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Impact of Cluster Thinning and Basal Leaf Removal on Fruit Quality of Cabernet Franc (*Vitis vinifera* L.) Grapevines Grown in Cool Climate Conditions

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***Abstract*. Achieving desired fruit quality at harvest in cool climate conditions is a challenge, especially for red varieties, and the typical inability of fruit to reach technological maturity is a critical contributing factor requiring examination. To probe this issue, this research investigated the impact of two levels of crop thinning and of basal leaf removal at three phenological stages in the 2011 and 2012 growing seasons in Michigan. Experiments were conducted at the Southwest Michigan Research and Extension Center (SWMREC) in Benton Harbor. Using 'Cabernet franc' (*Vitis vinifera* L.) vines, yield components (yield per vine, pruning weight, and cluster and berry weight) and basic fruit composition traits [total soluble solids (TSS), pH, titratable acidity, anthocyanins, and phenolics) were studied to investigate the effect of cluster thinning and basal leaf removal on vine performance and fruit quality at harvest. Neither of the treatments significantly impacted TSS in either of the two seasons. Cluster thinning treatment successfully altered cropload ratio, indexed as Ravaz Index (RI), independently of the time of application. Basal leaf removal increased exposed berry temperature, cluster light exposure, and subsequent anthocyanin and phenolic content of the berry in both seasons, again independent of application date, whereas cluster thinning was effective only in 2012. Crop thinning coupled with basal leaf removal resulted in an increased efficiency in anthocyanin accumulation in relation to TSS accumulation, expressed as anthocyanin:sugar, in both years. This is significant because it offers potential for vineyard management practices aiming to improve fruit quality in cool climates where the onset of anthocyanin accumulation could be reduced and decoupled from sugar accumulation.**

The concentration levels of several chemical components of wine grape, including TSS, organic acids, polyphenols, and flavor compounds, determine technological maturity of fruit at harvest (Mattivi et al., 2006). Together, they are the critical contributors to final wine quality and each constituent responds differently to various environmental factors like sunlight, temperature, humidity, water stress, and soil nutrition (Downey et al., 2006; Lakso and Kliewer, 1975, 1977; Parra et al., 2010; Ryona et al., 2008; Smart and Robinson, 2008; Spayd et al., 2002).

Vineyard management is critical to achieve optimal fruit maturity and many viticultural practices have been studied including canopy management, e.g., basal leaf removal, irrigation, soil conditioning, and crop thinning, in cool and hot–warm climates (Bavaresco et al., 2008; Bledsoe et al., 1988; Keller et al., 2005; Reynolds et al, 1996; Sadras and McCarthy, 2007). However, climate often influences cultural treatments and, in viticultural regions like Michigan characterized by cool and short growing seasons of considerable annual variability, the most important tools to achieve desired fruit chemistry and maturity are optimizing vine balance and managing the vine canopy to improve the fruit zone microclimate (Howell, 2001). The ratio between vegetative growth and reproductive growth is manipulated to achieve targeted fruit characteristics and often indexed as cropload, the ratio between fruit yield and 1-year-old cane pruning weight (Ravaz, 1911) or referred to as the RI. Generally, a cropload with an RI from 5 to 10 is considered indicative of a balanced vine (Kliewer and Dokoozlian, 2005). Similar to RI, a ratio of leaf area to fruit weight from 8 to 12 $cm^2 \cdot g^{-1}$ is also regarded as the hallmark of a balanced vine (Kliewer and Dokoozlian, 2005). For wine grape, an RI exceeding 12 indicates overcropping and less than 5 undercropping vine status, corresponding to leaf area to fruit weight of less of 8 $cm^2 \cdot g^{-1}$ or more than 14 $cm^2 \cdot g^{-1}$, respectively (Kliewer and Dokoozlian, 2005), particularly for vines grown on a vertical shoot positioning trellis (VSP), most common used training system in cool climate viticulture. In a cool climate, with its low heat accumulation [growing degree-days (GDD)] and short growing seasons, a higher amount of leaf area and, therefore, a lower cropload is suggested to ripen the fruit to a desired level (Howell, 2001; Tozzini et al., 2013). Canopy management techniques such as basal leaf removal are frequently used to increase sunlight exposure and exposed berry temperature (Bledsoe et al., 1988; Smart and Robinson, 2008). Cluster exposure increases polyphenols and decreases acidity in cool climates (Cortell and Kennedy, 2006; Downey et al., 2004; Price et al., 1995; Spayd et al., 2002). However, the timing of basal leaf removal appears to be critical; when performed too early, it results in the loss of leaf area with a significant decrease in the amount of photosynthesis and the associated production of carbohydrates for berry development (Palliotti et al., 2011) and the potential of modify the relationship between total soluble solids (mostly sugars) and anthocyanin accumulation, inducing a decoupling effect as reported by Sadras and Moran (2012).

