Leaf Removal and Cluster Thinning Efficiencies Are Highly Modulated by Environmental Conditions in Cool Climate Viticulture

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Abstract: One of several challenges in cool climate viticulture with a short growing season is to consistently reach a uniform, optimal fruit technological maturity at harvest before the first autumn frost. Weather conditions in Michigan from veraison to harvest are highly variable and unpredictable among years, constraining the preharvest assessment of fruit quality for grapegrowers and wineries. Under these environmental conditions, cluster thinning and leaf removal are commonly adopted viticultural techniques to enhance fruit ripening. Cluster thinning consists of a selective elimination of clusters to optimize the source/sink ratio of the vine. Cluster zone leaf removal induces changes in the fruit microenvironment, particularly solar radiation, temperature, and aeration. In this work, we evaluated the effects of cluster thinning and cluster zone leaf removal, applied separately in combination at veraison, on Cabernet franc in two consecutive years, 2011 and 2012. The two seasons had very distinct weather patterns from veraison to harvest. Fruit maturity was enhanced at 15 to 20 days after veraison in both years by these viticultural techniques, but with very different dynamics. The combination of leaf removal and cluster thinning led to greater fruit uniformity and better chemical composition at harvest in 2011, a year characterized by low heat accumulation after veraison. In 2012, when heat accumulation and mean temperatures after veraison were higher than in 2011, no differences were observed among treatments.

Key words. anthocyanin to Brix ratio, crop load, fruit composition, fruit ripening, technological maturity

Optimal fruit maturity at harvest strikes an ideal balance between chemical components such as sugars, acidity, aromatic and volatile compounds, color, and absence of pathogens. The concentrations of several chemical components of winegrapes, including total soluble solids (TSS), organic acids, polyphenols, and flavor compounds, determine technological, phenolic, and aromatic maturity of fruit at harvest and are critical contributors of wine quality (Jackson and Lombard 1993). Elevated temperatures and cluster exposure to direct solar radiation aid in sugar accumulation, organic acid degradation, and biosynthesis of color and aromatic compounds (Sadras et al. 2013, Matus et al. 2009, Diago et al. 2012), all essential to obtain optimal fruit quality, particularly in cool climates (Acimovic et al. 2016).

In cool viticulture regions, the achievement of fruit chemistry balance is challenged by different environmental factors that may affect the results of viticultural practices (Howell 2001). In viticultural regions characterized by cool, short growing seasons with considerable annual variability, the tools used to achieve desired fruit chemistry and maturity involve optimization of vine balance and management of vine canopy to improve fruit-zone microclimate conditions (Howell 2001). Additionally, an important role in fruit quality is played by the source/sink ratio and balance between vegetative vigor and reproductive activity of the vine, usually indexed as the ratio between leaf area and fruit yield per vine (Howell 2001, Kliewer and Dokoozlian 2005). Vine balance is achieved by manipulating the vine vegetative and reproductive growth (crop load) into a specific equilibrium to achieve targeted fruit characteristics (Howell 2001). Crop load has long been defined by the Ravaz Index (RI), or the ratio between fruit yield and one-year-old cane pruning weight, and was first used to assess vine balance (Ravaz 1911). Similar to RI, leaf area to fruit weight ratios of 8 to 12 cm^2/g are also regarded as a hallmark of vine balance (Kliewer and Dokoozlian 2005). However, these benchmarks for balanced vines and their components can vary due to the integrated impact of climate, fertilization plan, trellising system, and choice of cultivars (Kliewer and Dokoozlian 2005). In a cool climate,

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where low heat accumulation (growing degree days [GDD]), short growing seasons, and broad seasonal variation are common, a higher amount of total vine leaf area is required to ripen fruit to a specific level (Howell 2001, Tozzini et al. 2013). Adjustments to crop load or vine balance are achieved with vine and canopy management techniques.

Cluster thinning is widely used to obtain a desired crop load (Palliotti and Cartechini 2000, Guidoni et al. 2002). The selective removal of excessive clusters, despite additional labor time, allows vine productivity to be calibrated, eliminating defective components of the yield and avoiding excessive delays in ripening (Jackson and Lombard 1993, Palliotti and Cartechini 2000, Guidoni et al. 2002, Dami et al. 2006, Tardaguila et al. 2008a, Santesteban et al. 2011, Palliotti et al. 2014). However, cluster thinning effects can be compounded by environmental conditions, vine physiological status, and other viticultural practices, and its economical sustainability is being debated (Ough and Nagaoka 1984, Palliotti and Cartechini 2000, Keller et al. 2005, Gatti et al. 2012).

The removal of photosynthetically active leaves at different stages of vine growth is a technique used for many purposes (Bledsoe et al. 1988, Palliotti et al. 2013, Poni et al. 2013, Sivilotti et al. 2016). In particular, the timing of basal leaf removal is critical. When performed too early (e.g., before flowering), it results in the loss of leaf area and decreases photosynthesis necessary to produce carbohydrates for berry development (Palliotti et al. 2011). Basal leaf removal is frequently used to increase sunlight exposure and cluster temperature (Bledsoe et al. 1988, English et al. 1989, Smart and Robinson 1991). Cluster exposure to direct sunlight increases polyphenols and decreases acidity in cool climates (Price et al. 1995, Spayd et al. 2002, Downey et al. 2006). It also has the potential to modify the relationship between TSS (mostly sugars) and anthocyanin accumulation, inducing a decoupling effect (Sadras and Moran 2012). Prebloom leaf removal can lead to a looser cluster, with better penetration of light and air and, consequently, improved fruit composition (Sabbatini and Howell 2010, Acimovic et al. 2016, Sivilotti et al. 2016). When the technique is executed at the onset of veraison, it exposes the cluster to light and temperature and limits the spread of disease (Howell 2001). Leaf removal is an adjustment of total vine leaf area and affects the relationship between photosynthetically active leaf area and yield (Howell 2001, Palliotti et al. 2013).

Cluster zone leaf removal and cluster thinning, performed together or separately, and their interactions, could provide insights into physiological and metabolic processes under the influence of different viticultural practices in cool climate regions. The two techniques were recently proposed in Italy and Spain for Sangiovese and Tempranillo grapevines: cluster thinning was precise and effective in reducing yield, although early leaf removal (before flowering) was a simpler, less expensive, but less efficient practice (Gatti et al. 2012, Tardaguila et al. 2012). Cluster thinning, applied with early leaf removal, improved fruit (Bogicevic et al. 2015). In Ontario, the combined treatments of leaf removal and cluster thinning enhanced accumulation of TSS and increased the concentration of anthocyanins at harvest, while the single application of one of these techniques led to inconsistent effects (Di Profio et al. 2011). In Michigan, cluster thinning and leaf removal applied at different times during the growing season enhanced fruit maturity more effectively when applied in cool seasons (Zhuang et al. 2014).

