine (Koruza et al., 2012), there is no literature on the influence of reduced yields on grape and wine composition. Therefore, this study on 'Welschriesling' was conducted over three years with the aim of evaluating the effects of cluster thinning at veraison on yield and the composition of grape juice and wine.

Materials and Methods

Vineyard characterization

Vitis vinifera L. cv. 'Welschriesling' grafted on SO4 was grown in Litmerk, Ivanjkovci, Štajerska Slovenija (46°27'3''N, 16°8'44''E). Vines were planted in 2005 at 2.4 m (row) x 1.2 m (vine) spacing in a NE row orientation. Vines were trellised to a double Guyot and not irrigated during the season. To investigate the effects of yield reduction on yield parameters, grape juice and wine parameters, cluster thinning was carried out in the 2017, 2018 and 2019 vintages. Two treatments were randomly established on the vines in three replicates in three parallel rows with one replicate consisting of 15 vines. The treatments were control without yield reduction (C) and cluster thinning treatment (CT) with 33 to 53 % clusters removed at veraison (BBCH 83 to 85) (Lorenz et al., 1995). There were at least

three buffer rows on each side of the experimental plot and five buffer vines at the beginning of the row. For CT treatment most second and third clusters on a single shoot or clusters entering veraison late were removed. Grapes were harvested at full maturity; a few days (2017 and 2018) or one day (2019) after the phenophase 'Harvest' (BBCH 89) was recorded, defined by total soluble solids (TSS) content; the dates were 13.9. (2017), 17.9. (2018), and 16.9. (2019). During the growing seasons, vine phenology was monitored using the BBCH scale (Lorenz et al., 1995).
Meteorological data were collected from the Agrometeorological Portal of Slovenia (UVHVVR, Agrometeorološki portal Slovenije http://agromet.mko.gov.si/APP/Tag/Export/155 , 13.2.2020) from the official weather station Litmerk (27388), located in the experimental vineyard, to calculate the average temperatures and precipitation for the period from April, 1st, to September, 30th, in 2017, 2018 and 2019 vintages. Growing degree days (GDD) index was calculated according to literature reports (Hall and Jones, 2010) using 10 °C as the base temperature for the vine, which is subtracted from the average temperature recorded from April, 1st, to September, 30th, in Figure 2 or from April, 1st, till phenological stage 'Harvest' in Table 1.

Yield and grape parameters

The average yield per vine and the average number of clusters per vine was recorded at harvest on 10 randomly selected vines per treatment. Average cluster weight was calculated from recorded yield per vine divided by number of clusters per vine. Berry fresh weight was determined on 100 berries, which were carefully excised from randomly selected clusters.

Grape harvest and microvinifications

Approximately 30 kg of grapes for each of three replicates per treatment were harvested and transported to the institutional experimental cellar. Grapes were de-stemmed and crushed, with the addition of 0.25 g/1 Suprarom (Laffort, Bordeaux, France) to prevent juice oxidation. Grapes were immediately pressed with a 55 l-water bladder press to maximum pressure 1.5 bar (Lancman VSX 55, Gomark d.o.o., Vransko, Slovenia). Pressed juice was collected in 20l-demi-johns and 50 ml of juice was sampled for TSS, titratable acidity (TA) and pH analyses. Juice was left overnight at +4 °C for cold settling. The following day, clear juice was racked in the presence of N_2 gas in 15l-demi-johns, one per replicate. Juice was inoculated with 0.3 g/l *Saccharomyces cerevisiae* QA23 (Lallemand, Montreal, Canada), VL3 (Laffort, Bordeaux, France) and VIN13 (Anchor Oenology, Cape Town, South Africa) in ratio 1:1:1. Fermentations were carried out in a temperature controlled room at 15 to 18 °C. Fermenting juice was supplemented with yeast nutrient Nutri start Org (0.3 g/l) (Laffort, Bordeaux, France) at approximately 1/3 of fermentation, determined by refractometric TSS measurements. Progress of fermentation was monitored by refractometric measurements of density in Oechsle degrees (°Oe). All the treatments fermented dry, to residual sugar levels below 1.6 g/l in all three vintages, which was confirmed by enzymatic measurements. When fermented dry, 5 to 6 % aqueous solution of sulphurous anhydride were added in amount of 50 mg/l SO_2 and the wine was racked. Wines were bottled 8 to 10 weeks after completed fermentation in 0.75 l screw cap bottles.

