EFFECT OF VITICULTURAL PRACTICES ON FRUIT COMPOSITION OF CABERNET FRANC GROWN UNDER MICHIGAN CLIMATE

By

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ABSTRACT

EFFECT OF VITICULTURAL PRACTICES ON FRUIT QUALITY OF CABERNET FRANC GROWN UNDER MICHIGAN CLIMATE

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Vitis vinifera planting in Michigan is increasing by 10% per year. Red varieties are currently the main focus and challenge for the Michigan wine industry, since it is hard to get these varieties to reach full maturation at harvest. The objectives of this study were to improve the fruit quality and subsequent wine sensory characteristics of Cabernet Franc by applying different viticultural practices aimed at reducing typically high levels of 3-isobutyl-2-methoxypyrazine (IBMP) in this region.

Experiments conducted in 2010 and 2011 evaluated different levels and timings of cluster thinning and leaf removal from the basal nodes. In 2010, cluster thinning increased the pruning weight and cluster weight, and had insignificant impact on basic fruit chemistry at harvest. In both years, neither cluster thinning nor leaf pulling were significantly affecting vegetative growth. In 2011, cluster thinning did not have a significant impact on vine vigor and basic fruit chemistry, but did reduce the IBMP level by 17%. Leaf removal decreased the titratable acidity by 10% and IBMP level by 35%, but had no effect on other basic quality parameters. Leaf removal and cluster thinning not only increased the anthocyanins content, but also the efficiency of anthocyanins accumulation expressed as the ratio of anthocyanins to sugar. In addition, more uniform fruit quality has been achieved by early leaf removal.

DEDICATION

Dedicated to good friends, good times, and good or bad Michigan wines.

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CHAPTER I
LITERATURE REVIEW

Grapes: General Information and Economic Importance

Grapes today are one of the world's most economically important fruit crops. According to 2009 statistics by the Food and Agriculture Organization of the United Nations, globally, grapevines were planted on almost 7.3 million hectares producing more than 67 million metric tons of fruit in 2007. This makes grapes the number 25 food crop in terms of planted area and number 16 in terms of tonnage. More than 70% of this crop was used to make wine, 27% consumed as fresh fruit (table grapes), 2% as dried fruit (raisins), and less than 1% was processed to grape juice or distilled to brandy (Keller 2010).

Grape production is largely limited to climatic areas similar to those of the indigenous range of *Vitis vinifera*. This zone approximates the regions between the 10°C and 20°C annual isotherms. Furthermore, grape culture is mainly restricted to regions characterized by Mediterranean-type climates. It is possible for the extension of grape growing into cooler, warmer, or moister environments when the local conditions or microclimate favor grape growing. Viticultural practices can compensate to some degree for less than ideal conditions.

Vitis vinifera planting in Michigan is increasing by 10% per year. Red varieties are the main focus and challenge for the Michigan industry currently since it is hard to get these varieties to full maturation at harvest. The cold winter and relatively short growing season in Michigan are the limiting elements for Michigan growers and force them to experiment with red *Vitis vinifera* varieties as well as hybrids and native cultivars. Consequently, few red *Vitis vinifera* varieties were planted in relatively large acreages in Michigan. Among those, Cabernet

Franc is an important red variety for Michigan growers, second only to Pinot noir in acreage (155 vs 235 in 2011, an 82% increase compared to 2006 [USDA's NASS Michigan Fruit Inventory 2011 to 2012]); however, the optimal ripeness of Cabernet Franc in Michigan is typically not achieved every year. Harvested unripe grapes associated with poor fruit quality and strong unpleasant green herbaceous flavor are the biggest challenges for Cabernet Franc. Thus, it is essential for Michigan growers to know how to fully ripen this variety and how the optimal fruit quality can be reached by implementing specific vineyard management techniques to improve fruit and wine quality. Consequently, my studies were focusing on how to achieve optimal fruit quality of Cabernet Franc (sugar, pH, organic acids, anthocyanins, phenolics and flavor compounds) through the manipulation of crop load and canopy microclimate by applying cluster thinning and basal leaf removal at different timings during the season in both 2010 and 2011.