Weather conditions after veraison are fundamental to reach optimal fruit maturity, especially in cool climate viticulture, when winter and spring minimum temperatures are favorable and vines reach the veraison stage with little or no damage (Jackson and Lombard 1993, Schultze et al. 2014). The aim of this study was to evaluate the effects of cluster thinning and cluster zone leaf removal, applied in combination at veraison in two seasons. Different weather conditions after veraison in the two years provided a guide to understand fruit ripening dynamics and fruit composition at harvest in Cabernet franc grapevines, a mid- to late-ripening cultivar for Michigan, and a pivotal red cultivar for cool climate viticulture.

Materials and Methods

Plant material. The experiment was conducted in 2011 and 2012 on *Vitis vinifera* L. Cabernet franc vines (clone FPS 01), grafted on 3309C rootstock and planted in 1993 at the Southwest Michigan Research and Extension Center (SWMREC) of Michigan State University (Benton Harbor, 42°05′N; 86°21′W). SWMREC soils are defined as Spinks sandy loam soil (USDA 1957). In the experimental vineyard, each row (north-south orientation) is composed of 48 vines with spacing of 2.4 m between vines and 3.0 m between rows, trained to a vertical shoot-positioned system and spur-pruned to ~48 nodes per vine during the winter. Vines were trained with multiple trunks to ensure survival at low winter temperatures (Pool and Howard 1984). Hedging was carried once during the season, when shoots were 30 cm above the top catch wire, to maintain a canopy free of excessive shading and, subsequently, to avoid eventual reduction in passive heating capacity (Sadras and Moran 2012). Standard commercial pest-control practices were applied based on scouting, experience, and weather conditions (Wise et al. 2003). Rainfall and cumulative GDD (Baskerville and Emin 1969) during the growing season were measured at the Michigan Automated Weather Network station at SWMREC, located 300 m from the site of the experiment (http://www.enviro-weather.msu.edu/weather.php?stn=swm).

Field experimental design and treatments. The experiment was a randomized complete block design consisting of four blocks and three factors (cluster thinning, leaf removal, and year) with 12 vines per treatment. The phenological stages were identified during the season as described (Pearce and Coombe 2004). After fruit set, ~80 to 90 clusters were left on each vine. Before the onset of veraison, the two factors were assigned, dividing the vines into four treatments: no thinning no leaf removal (C), no thinning with leaf removal (LR), thinning with no leaf removal (TH), and thinning with leaf removal (TH + LR). Cluster thinning was applied when clusters reached 50% berry color change, leaving ~50 clusters on TH and TH + LR vines (53 ± 11 in 2011 and 47 ± 10 in

2012), and ~85 clusters on C and LR vines (92 ± 16 in 2011 and 78 ± 14 in 2012). Leaf removal was performed at the same time as cluster thinning, removing ~6 basal leaves in LR and TH + LR vines; no leaves were removed in C and TH vines.

Daily cluster temperature and radiation measurement. Cluster zone light intensity and temperature were measured using photosynthetically active radiation (PAR) sensors (model SQ-110, Apogee Instruments) and a fine-wire (American Wire Gauge [AWG]) thermocouple (Type T [copper-constantan]) in contact with the berry skin. Six light sensors were placed horizontally, corresponding to the fruit zone of the canopy on three representative vines for each treatment. Both PAR sensors and thermocouples were connected to data loggers (CR-10, Campbell Scientific) that also controlled multiplexers designed specifically for thermocouples and quantum sensors (AM18/32A, Campbell Scientific). Ambient air temperature was also tracked by shielded, aspirated, fine-wire thermocouples (AWG; type T) placed at the same height of the fruit zone. All signals were scanned at 30 sec intervals with the values recorded every 20 min continuously from veraison to harvest. Mean diurnal PAR (0800 to 2000 hr) and temperature (0800 to 2000 hr) patterns were based on the average 60 min values calculated from data collected over the full season. Cluster zone diurnal temperature in 2011 and 2012 from veraison to harvest were calculated using the average 60 min value (hourly temperature) to generate the cluster zone temperature distribution during the day (from 0800 to 2000 hr) and night (from 2000 to 0800 hr) for each treatment.

Sampling procedures and harvest data collection. At the beginning of veraison, 60 berries from six vines per treatment were periodically sampled from clusters on previously tagged shoots (six per vine) to track fruit maturation until harvest. Harvest was fixed when samples collected reached an average of 21 Brix. At harvest, vine yield and cluster number per vine were recorded and clusters from the tagged shoots of each vine were harvested and immediately placed in coolers, transported to campus, and stored at -20°C. Each cluster was weighed and the berry number per cluster was used to calculate average berry weight. Fruit chemistry components (TSS, pH, titratable acidity [TA], anthocyanins, and total phenolics) were then analyzed. Pruning weights were collected the following winter.

Fruit chemistry measurements. Harvested frozen grapes were thawed to room temperature before chemical analysis. Berries were crushed with a manual press and free-run juice was decanted into 50 mL tubes. Juice Brix was measured using a digital refractometer (ATA-3810 PAL-1, Pulse, Inc.). A 370 Thermo Orion pH meter (Thermo Fisher Scientific, Inc.) was used to measure pH. TA was measured using a Multi-T 2.2 digital titrator (Laboratory Synergy, Inc.) with each sample consisting of 10 mL clear juice diluted to 100 mL with distilled water and titrated with 0.1 M sodium hydroxide to pH 8.2 using an equation to yield the TA (g/L) as described (Iland et al. 2004). Anthocyanins and total phenolic compounds were measured by the total phenol assay using UV-vis as described (Iland et al. 2004). Berries stored at -20°C were thawed and ground using a tissue homogenizer (Brinkmann Instruments) at a speed of four on the manufacturer's scale for ~1 min. Samples were ground while maintained in an ice bath to minimize oxidation. The homogenate included flesh, skins, and seeds. Homogenized samples (~1 g ± 0.05 g) were added to a tared 15 mL centrifuge tube and the mass was recorded. Ten mL of 50% v/v aqueous ethanol acidified to pH 2 (~1 mL 12.1 M HCL) was added to the 1 g sample and manually mixed once every 5 min for 1 hr. The sample was then centrifuged at 1800 g_n for 20 min. One mL of extract (supernatant liquid) was pipetted into a 15 mL centrifuge tube. Ten mL 1 M HCL was added to the mixture and equilibrated for 3 hrs. Absorbance values were read using a UV-vis spectrophotometer (Model UV-1800, Shimadzu Corporation) at 280 nm (total phenolics), 520 nm (anthocyanins), and 700 nm (turbidity control).