This 2-year research project was designed to study the effects of cluster thinning and cluster microclimate (temperature and incident solar radiation) modified by basal leaf removal on vine growth and fruit composition of mature 'Cabernet franc' grown in the cool Michigan climate. Specifically, it was our goal to identify the efficacy of selected timings of the two major cultural practices for advancing fruit ripening and achieving targeted quality traits, especially with respect to the concentrations of sugars, anthocyanins, and phenolics at harvest.

Materials and Methods

Plant material. Vitis vinifera L. cv. Cabernet franc vines (clone FPS 01), grafted on rootstock 3309 C and planted in 1993 at SWMREC of Michigan State University

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(Benton Harbor, lat. 42°05′10″ N, long. 86°21′36″ W), were used for these field experiments in 2011 and 2012. The climate here is characterized by a short growing season (145 to 175 d) with cool-climate summer conditions (1300 ± 300 GDD, calculated beginning 1 Apr. to 31 Oct. using base 10 °C). The experimental vineyard consisted of 10 rows and 48 vines per row with spacing of 2.4 m between vines and 3.0 m between rows. Rows were planted in a north-to-south orientation and vines were trained with a VSP trellis system. Vines were planted in Spinks sandy loam soil (U.S. Department of Agriculture, 1957) and spur-pruned to ≈48 nodes per vine during the winter. Vines were trained with multiple trunks to ensure survival of at least part of the vine through damaging winter temperatures (Pool and Howard, 1984). During the growing season, shoots were hedged when the tips were 30 cm above the catch wire to maintain a canopy free of excessive shading and, subsequently, to prevent a reduction in heat accumulation (Sadras and Moran, 2012). After bloom, ≈90 clusters were left on each vine considered in the study. Standard commercial pest-control practices were applied during the season based on scouting, experience, and weather conditions (Wise et al., 2008). Monthly rainfall and cumulative GDD during the growing season were obtained from the Michigan Automated Weather Network station at SWMREC, located 300 m from the site of the experiment. Additional weather data details and parameters can be accessed at <http://www.enviro-weather.msu.edu/weather.php?stn=swm>.

Field experimental design and treatments. The experimental design was a randomized complete block with eight blocks and three factors (cropload, leaf removal, and timing of treatment application) and four single vine replications per each treatment. The crop was manually adjusted according to a low crop and high crop level with ≈40 (40 ± 11 SD in 2011 and 40 ± 10 SD in 2012) and 80 (80 ± 16 SD in 2011 and 80 ± 14 SD in 2012) clusters per vine, respectively. To study the effect of cluster microclimatic conditions on fruit composition and quality, two levels of cluster exposure to sunlight were designed: on selected vines, the first six nodes of each shoot were defoliated (leaf removal), whereas the other treatment was left undefoliated (no leaf removal). The simultaneous application of the yield level and cluster exposure treatments was assigned to three different timings: fruit set, 3 weeks before veraison, and veraison.

Canopy growth measurement. Shoot length was monitored weekly from June until hedging, which was performed before veraison (end of July) in both seasons. Five modal shoots per vine were selected and total leaf area was estimated based on the regression between shoot length and shoot leaf area using a non-destructive method. Weekly, 20 shoots were sampled randomly from guard vines from bloom to veraison and shoot length was recorded and leaf area per shoot was measured using a leaf area meter (LI-3100 area meter; LI-COR, Lincoln, NE). The equation of the regression between leaf area and shoot length ($y = 23.0\,x - 510.7$, $r^2 = 0.97$) was used to estimate leaf area for the five tagged shoots according to Mabrouk and Carbonneau (1996). At the three times of treatment application, six basal leaves were removed from each tagged shoot, placed in zip-lock sampling bags, and transported to the laboratory where their total leaf area was measured and recorded to estimate the percentage of leaf area removed through the treatments.

Daily exposed berry temperature and radiation measurement. Cluster light intensity and temperature was measured using photosynthetically active radiation (*PAR*) sensors (Model SQ-110; Apogee Instruments, Logan, UT) and with a fine-wire [American Wire Gauge (AWG)] thermocouple (Type T (copper-constantan)] in contact with berry skin. Three representative vines were selected for each cluster exposure treatment and six temperature and light sensors were positioned horizontally immediately adjacent to the fruit zone of the canopy.

Both *PAR* sensors and thermocouples were connected to data loggers (CR-10; Campbell Scientific, Logan, UT) 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 in the fruit zone. All signals were scanned at 20-s intervals with the values recorded every 20 min continuously from July first to harvest (21 Oct. and 4 Oct. in 2011 and 2012, respectively). Mean diurnal *PAR* (0500 to 2100 HR) and temperature (0000 to 2400 HR) patterns were based on the average 60-min values calculated from data collected over the full season (July to harvest) and expressed as seasonal mean.