Physiology of Berry Growth

Berry growth in grapevines follows a typical two successive sigmoid curve pattern and each of three stages has distinctive characteristics (Figure 1) (Coombe and McCarthy 2000). The first stage is mainly berry formation with cell division in the pericarp tissue. During this stage, cell division largely determines the final berry size, and the rate of cell division is positively correlated with the growth rate of the seeds (Coombe and McCarthy 2000). At the end of this stage, berry volume increases up to a lag phase before veraison when the berry remains hard and green. During this stage of cell division, malate was the main solute transported into berries via both xylem and phloem, especially the xylem. The second stage of berry growth, also known as veraison, starts with sugar accumulation, berry softening, or berry coloring. The primary changes

at this stage include the start of ripening, signaled by accumulation of sugars in the flesh and skin, and the collection of potassium and phenolics (anthocyanins in red grape varieties) in the skin. After veraison, the berry continues its evolution in stage III during which berry growth accelerates from cell expansion. At the end of stage III, flavor compounds start to accumulate. Since the stage of flavor accumulation is not coincident with the berry ripening stage or stage III, a new term of "engustment" is used to characterize the start of the biosynthesis of flavor compounds in the berry (Coombe and McCarthy 1997). The flow through the xylem is impeded during stage III and water with sugar is largely transported through the phloem into the berry during this stage. The intake of water and sugar happens during the period from veraison to 18 to 20 °Brix in berries. During this stage, the solute, which mainly accumulates in pulp, is sugar. At the same time, anthocyanins increase in the skin with potassium. From the stage of 18 to 20 °Brix to harvest, it has been suggested that different varieties perform differently in terms of berry development, and certainly when it comes to Muscat versus Shiraz (Coombe and McCarthy 2000). For Muscat, berry weight still follows the sigmoid growth at the end of stage III. Also, an increase of solutes per berry was found during this phase. As a contrast, Shiraz berries start to decrease in weight at 18 to 20 ° Brix. Water and sugar are cut off because of the blockage of phloem transport. This results in a decrease of volume due to a decrease in intake of water, but transpiration is still simultaneously occurring. Sugar is increased during this stage, not because of more sugar accumulation from phloem sap, but from increased concentration due to evaporation. Shiraz berries have little vascular connection to vines during this phase since the phloem is blocked; yet, the accumulation of non-anthocyanin glucosides in berries, both for Shiraz and Muscat, continued without the existence of phloem sap. This conclusion, drawn by Gholami, et al. (1995), was founded on work using bunch grafting. It has been proven that the

genotype of the grafted bunch determines the accumulation of glucosides of aroma compounds (glycosylated terpenes), rather than any influence of the scion tissues.

Impact of Vine Balance on Fruit Composition

Vine balance is the concept of balance between vegetative growth and reproductive growth. Theoretically, this concept is used to determine how much fruit can be sustainably cropped to reach the desired fruit quality according to canopy characteristics (Howell 2001). Crop load, the ratio between yield and one year-old cane pruning weight, was first used by Ravaz to measure vine balance (Ravaz et al. 1911). Ravaz applied the concept of crop load to balanced pruning to determine how many buds to leave for next year's crop. After this first introduction of the technique, Partridge mentioned a similar concept in using the balance between reproductive growth, e.g., crop weight or yield, and vegetative growth, e.g., shoot growth or pruning weight, to understand how much fruit should be cropped (or how many buds should be left) according to the vine canopy (Partridge 1925). Partridge even used the one year-old cane pruning weight from the previous year to guide the range of crop in the following year and, with this data, growers could estimate how much fruit would be cropped for the next vintage. After Partridge, Shaulis reconfirmed crop load as a reliable method for estimation of vine balance and refined the theory even further (Shaulis 1953).

The ratio between yield and one year-old cane pruning weight is referred to as crop load, and this unitless ratio is widely accepted as a tool to assess vine balance and the range of crop load (Ravaz Index) from 5 to 10 was considered indicative of a balanced vine (Bravdo et al. 1984, 1985).

Later, the ratio between leaf area and yield, expressed as m^2/ kg or cm^2/ g, was used as another tool, rather than crop load, to assess vine balance (Kliewer and Dokoozlian 2005). The theory, in using leaf area instead of pruning weight, is that photosynthesis of the sun-exposed leaves triggers production of carbohydrates that are then transported to the berries. Trellising systems with different canopy architectures have different photosynthetic efficiencies (Kliewer and Dokoozlian 2005). A single canopy trellising system, like vertical shoot position (VSP), has relatively low efficiency in capturing sunlight compared to a divided canopy system, like geneva double curtain (GDC). Accordingly, in VSP, the leaf area required to ripen 1 g of fruit ranges from 8 to 12 cm^2, but in GDC the leaf area/fruit ratio is reduced to 5 to 8 cm^2/ g (Kliewer and Dokoozlian 2005). Consequently, crop load and leaf area/fruit ratio are essentially the same concept as vine balance though they are expressed in different ways. Both indexes have been shown to be significantly correlated (Kliewer and Dokoozlian 2005).