Statistical analysis. Results were tested for normality and homogeneity of variance and subjected to a three-way (cluster thinning, leaf removal, and year) analysis of variance (ANOVA) using the PROC MIXED in SAS (version 9.1.3; SAS Institute, Inc.). However, for the variables analyzed, year was not significant and did not interact with other factors; therefore, results were analyzed with a reduced two-way factorial statistical model. Regression analysis for selected variable was performed, separating data for each parameter collected during the two experimental years using Sigma Plot (version 10; Systat Software). Data frequency distribution was also analyzed. The distribution of the harvest data for Brix, anthocyanins, and relative ratio was evaluated by dividing samples into 11 classes. For sugars, classes were divided into intervals of 1 Brix, with class 1 representing samples between 15.5 and 16.5 Brix, and class 11 representing samples between 25.5 and 26.5 Brix. For anthocyanins, classes were divided into intervals of 0.1 mg/g, with class 1 representing samples between 0.3 and 0.4 mg/g, and class 11 representing samples between 1.3 and 1.4 mg/g. For the anthocyanins to Brix ratio, classes were divided into intervals of 0.005 mg/g/Brix, with class 1 representing samples between 0.015 and 0.02 mg/g/Brix, and class 11 representing samples between 0.065 and 0.07 mg/g/Brix.

Results

Weather conditions and phenological stages. Weather conditions for the experimental site in 2011 and 2012 are reported (Figure 1). The max, min, and average temperatures recorded during the two growing seasons were particularly different in May, in the second part of August, and later in September and October. In 2011, there was more rainfall and it was more uniformly distributed throughout the first part of the season than in 2012 (+174 mm in 2011 from 1 April to 20 Aug). In September, rainfall was slightly higher in 2012 (79 mm in 2011, but 90 mm in 2012). In 2012, rains were more concentrated in the last week of August and the second and third week of October, which were after harvest. In 2011, from veraison to harvest, the temperatures were lower than in 2012. September 2011 was characterized by repeated fluctuations in temperature, with the min falling below 5°C four times. In 2012, September was much warmer, with max

temperatures below 20°C on only eight days during the entire month. This was in contrast with September 2011, which had 16 days with temperatures under 20°C. Therefore, in September 2012, vines accumulated 52 GDD more than in September 2011. Conversely, October was warmer in 2011. The mean day (0800 to 2000 hr) air temperature from veraison (25 Aug 2011 or 22 Aug 2012) to harvest (21 Oct 2011 or 3 Oct 2012) was 2.6°C higher in 2012 than in 2011, as was the average night (2000 to 0800 hr) air temperature, which was 1.9°C higher in 2012 (Table 1). As a result, between 1 April and 31 Oct, vines accumulated 1587 GDD in 2011, but 1680 GDD in 2012. Vine phenological stage development was related to heat accumulation during the two seasons (Table 2). No substantial difference was found in timing of anthesis between 2011 and 2012, despite the higher GDD accumulation in May 2012. After full bloom, active temperatures followed a similar pattern in both years until mid-August, and phenological evolution respected this trend. Vines reached veraison on 25 Aug 2011 and 22 Aug 2012 at 1241 and 1288 GDD, respectively, at close days during the season with similar GDD accumulation. After veraison, grape maturity occurred very late in 2011, when criteria fixed for harvest were satisfied only on 21 Oct. In 2012, harvest fell much earlier in the season and grapes satisfied the same criteria on 3 Oct. Despite harvest occurring 18 days earlier in 2012, in both years, 338 GDD were accumulated from veraison to harvest, but this took 57 days in 2011 and 42 days in 2012.

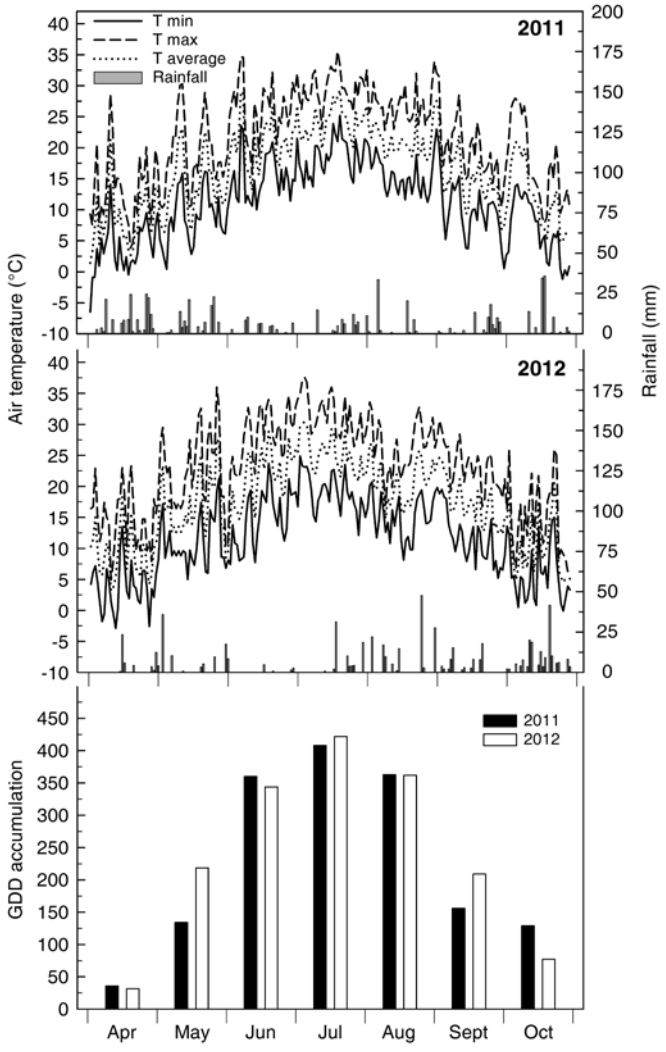

Figure 1 Weather data for the Southwest Michigan Research and Extension Center in Benton Harbor, MI, in 2011 and 2012 (1 April to 31 Oct). Daily max temperature (T max), min temperature (T min), average temperature (T average), rainfall, and monthly growing degree days (GDD) accumulation.