Sampling procedures and harvest data collection. At the beginning of veraison, 20 berries from six vines were sampled randomly on biweekly basis in 2011 and weekly basis in 2012 from clusters on non-tagged shoots to track fruit maturation until harvest. At harvest, vine yield and cluster number per vine were recorded. 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, e.g., TSS, pH, titratable acid (TA), anthocyanins, and total phenolics, were then analyzed. Finally, pruning weight per vine was collected the next 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 TSS (°Brix) was measured using a digital refractometer (ATA-3810 PAL-1; Pulse Inc. Van Nuys, CA). We used a 370 Thermo Orion pH meter (Thermo Fisher Scientific Inc., Logan, UT) to measure pH. TA was measured using a Multi-T 2.2 digital titrator (Laboratory Synergy Inc., Goshen, NY) with each sample consisting of 10 mL clear juice diluted with distilled water to 100 mL and titrated with 0.1 M sodium hydroxide

Table 1. Climatic data from the Michigan Automated Weather Network station at the Southwest Michigan Research and Extension Center, Benton Harbor, MI.[z]

Yr	Anthesis	Fruit set	Veraison	Harvest
2011	18 June (169)[x]	30 June (181)	25 Aug. (237)	21 Oct. (294)
2012	21 June (173)	28 June (180)	22 Aug. (235)	4 Oct. (278)
2011	403 GDD[y]	529 GDD	1241 GDD	1579 GDD
2012	481 GDD	566 GDD	1288 GDD	1626 GDD
2011	354[w]	374	517	683
2012	202	204	352	506

[z]Dates of phonological stages (anthesis, fruit set, veraison, harvest) and corresponding growing degree-days (GDDs) in 2011 and 2012.
[y]GDD calculated from 1 Apr. to 31 Oct. with base temperature of 10 °C.
[x]Day of year is in parentheses.
[w]Annual precipitation (mm) calculated up to the phenological stage.

Table 2. Leaf area removed during defoliation treatment applications.[z]

Timing	GDD	Removed shoot leaf area (cm^2)	Total shoot leaf area (cm^2)[y]	Removed shoot leaf area
Fruit set	482	781 ± 69 SD	2134 ± 102 SD	35%
Pre-veraison	961	640 ± 35 SD	2573 ± 146 SD	28%
Veraison	1183	797 ± 49 SD	2576 ± 150 SD	33%

[z]The means of 2011 and 2012 have been pooled.
[y]Total leaf area per shoot estimated at each time of treatment.
GDD = growing degree-days.

Table 3. Seasonal mean daily exposed berry temperature (from July first to harvest) calculated from 0800 HR to 2000 HR of clusters exposed at different phenological stages (fruit set, pre-veraison, and veraison) and not exposed for 2011 and 2012.

	Temperature (°C)	
	2011	2012
Undefoliated	20.1 ± 0.1[z]	22.8 ± 0.5
Fruit set	20.9 ± 0.3	24.8 ± 1.4
Pre-veraison	20.8 ± 0.2	24.2 ± 1.1
Veraison	20.5 ± 0.2	23.6 ± 1.0

[z]SD for four temperature sensors.