Analyses of total soluble solids, titratable acidity and pH and other basic wine parameters

The TSS was determined using a digital refractometer WM-7 (Atago, Saitama, Japan). Juice and wine pH were measured with Meterlab PHM 210 (Radiometer Analytical, Lyon, France) and TA was determined by sodium hydroxide titration and indicator bromothymol to the colorimetric change (European commission regulation (EEC) No. 2676/90, 1990). Alcohol content was measured using an alcohol meter Alcolyser Wine M (Anton Paar, Graz, Austria), reducing sugars and volatile acidity (VA) were quantified using enzymatic robot BS-200 (Mindray, Nanshan, Shenzhen, China) and total dry matter using the OIV-MA-AS2-03B method (OIV, 2019).

Analyses of varietal thiols

Thiols in wines were quantified as published according to Jenko et al. (2013), Tominaga and Dubourdieu (2006), and Tominaga et al. (1998).

Samples were analysed using gas chromatograph (GC) (Agilent Technologies 7890A, Palo Alto, Santa Clara, CA, USA) coupled to mass spectrometer (Agilent Technologies 5975C, Palo Alto, Santa Clara, CA, USA). Sample preparation followed the protocol adopted from Tominaga and Dubordieu (2006), and Tominaga et al. (1998) as described in Jenko et al. (2013). Briefly, 50 ml of wine was spiked with 4-methoxy-2-methyl-2-mercaptobutane (Sigma Aldrich, Schnelldorf, Germany) for 4MMP quantification, [2H2]-3-mercaptohexyl acetate (Auckland University, New Zealand) for 3MH quantification and [2H2]-3-mercaptohexan-1-ol (Auckland University, New Zealand) for 3MH quantification. Wine pH was adjusted to 7 and passed through previously activated Dowex raisin. Thiols were eluted from Dowex raisin using cysteine buffer and thereafter extracted using liquid-liquid extraction with ethyl acetate and dichloromethane (Tominaga and Dubourdieu, 2006; Tominaga et al., 1998). Organic phase was dried over anhydrous sodium sulfate and concentrated to 50 µl (Jenko et al., 2013). Chromatographic conditions were identical to those published in Jenko et al. (2013). One-point calibration was performed using calibration standards in water solution with a final concentration of 88 ng/l of 4MMP, 651 ng/l of 3MHA and 1624 ng/l of 3MH, and injected after every ninth sample. The limit of quantification (LOQ) was 2, 5 and 60 ng/l for 4MMP, 3MHA and 3MH, respectively.

Analyses of monoterpene

Monoterpene alcohols were analysed in wines only in the 2019 vintage. Analyses were performed by headspace-solid phase micro extraction (HS-SPME) as described by Bavčar et al. (2011) using GC (Agilent Technologies 7890A, Palo Alto, Santa Clara, CA, USA) coupled to MS (Agilent Technologies 5975C, Palo Alto, Santa Clara, CA, USA) and equipped with Gerstel MPS Autosampler (Gerstel, Mülheim an der Ruhr, Germany). Wine samples were diluted in ratio 1:4 with MilliQ water. In a SPME vial 5 ml of diluted wine was spiked with international standard 4-nonanol, followed by 1.7 g NaCl. Compound separation was achieved on INNOWax 30 m x 0.25 mm, film thickness 0.25 µm column (Agilent Technologies 7890A, Palo Alto, Santa Clara, CA, USA) coupled to a guard column fused silica deactivated 2 m x 0.25 mm (Agilent Technologies 7890A, Palo Alto, Santa Clara, CA, USA). The ions used for monoterpene alcohols quantification and method validation parameters are reported elsewhere (Bavčar, 2011; Bavčar et al., 2011).