Theoretically, crop load greater than 12 has been considered to be "overcropping" and less than 5 "undercropping" (Bravdo et al. 1984, 1985). Similarly, leaf area/fruit ratio more than 14 cm^2/g has been regarded as "overcropping" and less than 8 cm^2/g as "undercropping" (Kliewer and Dokoozlian 2005). Overcropped vines have difficulty in reaching the desired fruit quality at harvest, while undercropped vines are not economically desirable.

There are limits to using this classic concept of vine balance in cool-climate growing regions, like New York, Michigan, and New Zealand. In cool climates, the description of crop load using the range from 5 to 10 as balanced has proven less useful, as was also true for leaf area/fruit ratio in using the range from 8 to 12 cm^2/g. When considering the growing degree days (GDD), light intensity, and the potential of early frost close to harvest, viticulturists in these

regions have had to maintain more leaf area to decrease the crop load ratio and to minimize the risk of unripe fruit at harvest (Howell 2001).

Sugar: Accumulation and Impact of Environment and Viticultural Practices

Sunlight to the leaves is the energy source for photosynthesis and the production of carbohydrates for the first and secondary metabolization in berries. However, photosynthesis has a negligible impact upon the berry itself, and since light and temperature are usually interacted with each other, an increase in temperature due to sunlight is shown to provide more substantial influence. Berry temperature increasing above a minimum threshold of 30°C (Coombe 1987) are key to metabolic activity, and it does not matter whether the increase is from direct sun exposure or from microclimate ambient temperature. Ultimately, berry enzymatic activities will be promoted for both the primary and secondary metabolic pathways.

Total sugar concentration in grape berries, usually measured as total soluble solids (TSS, expressed as °Brix), ranges from 18.7 to 27°Brix at maturity, including both table and wine grapes (Kliewer 1967). The source of sugar in the berry is sucrose, which is transported from the photosynthesizing leaves (Hale and Weaver 1962, Swansom and Elshishing 1958). The sugar composition in berries, determined largely by genotype, is mainly fructose and glucose in *V. vinifera* (Kliewer 1966). There is, however, a small amount of sucrose in other *Vitis* species as well, e.g., *V. labrusca* (Concord) (Richard and Herbert 1967). Generally, most wine grapes from *V. vinifera* develop a glucose-fructose ratio of 1 at the technological ripening stage (Kliewer 1967).

Environmental conditions and viticultural practices impact sugar accumulation in grape berries. In comparison with organic acids (Keller et al. 2005) and anthocyanins (Keller et al.

1998, Sadras et al. 2007), berry sugar accumulation has been found to be relatively stable for a given variety, irrespective of environmental conditions and viticultural practices in warm climate. Some experiments have found a significant relationship between sugar concentration and these two factors, but the results were neither consistent nor conclusive. Some studies have indicated that the reduction of crop by pruning or cluster thinning increased berry TSS (°Brix) as compared to the high-cropped vines (Chorti et al. 2010, Guidoni et al. 2002, Ollat and Gaudillere 1998, Petrie and Clingeleffer 2006, Petrie et al. 2000, Reynolds et al. 1994). But some studies have found that there was little or no impact of cluster thinning or defoliation on sugar accumulation at harvest (Keller et al. 2005, Nuzzo and Matthews 2006, Tardaguila et al. 2010). This inconsistency of results in terms of sugar could be due to the fact that the cluster thinning changed the vine balance or not. The intensity and timing of water stress has an inconsistent effect on sugar accumulation (Roby and Matthews 2004, Sadras et al. 2007, Cramer et al. 2007, Esteban et al. 1999, Esteban et al. 2002, Sivilotti et al. 2005). Therefore, the questions about the TSS (°Brix) due to environmental and viticultural impacts are complicated.