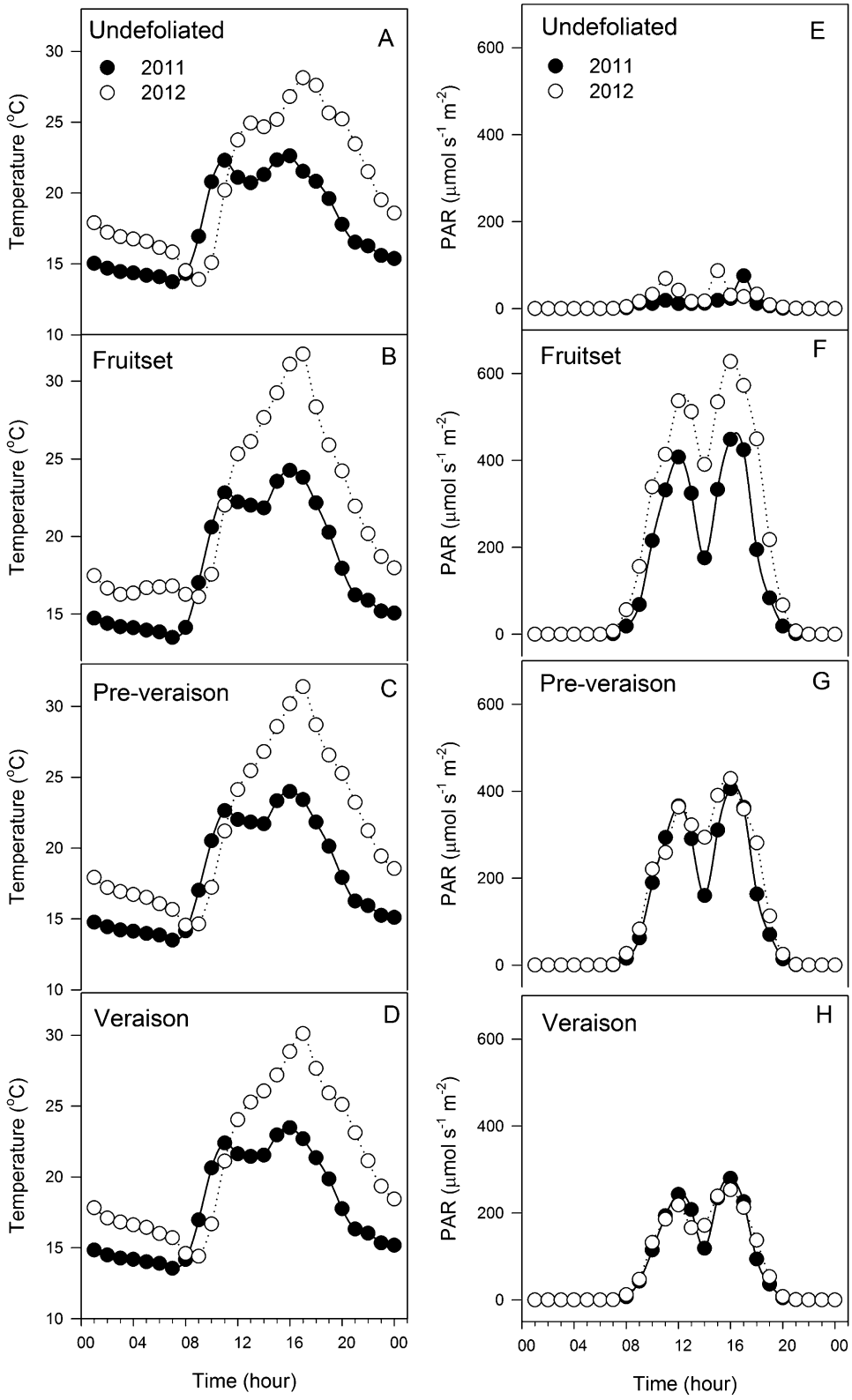

Fig. 1. Seasonal means (from July first to harvest) of diurnal temperature and photosynthetically active radiation (*PAR*) of clusters exposed at different phenological stages (fruit set, pre-veraison, and veraison) for 2011 and 2012.

(NaOH) to a pH of 8.2 using an equation to yield the TA ($g \cdot L^{-1}$), according to Iland et al. (2004).

Anthocyanins and total phenolics were measured by the total phenol assay using ultraviolet-Vis (Iland et al., 2004). One hundred berries stored at –30 °C were partially thawed before grinding in a tissue homogenizer (Brinkmann Instruments, Westbury, NY) at a speed of four on the manufacturer's scale for ≈1 min. Sample was ground while maintained in an ice bath to minimize oxidation. The homogenate included flesh, skins, and seeds. Approximately 1 g ± 0.05 g of homogenized sample was added to a tared 15-mL centrifuge tube and the mass was recorded. Ten milliliters of 50% v/v aqueous ethanol acidified to pH 2 (≈1 mL 12.1 M HCL)

Table 4. Crop level and leaf removal impact on yield and cluster components for 2011 and 2012.

	Yield (kg/vine)		Number of clusters		Pruning wt (kg/vine)		Ravaz Index[z]		Cluster wt (g)		Number of berries/cluster		Berry wt (g)	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Crop level														
Low crop	4.2 a[y]	3.8 a	52 a	48 a	1.4	1.0	3.0 a	4.7 a	98.4	102.3 a	71	73.7 a	1.32	1.27
High crop	6.6 b	6.2 b	92 b	78 b	1.5	1.0	4.7 b	7.5 b	102.5	121.1 b	75.7	86.0 b	1.29	1.32
Cluster exposure														
Leaf pulling	5.5	4.8	70 a	67 a	1.3 a	1.0	4.2	5.9	97.5	112.7	72.6	80.7	1.28	1.30
No leaf pulling	5.3	5.1	74 a	69 a	1.7 b	0.9	3.4	6.2	103.4	110.4	74.1	78.7	1.33	1.30
Interaction	NS[x]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[z]Ravaz Index = crop yield/pruning weight.
[y]Means within columns followed by different letters are significantly different at $P < 0.05$ by F test for main effects.
[x]Crop level × cluster exposure: *, **, NS indicate significant at $P < 0.05$, 0.01, or nonsignificant, respectively.

Table 5. Crop level and leaf removal impact on basic fruit chemistry for 2011 and 2012.