Statistical analysis

Analysis of variance (ANOVA) was performed using Statistica, Version 12 (StatSoft, Tulsa, OK, USA) and the means were separated using Stats-Fisher's LSD test (different letters account for significant differences at $p \leq 0.05$). All stated uncertainty of juice and wine parameters is the standard deviation of three replicates of one treatment. Principal component analyses (PCA) was built with factoextra package (R StudioTeam, version 1.4.1106) on unit variance scaled data.

Results and Discussion

Weather data and phenological stages

Average temperatures, precipitation, and GDD are shown in Figures 1 and 2. The GDD index is a heat summation and is used to provide information on the suitability of crops for cultivation in different climates (Hall and Jones, 2010). The calculated GDD in Figure 2 were 1674, 1876 and 1553 for 2017, 2018 and 2019 vintages, respectively, classifying the experimental site as Region II in 2019 vintage and as Region III in 2017 and 2018 vintages (Hall and Jones, 2010). The greatest differences in GDD accumulation between vintages were observed in April, May, and partially in June, while GDD accumulation in July and August followed a similar pattern between the three vintages (Fig. 2). In September, a month when harvest occurred, a much lower accumulation of only 147 GDD was calculated for the 2017 vintage, compared to 247.5 and 218 GDD in 2018 and 2019, respectively (Fig. 2). As also evident from the trend in average temperatures, the 2018 vintage was the warmest, particularly due to high average temperatures in April and May (Fig. 1). A similar GDD summation was obtained for the 2017 and 2019 vintages (until harvest; Table 1), but both vintages were very different. The 2017 vintage was notable for a very dry spring and summer, combined with warm weather in June and July, followed by one of the wettest and coldest Septembers on record (Fig. 1). Compared to the 2017 vintage, the 2019 vintage was characterized by a cold April and May and a late start of phenology. June 2019 was extremely warm, while the rest of the season was comparable to the 2018 vintage (Fig. 1 and 2). Anthesis (BBCH 61) and fruit set (BBCH 71) were about one week earlier in 2018 than in 2017 and about 14 days earlier than in 2019 (Table 1). There were no differences in the onset of veraison between the 2018 and 2017 vintages, whereas it was five days later in 2019, compared to 2018 vintage. Veraison occurred at 1143 GDD in 2018 and 1045 GDD in 2017, respectively. The differences in GDD required between vintages for vines to enter a particular phenophase are strongly related to vine water status in addition to temperature summation (Jones and Davis, 2000). As reported by Jones and Davis (2000), rainfall delayed floraison as well as veraison, which explains the lower GDD required for vines to enter veraison in the 2017 vintage compared to the 2018 and 2019 vintages in our study (Table 1).