Yield components, vegetative parameters, and cluster morphology. Harvest occurred on 21 Oct 2011 and 3 Oct 2012, which was 18 days earlier in the warmer year, 2012, but only 15 days earlier when the days from veraison are considered. As expected, cluster thinning reduced yield (-31 to -46%) (Table 3). Leaf removal did not affect vine yield: C vines were similar to LR and TH vines, to TH + LR. Cluster weight, average number of berries per cluster, and average berry weight were not affected by cluster thinning or leaf removal, as no difference was found among treatments (Table 3). Pruning weight at the end of the winter was not different among treatments. Due to the discrepancy in vine yield, the RI was consequently lower in the thinned vines, but still within the range of balanced vines for a cool climate region (RI 4 to 7; Howell 2001).

Cluster temperature and light exposure. In 2011, the mean night cluster temperature was higher in C (+0.3°C), but there was no difference during the day (Table 4). In 2012, cluster temperature was no different at night, but higher during the day in LR vines (+2.0°C). PAR was considerably higher in LR vines than in other treatments. PAR in C vines was 22.8% of the PAR recorded on LR vines in 2011, and 29.8% of PAR recorded on LR vines in 2012.

Table 1 Night (2000 to 0800 hr) and day (0800 to 2000 hr) air mean temperature in 2011 and 2012 from veraison to harvest (25 Aug to 21 Oct 2011 and 22 Aug to 3 Oct 2012, respectively).

Year	Night air temp (°C)	Day air temp (°C)
2011	13.6	17.1
2012	15.5	19.7
Significance[a]	*	*

[a] * means significance at $p < 0.05$ by F-test for main effect.

Table 2 Date, growing degree days (GDD) accumulation, and day of the year for phenological stages and harvest date of Cabernet franc grapevines grown at the Southwest Michigan Research and Extension Center in Benton Harbor, MI, in 2011 and 2012.

Development stage	Date		GDD[a]		Day of year	
	2011	2012	2011	2012	2011	2012
Anthesis	18 June	21 June	403	481	169	173
Fruit set	30 June	28 June	529	566	181	180
Veraison	25 Aug	22 Aug	1241	1288	237	235
Harvest	21 Oct	3 Oct	1579	1626	294	277

[a]GDD calculated from 1 April with a base temperature of 10°C (Baskerville and Emin 1969).

Table 3 Yield components, cluster morphology, and pruning parameters in 2011 and 2012.

	Yield (kg/vine)		Number of clusters		Pruning weight (kg/vine)		Ravaz Index[a]		Cluster wt (g)		Number of berries/cluster[b]		Berry wt (g)[c]	
Treatment	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
C[d]	6.7 a[c]	6.8 a	95 a	78 a	1.7	1.0	4.2 ab	7.5 a	72	89	84	83	1.30	1.31
LR[d]	6.5 a	5.8 a	89 a	78 a	1.3	1.0	5.2 a	7.4 a	73	78	70	90	1.29	1.33
TH[d]	4.0 b	3.8 b	55 b	47 b	1.7	0.9	2.5 b	4.8 b	77	82	69	75	1.36	1.28
TH + LR[d]	4.4 b	4.0 b	51 b	46 b	1.3	1.0	3.3 b	4.5 b	89	91	74	76	1.29	1.25

[a]Ravaz Index = crop yield/pruning weight.
[b]Number of berries per cluster and berry weight were calculated from five tagged shoots per vine.
[c]Means within columns followed by different letters are significantly different at $p < 0.05$ by F-test for main effect.
[d]C: no cluster thinning no leaf removal; LR: leaf removal without cluster thinning; TH: cluster thinning without leaf removal; and TH + LR: cluster thinning and leaf removal.

Table 4 Night (2000 to 0800 hr) and day (0800 to 2000 hr) cluster mean temperature and cluster mean daily light radiation in 2011 and 2012.

	Night cluster temp (°C)		Day cluster temp (°C)		Day cluster light radiation (PAR)[a]	
Treatment	2011	2012	2011	2012	2011	2012
LR[a]	12.3	14.8	18.7	21.2	906	1084
C[a]	12.6	15.1	18.1	19.2	207	323
Significance[b]	*	ns	ns	*	*	*

[a]PAR: photosynthetically active radiation; LR: leaf removal without cluster thinning; C: no cluster thinning no leaf removal.
[b] * and ns means significance or not at $p < 0.05$ by F-test for main effect.

Fruit chemistry. At harvest, no differences were found in Brix and pH among treatments in either 2011 or 2012 (Table 5). TA was instead lower in both years for LR and TH + LR vines when compared to C and TH vines (-0.4 to -1.1 g/L). In 2011, total anthocyanins were higher in TH + LR than in any other treatment (+14% over C), but there was no difference among treatments in 2012. Total phenols were notably higher in TH + LR than any other treatment: +10% over C in 2011 and +14% over C in 2012; however, the LR treatment contained similar values and was also different (+9%) from C.

Fruit ripening dynamics. In both years, TH quickly enhanced the TSS concentration of the berries. TH was the treatment with the higher TSS at 18 days from veraison in 2011 and at eight days from veraison in 2012 (Figure 2). In both years, LR had lower Brix than any other treatment between eight and 27 days after veraison. However, these differences disappeared later in the season. Total anthocyanins were quickly enhanced in TH + LR vines in both 2011 and 2012, and C had less than any other treatment at the first step of ripening in both 2011 and 2012. Later in the 2011 season, TH + LR showed more color until harvest, while no differences were observed among treatments beyond 25 days after veraison in 2012. In both years, all treatments had an enhanced anthocyanins to Brix ratio from one to 24 days after veraison, with a peak at 15 to 18 days after veraison (TH + LR was +13% over C in 2011 and +12% in 2012). There was a faster rate of anthocyanin accumulation in TH + LR, TH, and LR treatments than in C (TH + LR was +60% faster than C at 18 days after veraison) in 2011 (Figure 3). Later in the season, at 27 days after veraison, the daily increase in anthocyanin was less in TH + LR than in C and consequently, all treatments followed a similar pattern. A similar trend was found in 2012; however, the daily increase in anthocyanin peaked earlier in the season for TH and TH + LR vines (eight days after veraison or 10 days earlier than in 2011), and was slower and more prolonged in C and LR (never faster than 0.033 mg/g/day and continuing until 15 days after veraison).