	TSS (°Brix)		pH		TA ($g \cdot L^{-1}$)		Anthocyanin ($mg \cdot g^{-1}$)		Phenolics (au/g)[x]	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Crop level										
Low crop	22.3[z]	21.8	3.6	3.7	6.07	5.53	0.88	0.83 a	1.31	1.41 a
High crop	22.0	21.6	3.6	3.7	6.18	5.68	0.78	0.74 b	1.24	1.33 b
Cluster exposure										
Leaf pulling	22.1	21.5	3.6	3.7	5.71 a	5.34 a	0.86	0.83 a	1.30	1.43 a
No leaf pulling	22.2	21.8	3.6	3.7	6.54 b	5.90 b	0.80	0.73 b	1.25	1.31 b
Interaction[y]	NS[y]	NS	NS	NS	NS	NS	**	NS	**	NS

[z]Means within columns followed by different letters are significantly different at $P < 0.05$ by F-test for main effect.
[y]Crop level × cluster exposure: *, **, NS indicate significant at $P < 0.05$, 0.01, or nonsignificant, respectively.
[x]AU = absorbance unit.

was added to the 1-g sample and then mixed once every 5 min manually for 1 h. The sample was then centrifuged at 1800 g_n for 20 min. One milliliter of extract (supernatant liquid) was pipetted into a 15-mL centrifuge tube. Ten milliliters 1 M HCL was added and the mixture was clear equilibrated for 3 h. The absorbance values were obtained using a ultraviolet-Vis spectrophotometer (Model ultraviolet-1800; Shimadzu Corporation, Kyoto, Japan) at values of 280 nm (total phenolics), 520 nm (anthocyanins), and 700 nm (turbidity control).

Statistical analysis. Results of the two seasons were separately tested for normality and homogeneity of variance and initially subjected to three-way (crop level × cluster exposure × timing) analysis of the variance using the PROC MIXED in SAS (Version 9.1.3; SAS Institute, Cary, NC). For the variables analyzed, timing was non-significant and not interacting with other factors; therefore, results were analyzed with a reduced two-way factorial statistical model. Regression analysis for selected variables was performed combining data for each parameter collected during the 2 experimental years, using Sigma Plot (Version 10; Systat Software, San Jose, CA).

Results

Weather conditions and canopy microclimate. Weather conditions for the experimental site in 2011 and 2012 are summarized in Table 1. Both growing seasons were close to the 10-year averages for both heat accumulation (1382 GDD) and precipitation (567 mm, from April to October). The phenological stages tracked similarly in timing between 2011 and 2012; however, the harvest in 2011 was delayed by 2 weeks as a result of heavy rainfall (683 mm in 2011 and 506 mm in 2012).

The defoliation treatments (six basal nodes) removed a consistent percentage of leaf area (≈32%) in both years and for each time of treatment (Table 2). Shoot growth was measured from bloom to veraison, but no significant effect of the treatments applied at different stages were observed in both years because shoot total leaf area at fruit set were already 80% of the total leaf area that vines reached before hedging (data not shown). Nevertheless, the defoliation treatments impacted the fruit zone microclimate, exposing directly the cluster to solar radiation.

However, no detectable differences in seasonal mean of diurnal temperature were observed when comparing undefoliated and defoliated vines in 2011 (Table 3), ranging from 20 to 21 °C. Instead the defoliation treatments in 2012 increased the exposed berry temperature of ≈1.7 °C across the different times of application. The 2012 season resulted warmer than the 2011 in terms of GDD (Table 1) but also as cluster diurnal temperature calculated for the growing season (Fig. 1A). In particular and higher maximum temperature (≈1600 HR) was observed in defoliated vines in 2012 (Fig. 1B–D); moreover, the timing of cluster exposure to sunlight, even with 6 to 7 weeks of difference between fruit set and veraison, did not impact the seasonal mean of diurnal temperature (Fig. 1A–D) with minimal differences in mean daily maximum observed between the three times of treatment application (Table 3).

Contrarily, daily *PAR* in the fruit zone of undefoliated vines was dramatically lower than any defoliation treatments in both years (Fig. 1E–H). Additionally, we observed a reduction in *PAR* insisting on the clusters as a result of the time of defoliation. Early defoliation (fruit set) clusters reached ≈500 *PAR* and 400 *PAR* and 220 *PAR* in preveraison and veraison, respectively, as the average of the 2 years.

Yield components and cluster parameters. The two crop levels significantly impacted yield in both seasons (Table 4), irrespective of the timing of application, which effect was not significant (F test) also on the vine size, indexed as winter pruning weight. As a result of cluster thinning, the yield was reduced by 34% and 38% in 2011 and 2012, respectively. Cluster weight, berry number, and berry weight were not significantly impacted in 2011 regardless of crop level and cluster exposure treatments. Nevertheless, in 2012, a smaller cluster resulted with cluster thinning treatments with corresponding fewer berry numbers and equal berry weight as compared with vines with no cluster thinning (Table 4). Cluster exposures treatments did not impact any yield component analyzed, as expected, because cluster zone manipulation was performed after fruit set, when major yield components, with the exception of berry weight, are already established.

Fruit chemistry composition. TSS concentration was not impacted by crop level and cluster exposure treatments (Table 5). No matter whether or when cluster thinning or basal leaf removal was applied, no significant difference in pH was found within each year between the two seasons (Table 5). In addition, there was no difference in levels of TA from the crop level treatment. Basal leaf removal significantly reduced the amount of TA in both seasons (Table 5) and, like with cluster thinning, the timing of treatment application did not prove to be important.