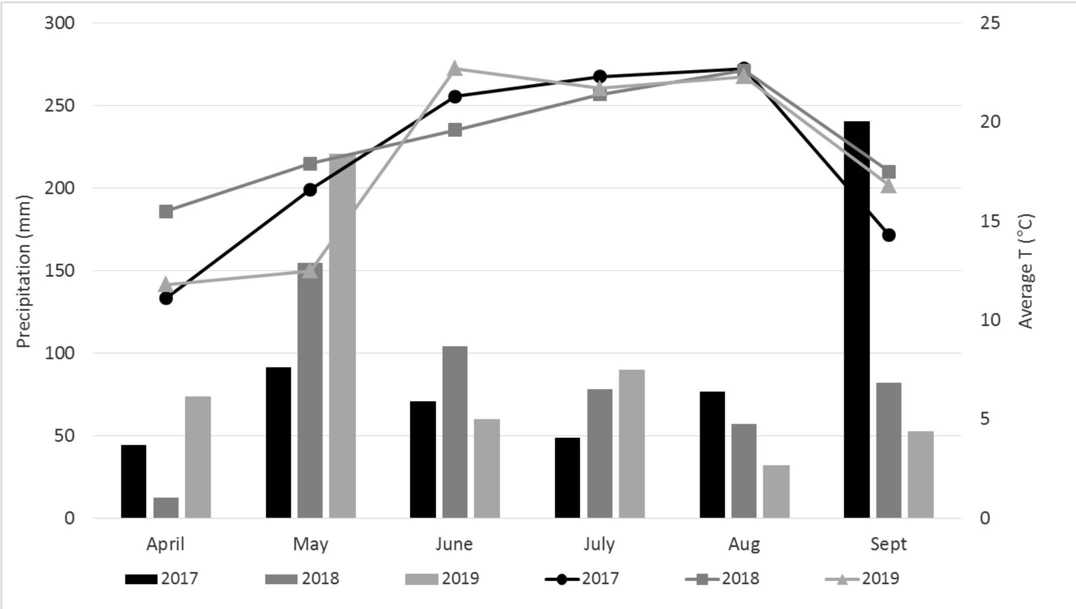

Fig. 1: Average monthly precipitation (mm) - histograms and average temperatures (°C) - lines from April 1 to September 30 for vintages 2017, 2018 and 2019

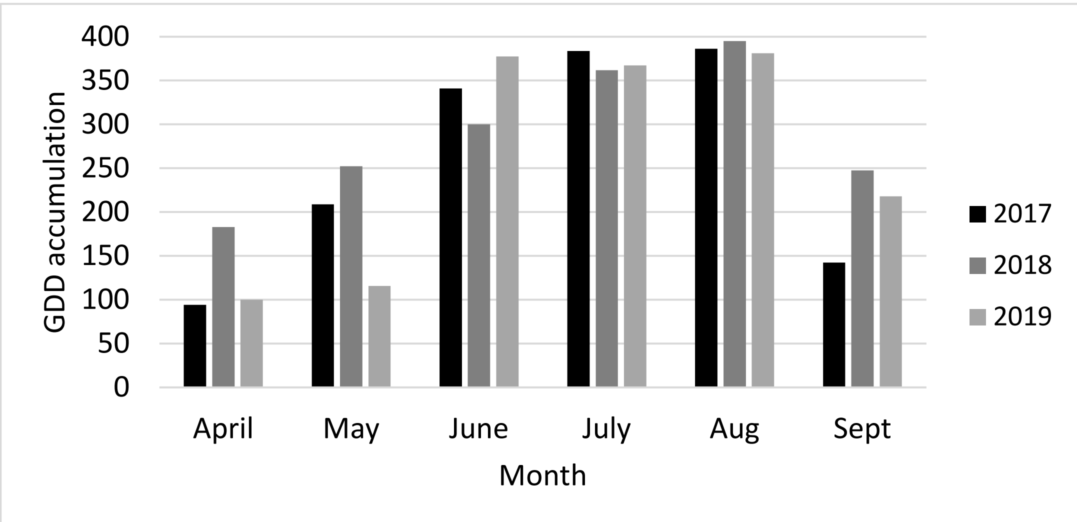

Fig. 2: Monthly growing degree days (GDD) from April 1 to September 30 for vintages 2017, 2018 and 2019

Table 1: Date, day of the year and growing degree days (GDD) accumulation for phenological stages of 'Welschriesling' grapevines in vintages 2017, 2018 and 2019

	Date			**Day of the year**			**GDD**[1]		
Phenological stages	2017	2018	2019	2017	2018	2019	2017	2018	2019
Budbreak (BBCH 05)	3.4.	14.4.	5.4.	93	104	95	19	56	16
Anthesis (BBCH 61)	3.6.	25.5.	12.6.	154	145	163	339	369	359
Fruit set (BBCH 71)	17.6.	9.6.	22.6.	168	160	173	477	537	486
Veraision (BBCH 81-85)	1.8.	3.8.	8.8.	213	215	232	1045	1143	1203
Harvest (BBCH 89)	10.9.	10.9.	15.9.	253	253	258	1482	1585	1462

[1] GDD calculated from April, 1st, until harvest date (base T > 10 °C)