Distribution of data. Normal distribution curves in percentage were drawn for Brix, anthocyanins, and their relative ratio at harvest in 2011 and 2012 (Figure 4). In 2011, TH + LR was the treatment with greatest homogeneity in Brix distribution ($R^2 = 0.99$), followed by TH ($R^2 = 0.84$), LR ($R^2 = 0.99$), and C ($R^2 = 0.96$), with all means close to the seventh and eighth class. In 2012, Brix distribution data were less uniform among treatments, without a clear trend (R^2 ranging from 0.67 to 0.99). Anthocyanin data in 2011 was distributed closer to the average in TH + LR than in any other treatment ($R^2 = 0.99$), with mean values falling in a higher class. Again in 2012, the anthocyanin data distribution was different and without a trend, and the treatment TH + LR ($R^2 = 0.49$) was

Table 5 Fruit chemical parameters at harvest in 2011 (21 Oct 2011) and 2012 (3 Oct 2012).

	Soluble solids (Brix)		pH		TA (g/L)		Anthocyanins (mg/g)		Phenolics (AU/g)[a]		Anthocyanins:Brix ratio	
Treatment	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
C[b]	22.2	21.7	3.5	3.7	6.5 a[c]	6.0 a	0.84 b	0.79	1.26 b	1.27 c	0.037 b	0.036
LR[b]	22.3	22.0	3.6	3.7	5.8 b	5.4 b	0.82 b	0.81	1.22 b	1.39 ab	0.037 b	0.037
TH[b]	22.0	21.3	3.6	3.7	6.6 a	5.7 ab	0.85 b	0.82	1.23 b	1.36 b	0.038 b	0.038
TH + LR[b]	22.5	21.1	3.6	3.7	5.5 b	5.3 b	0.96 a	0.81	1.39 a	1.45 a	0.043 a	0.038

[a]AU: absorbance unit.
[b]C: no cluster thinning no leaf removal; LR: leaf removal without cluster thinning; TH: cluster thinning without leaf removal; and TH + LR: cluster thinning and leaf removal.
[c]Means within columns followed by different letters are significantly different at $p < 0.05$ by F-test for main effect.

less uniform than 2011 and the other treatments, due to several values in the seventh class. The distribution of the ratio between Brix and anthocyanins had greater homogeneity in 2011 in TH + LR ($R^2 = 0.99$) than in other treatments and in 2012, there was an absence of clear trends.

Discussion

Cluster thinning and leaf removal are management techniques widely adopted in cool climates to avoid and/or mitigate the negative effects of low heat accumulation, high humidity, and rainfall on fruit ripening (Jackson and Lombard 1993, Howell 2001, Guidoni et al. 2002, Di Profio et al. 2011, Bogicevic et al. 2015). The efficacy of cluster thinning is subject to many external factors and there are additional costs associated with its adoption, which led growers and researchers to formulate alternative practices (Ough and Nagaoka 1984, Gatti et al. 2012). Leaf removal is a potentially fully mechanized operation and is considered essential in cool climates to achieve full ripening with limited fruit cluster rot complex at harvest (Howell 2001). In Montenegro, cluster thinning was proposed as an integrative canopy management of early leaf removal (Bogicevic et al. 2015). In cool climates, the simultaneous application of leaf removal and cluster thinning may have additive effects, improving color and sugars at harvest consistently over several seasons; when the techniques are applied singularly, the results are uncertain (Di Profio et al. 2011). Moreover, cluster thinning and leaf removal may have an indirect additional consequence, increasing the rate of clusters exposed to better microclimatic conditions over that seen with leaf removal alone. Cluster thinning may lead to the elimination of the distal part of the fruits, which are not particularly advantaged by leaf removal, being closer to the retained leaves. Recently, it was suggested that in cool climate viticulture, cluster