The inconsistent results of sugar accumulation, based on crop level and water stress, could be explained by different research conditions, the choice of varieties, the definition of over- or under-cropped vines, and/or the timing of water stress. Although a noticeable lack of consistency of sugar concentration results has been observed, one thing was clear: that water supply, crop level or other environmental and viticultural conditions influenced the period of sugar accumulation, but not the final sugar content at maturity (Petrie and Clingeleffer 2006, Roby and Matthews 2004, Sadras et al. 2007, Cramer et al. 2007, Esteban et al. 1999, Esteban et al. 2002, Sivilotti et al. 2005). Specifically, high crop level delayed the date of arrival of the targeted °Brix value without affecting the rate of sugar accumulation when it was compared to

low crop level. The most likely explanation is a delay of veraison date for unthinned vines (higher crop) compared to thinned vines (lower crop) (Petrie and Clingeleffer 2006). For example, berries targeted for 16°Brix from thinned vines got there approximately 9 days before the berries from vines with a higher crop level, without any difference in sugar accumulation rate, an approximate value of 0.182°Brix per day for both high and low cropped vines (Petrie and Clingeleffer 2006). Since the sugar accumulation rate was not affected, ultimate sugar concentration was eventually reached at the same level for different crop levels. However, further research was necessary to support this observation, especially the accumulation duration and the sugar accumulation rate. Both of these were found to be significantly modified by different environmental conditions and viticultural practices (Kliewer and Dokoozlian 2005).

There is another important issue related to sugar accumulation that has not been studied enough: distribution of sugar content. Besides the mean of sugar concentration, a limited number of research studies have examined the nature of variation in the distribution, including the skewness and kurtosis, and how it may be impacted by viticultural and environmental conditions, like shoot origin (the primary shoot and secondary shoot) and light exposure (Wolpert and Howell 1984, Wolpert et al. 1983). Higher variation in sugar content has been found in immature and non-irrigated berries, as compared to in mature berries and berries from irrigated vines (Rankine et al. 1962). Hand-cluster thinning has also been investigated and found to increase the uniformity of sugar concentration compared to unthinned or machine-thinned treatments (Petrie and Clingeleffer 2006). Although it is known that environmental conditions modify the mean value of a berry trait, like sugar content, they also appear to influence the distribution or variation of that trait. Further studies will be needed to understand how other environmental parameters

(light, temperature, humidity) and viticultural practices impact the distribution or variation of that trait in berries.

Organic Acids: Degradation and Impact of Environment and Viticultural Practices

In grape, Total titratable acidity (TA) mainly consists of tartaric and malic acids with the balance consisting of other organic acids, like citric, phenolic, amino, and fatty acids (Kliewer et al. 1967). Equilibrium between tartaric acid and tartrate exists in the grape berry as well, as is also true between malic acid and malate. Generally, the proportion of free tartaric acid is from 3 to 60% and malic acid is from 7 to 80% (Kliewer et al. 1967). The composition of tartaric acid and malic acid in berries largely depends on the genotype; in *V. vinifera* the concentration of tartaric acid ranges from 4.5 g/L to 7.7 g/L and malic acid ranges from 2.4 g/L to 3.8 g/L (Kliewer et al. 1967). Tartaric acid and malic acid are accumulating during the berry growth stage I and are stored mainly in cell vacuoles of the pulp (Coombe and McCarthy 2000). Tartaric acid is biosynthesized from Vitamin C (L-ascorbic acid) (Figure 2) (DeBolt et al. 2006). However, the specific enzymes that regulate the different steps are still not fully understood. Malic acid originates from the TCA cycle (Lakso and Kliewer 1975, 1977). It can be produced from sugar via aerobic respiration starting from glycolysis and then is modified further by the TCA cycle after veraison. This can explain why malic acid was easily metabolized through respiration (intermediate from TCA cycle) leading to a significant decline of malic acid content in berries after veraison along with accumulated sugars. Through another pathway, malic acid can also be biosynthesized via CO_2 fixation through phosphoenolpyruvate (PEP) during photosynthesis in the green berry stage. Furthermore, the metabolism of malic acid is similar for

pulp and skin. The concentration of malate in skin is relatively stable, while there is a significant decline of malate in pulp after veraison (Iland and Coombe 1988).

Environmental conditions have a significant impact on malic acid concentration but not on tartaric acid. Increased temperature (either from ambient or direct sunlight) significantly reduced the amount of malic acid in berries grown under cool climate conditions after veraison, which, in turn, leads to a lower TA at harvest (Keller et al. 2005, Alan and Kliewer 1975, 1977). This reduction of malic acid was due to the preference of berries to metabolize malic rather than 6-carbon sugars as the energy source for respiration after veraison (Alan and Kliewer 1975, 1977), and the activity of malate enzyme was increased during the ripening stage in a cool climate (Keller et al. 2005, Alan and Kliewer 1975, 1977). The impact of water stress experiments on malic acid has proved to be inconsistent and still require further study (Para et al. 2010, Koundouras et al. 2006). Conversely, the content of tartaric acid was not significantly influenced by temperature or water stress (Parra et al. 2010).