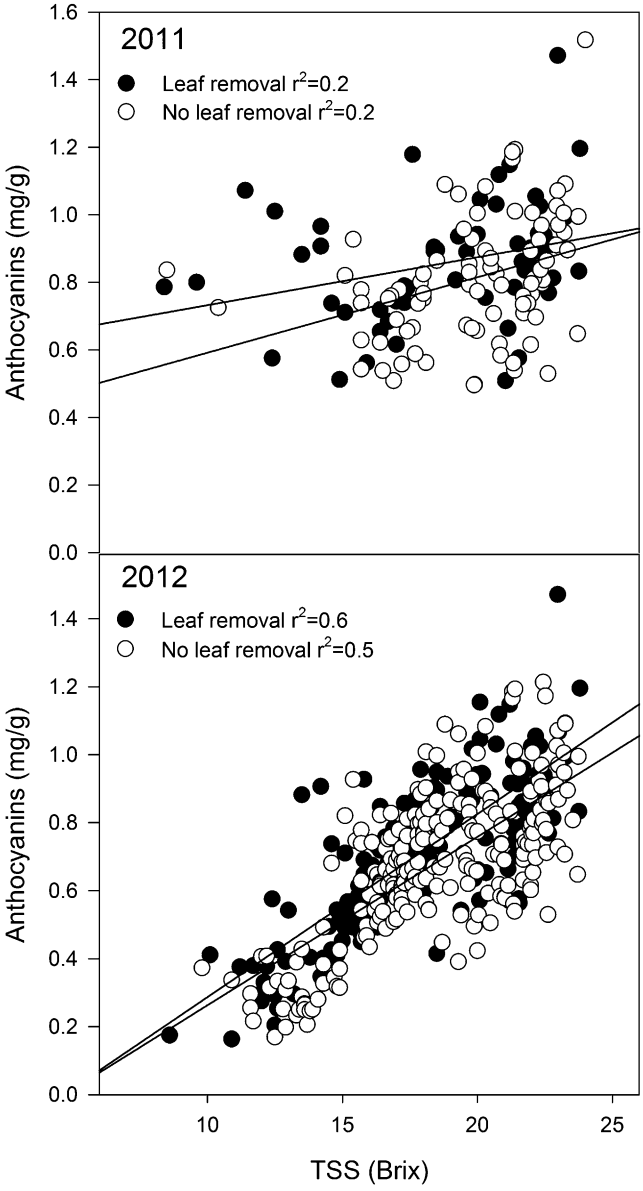

Fig. 2. Relationship between total soluble solids (TSS) and anthocyanin concentration on fruit collected from veraison to harvest in 2011 (above) and 2012 (below). Data were pooled across all treatments except the two levels of cluster exposure (leaf removal).

Anthocyanin content was not significantly affected by the crop level in 2011, whereas it was modified by cluster exposure to sunlight (Table 5). Lower crop per vine increased anthocyanin and phenolic content in 2012. The observed increase was ≈+8% when compared with the high crop level treatment. These parameters were also impacted by the basal leaf removal treatment; leaf removal increased anthocyanins and phenolics (≈+11%). In 2011 a significant interaction between crop level and cluster exposure was observed with a similar beneficial trend in increasing anthocyanin and phenolic content generated by low yield per vine and leaf removal.

Discussion

We monitored canopy growth, i.e., shoot growth rate, and found no significant difference resulting from any of our treatments (data not shown). It is noteworthy that maintenance of a higher yield per vine did not reduce canopy size; neither did pruning weight. Shoot leaf area had already reached 80% of the maximum leaf area (recorded at hedging) at fruit set before treatment application (Table 2), suggesting that the canopy of 'Cabernet franc' vines in Michigan grew rapidly at the start of the season. This might be a vine response to the short growing season, a phenomenon also seen in other cool climate growing regions (Silvestroni, 2014, personal communication), where plants allocate most of the resources in the vegetative structure before the fruit sink demand reaches its pick (Keller, 2010).

Yield was manipulated through cluster removal to similar levels in both years. Cluster weight, number of berries per cluster, and berry weight were not significantly different, but cropload (RI) was affected by cluster thinning. However, all of the RI values generated by the two levels of cluster thinning fell into the range of 5 to 10, a range defined as optimal for grape quality (Kliewer and Dokoozlian, 2005). Yield did not impact the final sugar (TSS) accumulation. Similarly, the lack of an effect of cluster thinning on other parameters of fruit composition, e.g., pH, TA, could be explained by the ability of balanced cropped vines to successfully deliver mature fruit at harvest. This confirms what was found in previous studies conducted in warm climate conditions indicating that crop level does not affect TSS or other parameters of fruit composition when the following index ranges were maintained: a cropload ratio of 5 to 10 and a leaf area-to-fruit weight ratio of 8 to 12 $cm^2 \cdot g^{-1}$ (Petrie and Clingeleffer, 2006).