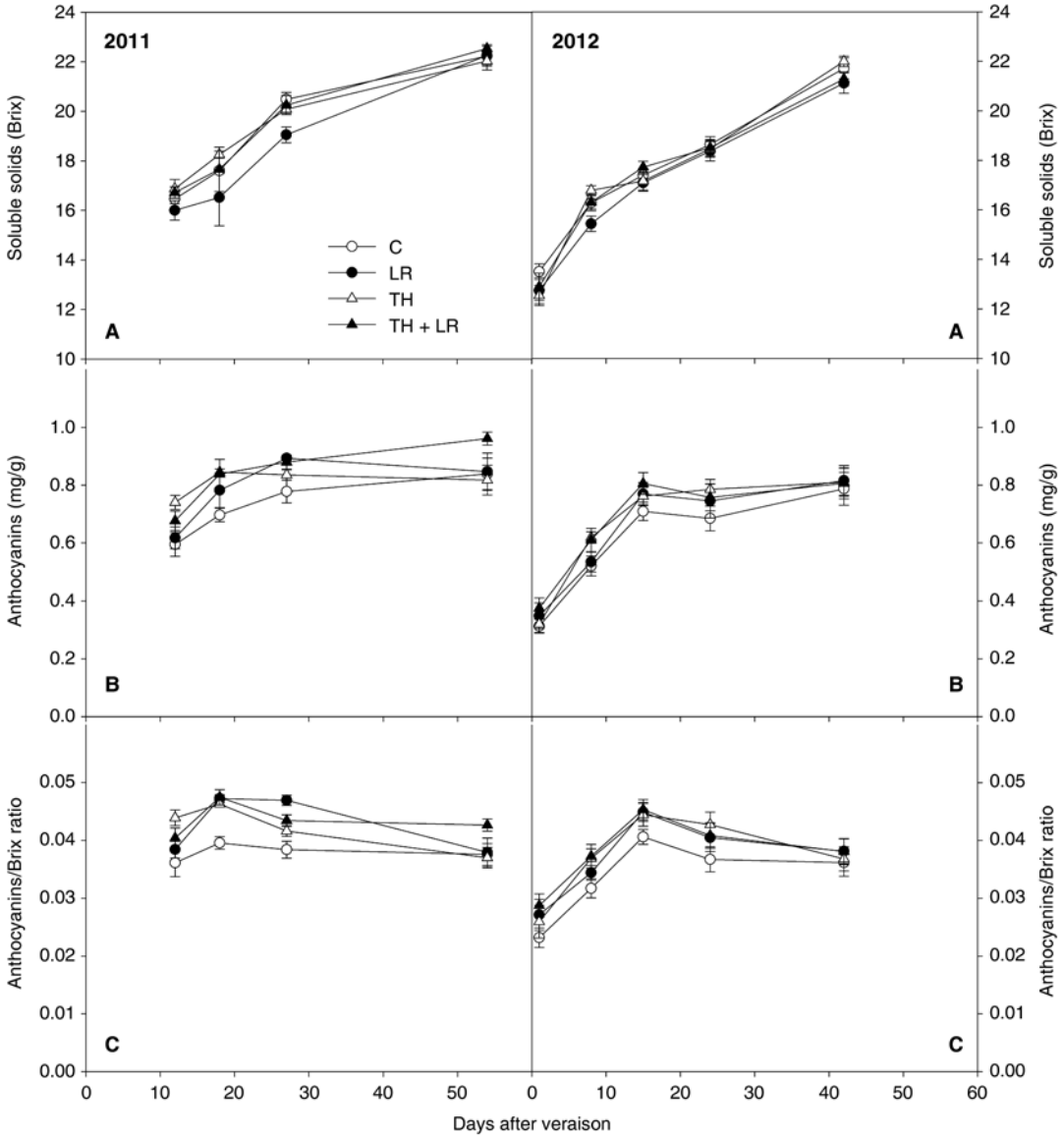

Figure 2 Profiles of (**A**) soluble solids, (**B**) anthocyanins, and (**C**) the ratio of anthocyanins to soluble solids during ripening in 2011 (left panels) and 2012 (right panels) in relation to cluster thinning and leaf removal. Vertical bars represent standard errors around means. C: no cluster thinning no leaf removal; LR: leaf removal without cluster thinning; TH: cluster thinning without leaf removal; and TH + LR: cluster thinning and leaf removal.

thinning and leaf removal efficacy was affected by seasonal heat accumulation, and not just by the time of execution (Zhuang et al. 2014). Our two-year experiment revealed that TH + LR effects could be different in relation to the weather patterns during ripening.

In Michigan, 2011 and 2012 had very different weather during the growing season, with 93 GDD less in 2011 than in 2012. This was caused by unstable temperatures in early May and later from veraison to harvest, as described by air T max and T min, by monthly GDD accumulation, and by cluster temperature recorded during ripening. Conversely, October was warmer in 2011, allowing a partial recovery in seasonal active temperatures. Rainfall was much more homogenously distributed in 2011 than in 2012, when rains were concentrated on specific days and during October, once grapes were already harvested. Considering all environmental parameters, 2011 and 2012 were a typical cool and warm season for the region, respectively, both contributing to the eventual determination of a long-term average for the area. Despite warm temperatures in May 2012, anthesis occurred between 18 and 20 June in both years, and there was comparable development recorded in the two seasons from budburst to veraison. After veraison, grape ripening followed different patterns in the two seasons, with fast sugar accumulation and organic acids degradation in 2012, when temperatures were higher, and a slower progress in 2011, when weather was less favorable.

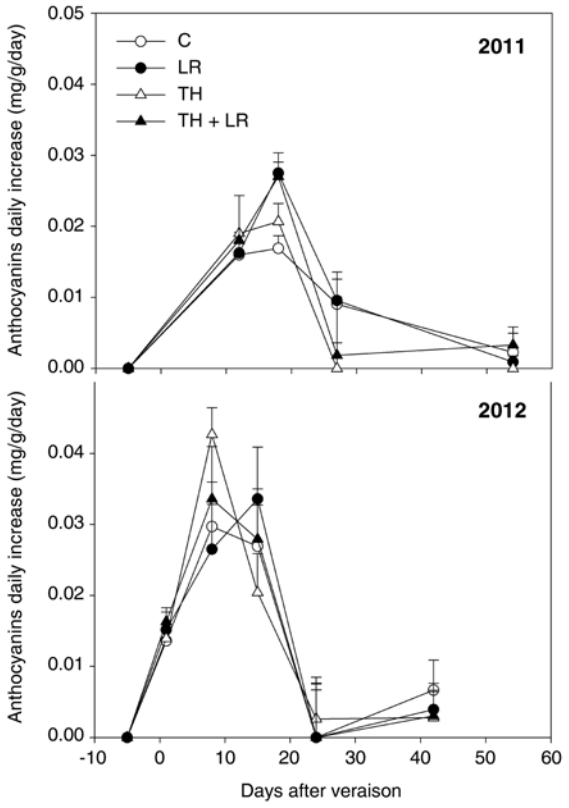

Figure 3 Daily increase in anthocyanins during ripening in 2011 (upper) and 2012 (lower) in relation to cluster thinning and leaf removal. Vertical bars represent standard errors around means. C: no cluster thinning, no leaf removal; LR: leaf removal without cluster thinning; TH: cluster thinning without leaf removal; and TH + LR: cluster thinning and leaf removal.

As a result, independent of treatment, even though grapes reached veraison between 22 and 25 Aug in both years, fruit from all Cabernet franc vines reached ~21 Brix 15 days earlier in 2012 than in 2011. Even if October 2012 was cooler and had more rainfall than the same month in 2011, stable temperatures in September 2012 favored earlier ripening, so that harvest fell on 3 Oct 2012, but in 2011, it occurred only on 21 Oct. Interestingly, higher active temperatures early in the season did not modify the date of occurrence of the main phenological stages, but when favorable conditions occurred after veraison, ripening was sensibly accelerated.

At harvest, TH led to a yield reduction consistent with the lower number of clusters per vine, while cluster morphology and berry size were not changed in TH or LR, as previously reported for this technique applied at veraison (Palliotti and Cartechini 2000, Zhuang et al. 2014). Leaf removal can lead to reduced fruit set and altered cluster morphology, but only when applied before or around bloom (Poni et al. 2009, Tardaguila et al. 2008b, Gatti et al. 2012, Sternad Lemut et al. 2015, Acimovic et al. 2016). Pruning weights were greater in 2011 than in 2012, but were indifferent among treatments, most likely due to the greater amount and even distribution of rain which occurred throughout the season. The RI difference between TH and C treatments is consistent with the yield reduction, confirming that vines were physiologically balanced, as defined elsewhere (Howell 2001, Kliewer and Dokoozlian 2005).

The primary objective of leaf removal is to improve the cluster microclimate by taking advantage of additional sunlight penetration, and consequently, higher temperature in the cluster (Sabbatini and Howell 2010). Cluster temperature and light exposure were modified by the leaf removal treatment. In 2011, LR vines had lower cluster temperatures at night, as previously reported, and can be explained by the greater temperature dispersion that occurred in the absence of leaves around the cluster (Zhuang et al. 2014). In the same year, no differences were found in day cluster temperature, while in the warmer 2012, LR resulted in higher cluster day temperature. In both years, LR exposed clusters to more irradiative energy than in C vines, which was more effective in 2012 than 2011 due to the warmer year. Light and temperature are known to directly regulate ripening parameters like sugars, acidity, anthocyanins, and phenolics (Sadras et al. 2013, Matus et al. 2009).