Grape yield, juice and wine parameters

A PCA was applied to the measured variables (i.e. yield parameters, grape juice and wine parameters) across three vintages explaining 65 % of the variation with the first two dimensions. Figure 3A shows that the 2017 vintage was separated from the 2018 and 2019 vintages by principal component (PC) 2, which explained 31.7 % of the variation in the data set. PC1 separated the warmer 2018 vintage, which was positively related to 4MMP, yield/vine, and number of clusters/vine, from the cooler 2017 and 2019 vintages (Fig. 3A, B). More so, imposed 95 % confidence ellipses showed that significant differences were observed between CT and C treatments in the 2017 and 2019 vintages, while no differences were observed between the two treatments in the 2018 vintage (Fig. 3A). Wine 3MH, 3MHA, total dry matter, juice, and wine TA were positively associated with CT treatment in the 2019 vintage, whereas the 2017 samples were associated with wine alcohol content, juice TSS, and wine pH (Fig. 3A, B). It could be postulated that yield reduction by cluster thinning is an efficient tool to modify grape and wine composition in cooler vintages, while it may not have a significant effect in warmer vintages such as 2018 in our study. These observations are strengthened also by Frioni et al. (2017) who observed effects of cluster thinning on 'Cabernet Franc' only in one cooler season out of two studied. However, in 2018 vintage cluster thinning resulted in 30 % yield reduction, which is less than 41 % and 45 % as in 2017 and 2019 vintages, respectively. The yield reduction was proportional to the number of clusters removed, i.e. 42, 33 and 53 % in 2017, 2018 and 2019 vintages, respectively, at veraison in CT treatment compared to control. Therefore, the severity of yield reduction could also have affected the outcome of cluster thinning in 2018 vintage. A compensatory effect such as an increase in bunch or berry fresh mass is often reported as a consequence of early bunch thinning (pre-veraison) (Dokoozlian and Hirschfelt, 1995; Palliotti and Cartechini, 2000). In our study, no significant differences were observed in bunch weight or berry fresh mass (Table 2), which is in agreement with previous studies (Esperanza Valdés et al., 2009; Xi et al., 2020), which performed bunch thinning at a similar phenological stage (around veraison). According to Ollat et al. (2002) maximum cell division of the mesocarp is observed around 8 days after flowering and lasts up to 30 to 40 days for berry skin cells, while Coombe (1962) and Kliewer and Ough (1970) reported that berry size is regulated by carbohydrate availability at early stages of berry development. Therefore, a possible explanation could be that the final berry size is determined by berry cell number and its maximum size, which is regulated at the early stages of berry development, and that the later source-sink ratio does not significantly affect berry growth. No significant differences in TSS in grape juice were measured between C and CT treatments (Table 2). Although it was reported in several studies for red and white varieties that cluster thinning increases TSS in grape juice (Bubola et al., 2011; Esperanza Valdés et al., 2009; Ferree et al., 2003; Reščič et al., 2015), this was not confirmed in this study. Environmental conditions (temperatures, rainfall, grape diseases) can mitigate or overlap with the effects of cluster thinning (Frioni et al., 2017; Reeve et al., 2018; Zhuang et al., 2014) and therefore no or inconsistent effects of cluster thinning on yield and grape composition may occur (Keller et al., 2005; Preszler et al., 2013). Also, in our study, the majority of the measured variables were significantly influenced by vintage (Table 2 and 3), while significant influence of cluster thinning was evident only for a limited number of variables, i.e. number of clusters/vine, yield/vine, TA in grape juice, total dry matter and 3MHA in wine (Table 2 and 3).