Conversely, an improved fruit zone microclimate (temperature and light exposure) resulting from basal leaf removal affected fruit composition. In both seasons, basal leaf removal significantly resulted in a lowering of TA also if that overall the berry temperature regimes were not remarkably different (Table 5). However, the level of organic acids did not prove to be sensitive to the timing of early leaf removal in the timeframe spanned by our treatments (from fruit set to veraison). Increased exposed berry temperature caused by leaf removal around the fruit zone likely accelerated the degradation of malic acid by stimulating an increase in respiration. It is known, however, that elevated temperature does not affect either the tartaric or citric acid levels (Ruffner, 1982). When the levels of these acids are tracked over the growing season, it is the respiration of malic acid that is the main cause of the significant decline of TA in berries after veraison (Coombe and McCarthy, 2000). Additionally, Smith et al. (1988) observed that basal leaf removal was associated with a reduction in potassium uptake and that a reduced amount of potassium in berries was correlated with a decrease in berry organic acids. Consequently, basal leaf removal might be pivotal to decreasing the levels of organic acids. However, the timing of basal leaf removal was not crucial for determining the TA in our experiment.

Basal leaf removal increased the amount of anthocyanins and phenolics in berries in 2012, whereas in 2011, they were increased only in interaction with cluster thinning treatments. Again, the influence of leaf removal on anthocyanins and phenolics was not sensitive to the timing of treatment application, contrary to a recent report (Sternad Lemut et al., 2013). In grapevine, exposed berry temperature proved to be more effective than cluster exposure to light in increasing the biosynthesis of anthocyanins (reviewed in Downey et al., 2006), but high temperatures might be detrimental for anthocyanin production in some red grape varieties (Kliewer and Torres, 1972; Mori et al., 2007; Sadras and Moran, 2012). In grape, day

temperatures lower than 35 °C and cool night temperatures lower than 15 °C result in greater anthocyanin accumulation than constant high temperatures during the day and night (Mori et al., 2005). In contrast, temperatures ≈35 °C resulted in a decrease of anthocyanin accumulation (Downey et al., 2006; Sadras and Moran, 2012), both inhibiting the biosynthesis and promoting the degradation (Mori et al., 2007; Sternad Lemut et al., 2013). In our study, cluster exposed by basal leaf removal did not significantly increase the average temperatures in comparison shade clusters, but light exposure was dramatically improved by several orders of magnitudes in both years. Moreover, cluster daily temperature on the defoliated treatments was ≈20.7 °C and 24.2 °C in 2011 and 2012, respectively, with the former below and the latter around the optimal temperature threshold (Kliewer and Torres, 1972; Mori et al., 2007; Sadras and Moran, 2012). By consequence, in this study, optimal exposed berry temperature in 2012 combined with improved cluster light exposure promoted the biosynthesis of anthocyanins resulting in higher levels of anthocyanins in the fruit. We observed that also cluster thinning can increase the anthocyanin content (Table 5). According to Keller (2010), increasing the sink-to-source ratio could favor the production of anthocyanins and other secondary metabolites (Keller, 2010). However, the result of cluster thinning on anthocyanins was not consistent across years, because in 2011, it was observed only in interaction with basal leaf removal.

As for phenolics, the impact of temperature and light on the fruit zone is still poorly understood. Flavan-3-ols monomers and tannins in skins and flavonols in berries have been found to be significantly impacted by sunlight exposure (Cortell and Kennedy, 2006; Downey et al., 2004; Price et al., 1995; Spayd et al., 2002). Flavonols were consistently induced by cluster light exposure (Downey et al., 2004; Price et al., 1995), whereas results on flavan-3-ols and tannins are contrasting: in Shiraz, cluster exposure did not affect their accumulation (Downey et al., 2004), whereas, in Pinot noir, clusters exposed to sunlight induced their accumulation in the skin and slightly reduced it in the seed (Cortell and Kennedy, 2006). Similar to previous consideration, in 2011, cluster exposure interacted with cropload in affecting the berry phenolic content, whereas in 2012, the positive effect on phenolic content was observed in both low yield per vine and leaf removal treatments independently.

Another important issue related to fruit maturity is the apparent parametric coupling between anthocyanin and sugars (Keller et al., 2005). After veraison, as TSS started to increase, the anthocyanin started to accumulate as well following a linear relationship (Fig. 2). In our results from both seasons, obtained using weekly berry samples from veraison to harvest, the coupling between sugar and anthocyanin accumulation was found to behave similarly in vines with