Viticultural practices, like varying applications of leaf removal treatments, have been studied for impact on TA. Conclusions about varieties and climate have also been inconsistent. In California, severe leaf removal for Sauvignon Blanc in geneva double curtain (GDC) significantly reduced the pH and TA, mainly malic acid, compared to the control (Bledsoe and Kliewer 1988). However, the same level of reduction in TA occurred without interference of leaf removal at different stages. In Virginia, leaf removal for Riesling and Chardonnay at fruit set in two-trellis systems, also gave a significant reduction of TA and malate content (Zoecklein and Wolf 1992). In Italy, inconsistent results for TA and pH due to leaf removal at veraison have also been observed in three different varieties (Bavaresco and Gatti 2008). Consequently, the impact

of viticultural practices, like leaf removal, on organic acids was complicated and might be interacted with the different environmental conditions.

Polyphenols: Composition and Impact of Environment and Viticulture

Anthocyanins

Anthocyanins are a class of compounds in the flavonoid family that are responsible for the color in red grapes and wines. It is biosynthesized through the pathway of flavonoid biosynthesis that is a combination of two metabolic pathways including shikimate and phenyl-propanoid (Figure 3) (Brenda Winkel-Shirley 2001). From the shikimate pathway carboxylated acetyl-CoA and amino acid phenylalanine are biosynthesized. These two components can go further through the phenyl-propanoid pathway to produce the flavonoids. We will discuss anthocyanins first and the details about other flavonoids, including flavonols and flavan-3-ols (proanthocyanidin or condensed tannin), will follow.

Recently, there has been a focus on anthocyanins for their benefit to human health. Examples include free radical scavenging and antioxidant activity, antimicrobial and antiviral activity, and anti-cancerigenic activity (He et al. 2010). Generally, anthocyanins have been found to be located in the hypodermal cell layers of berry skin after veraison (Mazza 1995). However, a few species of *V. vinifera* could accumulate the anthocyanins in their pulp as well, e.g. the Alicante Bouschet, a teinturier variety (Castillo et al. 2009).

The concentration of anthocyanins in grape berries is influenced by genotype, environmental conditions, especially temperature or sun exposure, and viticultural practices like leaf removal (Mark et al. 2006). The variety Casetta has been found to have the highest amount of anthocyanins at about 6.28 mg/g, while the lowest amount was detected in Muscat Rouge de

Madere at about 0.02 mg/g (Mattivi et al. 2006). The structures of anthocyanins in grape berry skins were 3-O-monoglucosides and 3-O-acylated-monoglucosides of five main anthocyanidins: delphinidin, cyanidin, petunidin, peonidin and malvidin (Figure 4). The differences between the five anthocyanidins were numbers and positions of the hydroxyl or methoxy groups located in the B-ring of the molecule (María Monagas and Begona Bartolomé 2009). At one time it was believed that there were only anthocyanidin monoglucosides in *V. vinifera*. However, recently anthocyanidin-3, 5-diglucosides were detected in berry skins in *V. vinifera* rather than non-*V. vinifera* species (Baldi et al. 1995, Vidal et al. 2004). The major component in the family of anthocyanidin is malvidin (quantified by malvidin-3-glucosides) and the contents of malvidin in berries representing characteristics of the variety ranged from 65% in Cannonau to less than 1% in Gewurztraminer (Mattivi et al. 2006). Similarly, the variation of acylated anthocyanins content occurs, and it depends on variety; Pinot noir, for example, is without them (Ribéreau-Gayón 2000).