Table 2: Yield and grape juice parameters at harvest for three consecutive vintages (2017 to 2019)

	Vintage [1]						p values [2]		
	2017		2018		2019				
	C	CT	C	CT	C	CT	T	V	T*V
Clusters/vine	27.0±0.42a	15.9±1.61b	32.1±3.56a	21.6±1.49b	29.6±1.23a	14.0±2.14b	***	***	ns
Yield/vine (kg)	3.83±0.55a	2.27±0.32b	4.86±1.74	3.41±1.44	4.70±0.64a	2.59±0.17b	**	ns	ns
Bunch weight (g)	153±12	173±5	153±58	154±55	161±27	187±23	ns	ns	ns
Yield reduction	0%	41%	0%	30%	0%	45%	**	ns	ns
100 berries fresh mass (g)	-	-	164±19	162±8	170±2	173±2	ns	ns	ns
TSS (°Brix)	22.3±0.3	22.8±0.4	19.7±0.7	19.7±0.3	22.1±0.8	21.5±1.2	ns	***	ns
TA (g/l)	5.92±0.23	6.06±0.14	5.97±0.25b	6.33±0.32a	6.07±0.51	6.83±0.21	*	ns	ns

[1] ANOVA was used to compare data. Means followed by a different letter in a row are significantly different at $p < 0.05$ (Fisher's LSD). All stated uncertainty is a standard deviation of three replicates per treatment.
[2] Significance of two-way ANOVA for T, treatment; V, vintage and T*V, interaction treatment*vintage; asterisks indicate level of significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, whereas ns indicates no significant differences.

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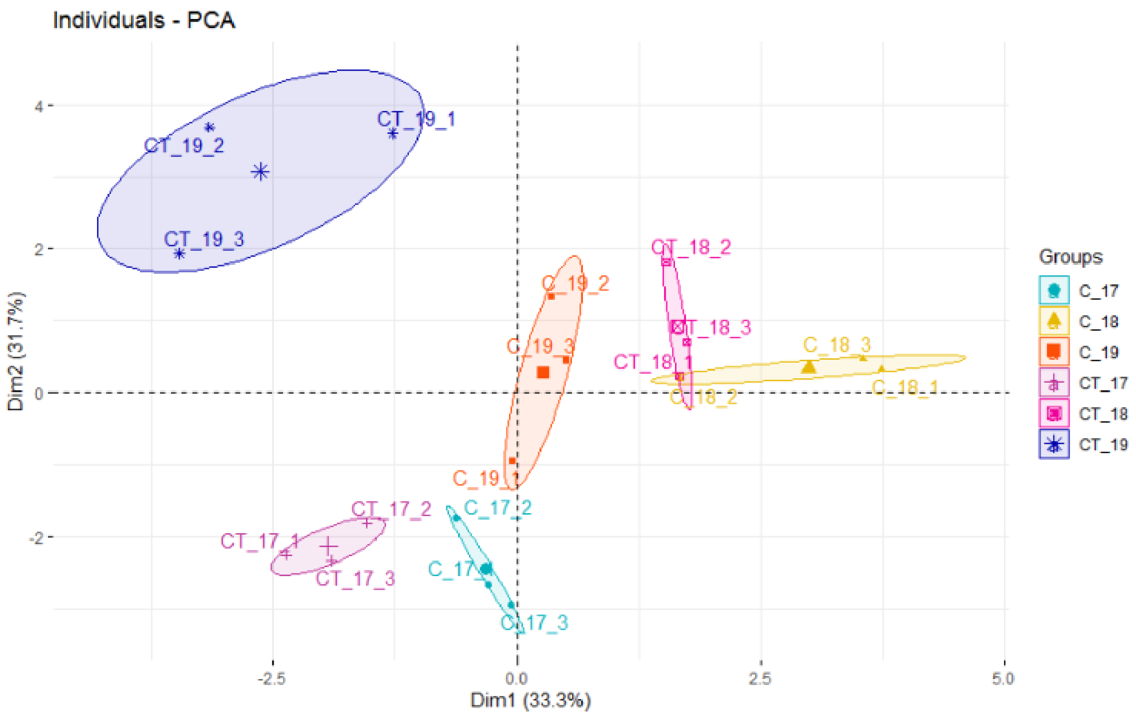