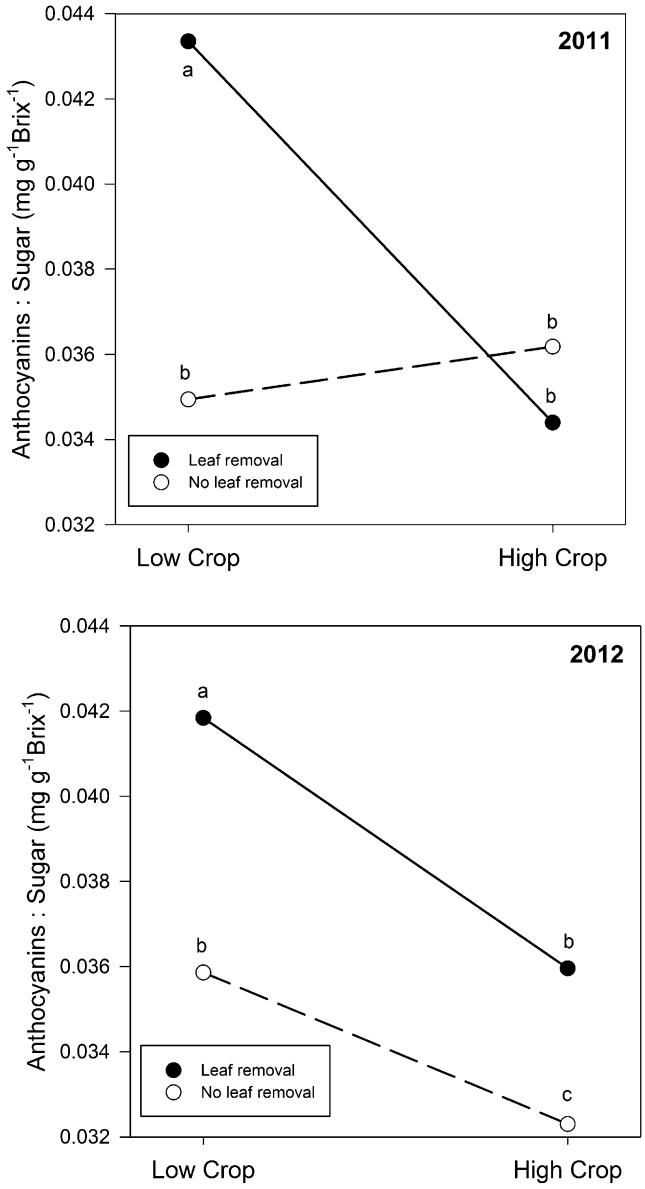

Fig. 3. Cluster thinning and basal leaf removal impact on the anthocyanin:sugar ratio in 2011 (above) and 2012 (below) at harvest. Values noted with different letters are significantly different at $P < 0.05$ by least significant difference test.

different cluster zone microclimate with stronger significance for the warmer year (2012) than the cooler year (2011). With the goal of clearly delineating the effect of treatments on the efficiency of anthocyanin accumulation, the ratio of anthocyanin:sugar (mg·g^{-1}:°Brix) was calculated (Fig. 3). At harvest in 2011, the ratio was impacted only by the combination of low crop and leaf removal resulting in a strong trend to a higher efficiency value when compared with high crop with leaf removal or just low crop and no leaf removal (Fig. 3). In 2012, when the leaf removal successfully improved the cluster microclimate, leaf removal significantly increased the efficiency of anthocyanin accumulation, although the effect was much stronger if it was coupled with low crop treatment. Contrary to Sadras and Moran (2012), we observed that besides the effect of cluster microclimate, the increase of the source sink ratio at lower crop level could significantly impact the anthocyanin:sugar ratio. Alterations of the source-to-sink ratio have now been studied extensively (Guidoni et al., 2002; Petrie and Clingeleffer, 2006; Sadras and McCarthy, 2007) and a variety of impacts on the anthocyanin:sugar ratio have been observed. Kliewer and Dokoozlian (2005) posit that the lack of a direct causal relationship between the source-to-sink ratio and accumulation of TSS makes this difficult to assess and forecast. Indeed, we did not find a difference on basic fruit chemistry at harvest in the 2 years and the source-to-sink ratio, manipulated by cluster thinning, did not consistently affect the anthocyanin:sugar ratio probably as a result of the year-to-year weather variation. In general, as a consequence of a cool climate viticulture, basal

leaf removal favors an increase in the anthocyanin:sugar ratio, especially in challenging years and when applied to low crop vines.

Conclusion

Cluster thinning proved to be a useful tool for the successful manipulation of cropload (ratio of leaf area to fruit weight); however, the two levels of cluster thinning applied in 2011 and 2012 seasons had no significant effect on basic fruit composition and just reduced yield and economic return. Basal leaf removal significantly improved the cluster microclimate (light and temperature) that probably contributed in promoting anthocyanins and phenolics in berries but most evidently in 2012. Similarly, increasing the source-to-sink ratio by cluster thinning had a positive effect on the anthocyanin and phenolic content of berries only in 2012. Importantly, we found that an increase of anthocyanins at harvest in relation to the sugar level of the berries (anthocyanin:sugar ratio) was achieved with cluster thinning only and leaf removal only in the cool year (2011). Those results may offer potential for vineyard management practices aiming to improve fruit quality in cool climates where the onset of anthocyanin accumulation could be reduced and decoupled from sugar accumulation as suggested by Sadras and Moran (2012).

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