Juice pH was not modified by any treatment, ranging from 3.5 to 3.7, even if the execution of analysis in previously frozen samples may have resulted in a slight increase in pH and decrease in TA. Acidity was lower at harvest in LR and TH + LR in both years. This is in line with previous findings and can be attributed to the cluster microclimate differences observed in LR (Spayd et al. 2002).

When considering the ripening profiles from the onset of veraison, our experiment found a slower Brix increase in LR than in any other treatment. This can be explained by a combination of the higher crop load together with the lower photosynthetic leaf area available for vines belonging to this treatment. Anthocyanin evolution was impacted after

veraison by both cluster thinning and leaf removal in both years. TH + LR, LR, and TH enhanced anthocyanin biosynthesis in response to the treatment between one and 24 days after veraison, with the differences peaking both years at 15 to 20 days after veraison. These results are exacerbated when anthocyanins were expressed as a ratio to Brix and can be explained by the higher light exposure in LR and TH + LR, which is related to color compound biosynthesis (Matus et al. 2009, Azuma et al. 2012, Diago et al. 2012) and the reduced crop of TH (Palliotti and Cartechini 2000). In our experiment, the combination of TH + LR was the only treatment that promoted higher amounts of color compounds until harvest in the cooler 2011. In 2012, when the mean temperatures and solar radiation were higher, all treatments reported similar anthocyanin values at harvest, despite TH + LR showing higher values at 15 days after veraison. In this season, the higher daily increase of anthocyanins was independent from the effect of leaf removal and/or cluster thinning. These results are consistent with other findings (Di Profio et al. 2011, Gatti et al. 2012, Zhuang et al. 2014, Bogicevic et al. 2015), but are still not fully described in the literature. Moreover, the ripening pattern observed between the two seasons can explain the variable efficacy of leaf removal and/or cluster thinning already observed in different years at harvest by Di Profio et al. (2011). Interestingly, in the two distinctive years, discarding the treatments, the amount of anthocyanins and phenolics in harvested grapes was substantially not different. If a higher content of anthocyanins can be expected in a warmer year, the prolonged hang time of fruit in 2011, when 21 Brix was achieved only in late October, is the probable cause of this lack of difference.

In our experimental condition, TH, despite the obvious reduction of vine productivity (2.1 to 2.6 kg/vine), did not improve fruit composition at harvest, unless if coupled with LR

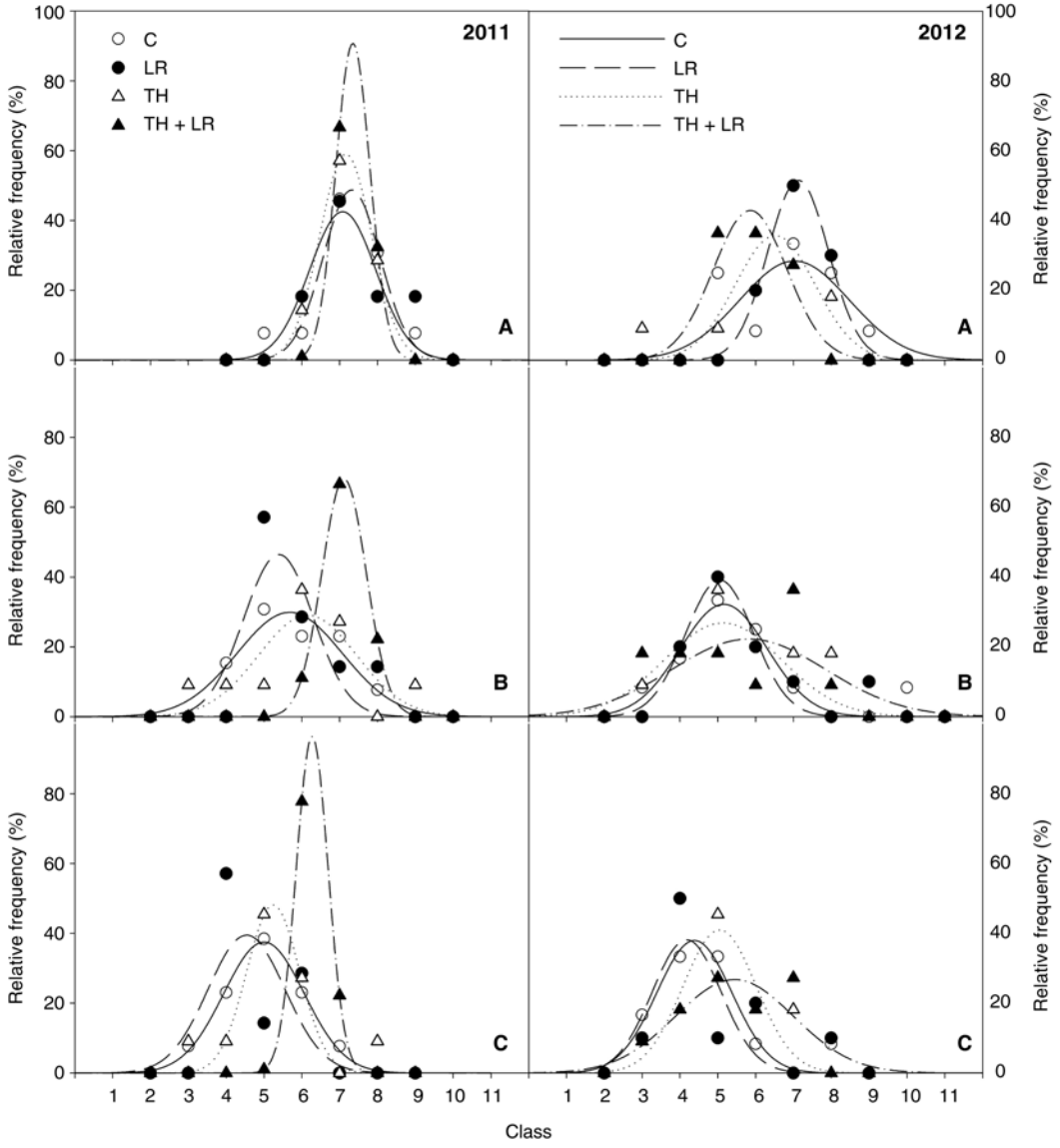

Figure 4 Relative frequency distribution of (**A**) soluble solids, (**B**) anthocyanins, and (**C**) the ratio of anthocyanins to Brix in 2011 (left) and 2012 (right). Data was divided into 11 classes, each corresponding to 1 Brix, 0.1 mg/g (anthocyanins), or 0.005 mg/g/Brix (ratio). C: no cluster thinning no leaf removal; LR: leaf removal without cluster thinning; TH: cluster thinning without leaf removal; and TH + LR: cluster thinning and leaf removal.

in a cool season. In the warmer 2012, high temperatures may have favoured evolution of ripening and, as suggested in Figures 2 and 3, TH was particularly efficient in both years right after veraison (18 to 27 days after veraison in 2011 and 8 to 15 days after veraison in 2012); meanwhile, later in the season, C and LR recovered the distance in quality parameters. This is partially in line with other studies which found cluster thinning efficiency to be directly dependent on vineyard characteristics, site, and environmental condition (Ough and Nagaoka 1984, Gatti et al. 2012, Santesteban et al. 2011, Zhuang et al. 2014).