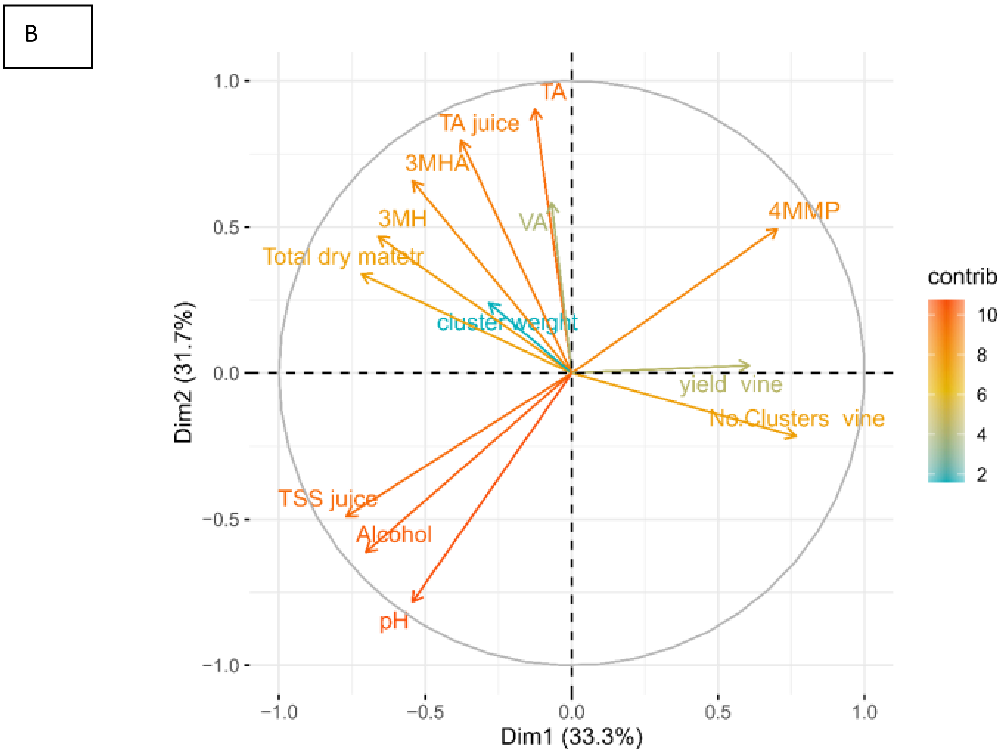

Fig. 3: Principal component analyses (PCA) conducted on measured grapevine yield, grape juice and wine parameters for the first two principal components; A) score plot for the first two principal components; ellipses represent 95 % confidence intervals for sample groups; C_17, control treatment in 2017 vintage; C_18 control treatment in 2018 vintage; C_19 control treatment in 2019 vintage; CT_17 cluster thinning treatment in 2017 vintage; CT_18 cluster thinning treatment in 2018 vintage; CT_19 cluster thinning treatment in 2019 vintage, B) loadings for the first two principal components; colour indicates the importance to the separation of samples for individual variable

Table 3: Effect of cluster thinning on 'Welschriesling' wine composition in three consecutive vintages (2017 to 2019)

	Vintage [1]						p values [2]		
	2017		2018		2019				
	C	CT	C	CT	C	CT	T	V	T*V
Alcohol (%vol.)	14.30±0.17	14.37±0.3	12.00±0.49	11.96±0.19	13.65±0.73	13.12±0.87	ns	*	ns
Total dry matter (g/l)	19±0.95	19.47±0.32	18.27±0.50	18.67±0.78	18.63±0.21b	20.27±0.57a	*	*	ns
TA (g/l)	5.27±0.21	5.3±0.2	5.77±0.21	5.77±0.21	6.03±0.32	6.7±0.60	ns	***	ns
pH	3.40±0.04	3.46±0.03	3.20±0.04	3.23±0.03	3.24±0.05	3.24±0.10	ns	***	ns
VA (g/l)	0.21±0.02	0.22±0.06	0.36±0.05	0.35±0.01	0.49±0.09	0.46±0.14	ns	***	ns
4MMP (ng/l)	1.7±1.49	2.5±2.5	9.03±3.13	9.13±3.23	4.67±1.15	4.33±1.07	ns	***	ns
3MH (ng/l)	633.5±100.4	779.3±63.6	520.3±125.2	481±89.16	1156.8±136.1	1591.06±599.43	ns	***	ns
3MHA (ng/l)	73.77±4.40	75.67±4.8	73.23±4.81b	91.83±6.67a	97.93±8.1	163.67±58.9	*	**	ns
linalool (µg/l)	-	-	-	-	12.3±1.5	9.7±3.2	-	-	-
α-terpeniol (µg/l)	-	-	-	-	26.3±7.1	22.3±7.2	-	-	-
Citronellol (µg/l)	-	-	-	-	8±1.0	7±1.0	-	-	-
Geraniol (µg/l)	-	-	-	-	16.7±3.8	12±5.3	-	-	-

[1] ANOVA was used to compare data. Means followed by a different letter in a row are significantly different at $p < 0.05$ (Fisher's LSD). All stated uncertainty is a standard deviation of three replicates per treatment.

[2] Significance of two-way ANOVA for T, treatment; V, vintage and T*V, interaction treatment*vintage; asterisks indicate level of significance: *p < 0.05, **p < 0.01, ***p < 0.001, whereas ns indicates no significant differences.

Cluster thinning did not significantly alter the majority of measured wine parameters in our study in any of the observed vintages. Variability between vintages had a significant effect and imparted wine composition (Table 3). On the other hand, only total dry matter and 3MHA concentrations were significantly affected by cluster thinning and had higher concentrations in wines from the CT treatment (Table 3). Cluster thinning did not affect basic wine parameters such as alcohol content, TA and pH in any of the vintages. There is no consensus in literature on the effect of cluster thinning on the above parameters (Gil et al., 2013; Reynolds et al., 2007; Šuklje et al., 2013). One explanation could be that the metabolic changes in grape composition induced by cluster thinning are strongly influenced by the developmental stage when cluster thinning is performed, the severity of yield reduction and also the initial yield potential of the vine. In addition, growth conditions such as water stress, nutrient availability, bunch light exposure, ambient temperatures, and others may interact or overlap with the changes induced by cluster thinning.

Varietal thiols have been previously identified in 'Welschriesling' wines (Čuš et al., 2017; Šuklje and Čuš, 2021). In the current study, a trend towards higher 3MH and 3MHA concentrations in wines from the CT treatment was observed. The obtained results are in agreement with a previous study on the relationship between leaf area/yield ratio, where bunch thinning resulted in higher concentrations of 3MHA, 3MH and 4MMP in 'Sauvignon blanc' wines (Šuklje et al., 2013). Higher amino acids concentrations were also reported in grape juice from cluster thinning treatments in 'Vilana' variety (Bena-Tzourou et al., 1999) and a positive correlation between several amino acids and 3MH and 3MHA concentrations was demonstrated (Pinu et al., 2014). Monoterpene alcohols were measured in the wines only in the 2019 vintage, but no significant differences were found between treatments. Our results are in agreement with those of Reynolds et al. (2007), who observed no effect of cluster thinning on free volatile terpenes in wine, regardless of the timing of cluster removal. However, several other studies report an increase in monoterpene concentrations with reduced yields (Rutan et al., 2018; Xi et al., 2020). It is well known that monoterpenes in grapes are strongly influenced by growing conditions, such as bunch exposure to sunlight (Marais et al., 1999), grape maturity (Marais and Van Wyk, 1986), water stress (Savoi et al., 2016), and also temperature (Šuklje et al., 2019).

Conclusions

Cluster thinning at veraison, removing up to 53 % of clusters/vine, resulted in a proportional reduction in yield/vine at harvest. A significant effect of cluster thinning on measured parameters was observed in cooler vintages, with a lower GDD summation and a yield reduction of over 40 % compared to the control. In a warmer vintage and with a yield reduction of 30 %, no effect of grape thinning was observed. Interestingly, varietal thiol concentrations, particularly 3MHA, were positively correlated with cluster thinning. In late vintages and with sufficient yield reduction, cluster thinning could be a tool to adjust wine styles already in the vineyard when adapted to the variety with its yield potential.

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