Analyzing the daily increase of anthocyanins reveals important dynamics resulting from the treatments and from the seasons. First, without taking into consideration the treatments, in a year with favorable temperatures after veraison, the max daily increase in anthocyanins can reach values of 0.04 mg/g/day, while in the cooler 2011, the daily increase was never over 0.028 mg/g/day. These peaks were recorded right after veraison in 2012 (eight days after veraison) and later in the season (18 days after veraison) in 2011, when temperatures after veraison were lower. Moreover, in both years, TH + LR and LR were more effective than any other treatment in accelerating anthocyanin biosynthesis. In detail, in the cooler season, C never reached an increase of 0.017 mg/g/day, staying stable between 12 and 18 days from veraison at slightly lower values. TH + LR and LR had a similar increase at 12 days from veraison, but then peaked at 0.0275 mg/g/day at 18 days from veraison. In contrast, in the warm season, C peaked at eight days after veraison, but both LR + TH and, particularly, TH had a higher daily increase at the same moment. Interestingly, TH and LR + TH determined the peak of the anthocyanin to Brix ratio soon after veraison, while leaf removal induced a later daily increase of this ratio. Therefore, the greatest contribution of the techniques to anthocyanin biosynthesis in 2011 resided in the higher accumulation in the first part of September, when vines experienced cooler temperatures. Before harvest, anthocyanin metabolism increased by over 100% in all the treatments when compared to the control (Supplemental Figure 1). However, no differences were found between TH, LR, and TH + LR, underlining how the adoption of viticultural techniques can improve anthocyanin metabolism in unfavorable environmental conditions. Furthermore, this suggests that cluster thinning and leaf removal applied together are more efficient in decoupling color and sugars in cool seasons, although they are highly modulated by weather conditions occurring immediately after veraison. It is well known that the composition of anthocyanins in ripe fruit are determined via the function of complex metabolic networks regulated by genetic, developmental, and environmental factors (Jaakola 2013). In particular, different light and temperature treatments have also been found to induce quantitative and qualitative biosynthetic changes in the anthocyanin profile of grapevine berries (Azuma et al. 2012). Despite the challenge of relating this information to vineyard field conditions, where several factors interfere, it is possible to speculate that cluster thinning and leaf removal enhance anthocyanin accumulation and have a potential additive effect in cooler seasons. LR and TH + LR take advantage of the synergistic combination of increased light radiation and temperatures. Meanwhile, in the warmer 2012, LR did not have a substantial effect in determining the early peak of daily anthocyanin increase because the source-sink balance was proven to have a pivotal role, deeming TH the most effective treatment in warm seasons. Cluster thinning and leaf removal were already supposed to have additive effects at harvest (Di Profio et al. 2011), but the evolution of anthocyanin accumulation and their relative daily increase rate during ripening was never reported before. Moreover, the different behavior of the treatment in the two distinctive seasons represents the demonstration that environmental conditions throughout ripening determine the techniques' efficacy.

Canopy management techniques such as leaf removal and, particularly, cluster thinning, can lead to higher fruit uniformity (Palliotti and Cartechini 2000, Guidoni et al. 2002, Matus et al. 2009). However, this aspect has never been investigated in seasons with two contrasting weather patterns. In the cooler 2011 season, cluster thinning and leaf removal improved fruit composition and uniformity, with TH + LR having lower variability in Brix, anthocyanins, and relative ratio than any other treatment. TH improved the homogeneity of sugars, and LR led to more uniform anthocyanin values in 2011. Under the warmer 2012 weather conditions, no clear trend was found among treatments in the distribution of sugars and color compounds. This could be due to the harvest threshold at 21 Brix, which is farther from the potential cultivar max. This threshold was reached quickly (42 days after veraison), causing a large data distribution in LR and TH + LR treatments and potentially leading to higher levels of fruit maturity. The prolonged fruit hang time in 2011 could be one cause of the generally more uniform quality, and slower achievement of the same maturity can improve the homogeneity of fruit. This suggests a possible relationship between the greater uniformity and lower max daily increase in anthocyanins found in 2011, unlike the fast accumulation with less uniformity found in 2012.

These observations warrant further investigation to clarify the physiological mechanisms whereby both leaf removal and cluster thinning interact with weather to affect fruit quality.

Conclusions

The efficacy of combined cluster zone leaf removal and cluster thinning applied at veraison was directly related to the seasonal temperature evolution from veraison to harvest. Both techniques, applied separately or together, improved anthocyanin concentration after veraison. However, this enhancement was more relevant in the cooler 2011 season, when low temperatures and reduced sunlight slowed the ripening processes. This improved physiological process was not related to the time period between veraison and harvest, but instead to the efficacy of the treatments, evident when indexed as daily color increase. In the cooler summer, cluster thinning and leaf removal improved fruit composition at harvest, while in the warmer summer, no difference was found among treatments at harvest because the vines synthesized anthocyanins efficiently, driven by optimal temperature and light conditions. In

the cooler summer, in addition to improved biochemical composition, the fruit was much more homogenous and uniform when cluster thinning and leaf removal were applied together. When the techniques were not applied together, sugars and color were still more uniform, although not to the degree as in the combined treatments. Temperature during the growing season is directly related to winegrape maturity; thus, the increased temperatures promoted by leaf removal are crucial to fulfill thermal requirements needed for fruit maturation in cool seasons. The combination of these viticultural practices improved the source-sink ratio, which is critical to speed accumulation of metabolites from veraison through maturation. The two techniques applied together can allow maturity of red grape cultivars in cool climates. When the growing season is short with varying temperatures, there is a conclusive positive effect on color and the ripening profiles of important chemical compounds in winegrapes.

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