Environmental conditions, like sunlight and temperature, play a significant role in the biosynthesis of anthocyanins. Experimental treatments, like basal leaf removal, artificial shading, and different trellis systems, have been used to investigate the effect of cluster sunlight exposure on fruit compositions (Kliewer et al. 1967, Kliewer and Antcliff 1970, Price et al. 1995, Hunter et al. 1995, Smart et al. 1988). However, these studies did not exclude the interference of temperature or humidity in the canopy along with the effect of light intensity. Recently, light and temperature have been separated to study their impact on fruit compositions, particularly on flavanoids, like anthocyanins. Spayed et al. (2002) applied the artificial heating and cooling of clusters under the control of light exposure and found that elevated temperature in warm climate inhibited the biosynthesis of anthocyanins and resulted in a noticeable lack of color in berries at

harvest. Also, Downey et al. (2004) used the lightproof box to prevent light penetration to separate the effect of light and temperature on clusters, and the similar result has been discovered. Both these studies indicated that temperature plays a more important role than light exposure in influencing the accumulation of anthocyanins in grape clusters, especially excessive amount of heat inhibits the biosynthesis of anthocyanins, and ultimately reduces the accumulation of anthocyanins in berries.

Flavan-3-ols

Flavanols are the other categories of chemicals in the family of flavonoid, which is also named flavan-3-ols. The main compounds in the flavanols group from grape are (+)-catechin, its isomer and (-)-epicatechin. Gallic ester of (-)-epicatechin and (-)-epicatechin 3-gallate exists in grape in much lower amounts compared to the previous three compounds (Figure 5) (Su et al. 1969).

The capacity of oligomers and polymers of flavan-3-ols interact with proteins gives the grape and wine astringency and tannic mouthfeel. Therefore, the oligomers and polymers of flavan-3-ols are also known as condensed tannins or proanthocyanidins. In the grape berries, the majority of flavan-3-ols are located in seeds and skins. On one hand, the flavan-3-ols start to accumulate a few weeks after bloom in skins. On the other hand, the accumulation of flavan-3-ols is relatively delayed in seeds and then reaches the peak a few weeks after veraison (Downey et al. 2003a). The total flavan-3-ols in berries ranged from 62.8 to 322.0 mg/kg, however, 85 to 98% of the total flavan-3-ols are located in seeds (Mattivi et al. 2008). The total amount of extractable tannins declined post-veraison (Downey et al. 2004).

The effect of environment and viticulture on flavan-3-ols content has been studied. Low vine vigor and sunlight exposure increase the contents of flavan-3-ols in the skins (Cortell et al. 2005, Downey et al. 2004), however, little impact on flavan-3-ols in the seeds has been found due to the sunlight exposure (Downey et al. 2004, Chacon et al. 2009). Water stress was also studied and the results have shown that there is little effect on flavan-3-ols (Ojeda et al. 2002). For seed flavan-3-ols, the level is hardly influenced by environmental conditions, with the exception of an increase in concentration of seed flavan-3-ols and other polyphenols in Merlot when water stress is applied in a warm growing climate (Chacon et al. 2009).

Flavonols

Flavonols are another category of compounds in the flavonoid family, but contain a keto group positions phenolic –OH (Figure 6). They are well known for their critical ability to protect vines from UV damage. Flavonols were mainly found in grape skins and leaves, while little was found in either the pulp or the seeds (Pereira et al. 2006, Rodriguez Montealegre et al. 2006, Downey et al. 2003b). It has been found that flavonols start to accumluate at bloom; however, only a small amount of flavonols can be quantified from bloom to veraison; a majority of flavonols accumulate from veraison through to the ripening stage (Downey et al. 2003b). This conclusion was drawn based on the application of mg/berry to express the concentration of flavonols. The level of flavonols in Shiraz is from 0.17 to 0.23 mg/g FW and a similar level is detected in other red *V. vinifera* grown in a warm region (Rodriguez et al. 2006). Generally, the concentration of flavonols in red varieties is higher than it is in white varieties. However, due to the significant effect of environmental factors on flavonols (Rodriguez et al. 2006), there are

some exceptions; for example, sunlight exposure was regarded as the most important environmental factor affecting flavonol content (Downey et al. 2004).

Flavonols concentration in berries of several varieties (Pinot Noir, Merlot and Syrah) increases in response to sunlight exposure (Downey et al. 2004, Spayd et al. 2002, and Price et al. 1995). Temperature was less effective in changing flavonols than sunlight exposure (Spayd et al. 2002). These results indicate that the biosynthesis of flavonols is induced by light, consistent with its function as a UV-protector.

Phenolic Acids and Stilbenes (Resveratrol)

In grapes, there is another group of phenolic compounds that is not a part of the flavonoid family. The major two categories in these non-flavonoid phenolic compounds are: 1) phenolic acids and 2) stilbenes.

Phenolic acids can be further classified into two groups: hydroxybenzoic acids (HBA) (Figure 7) and hydroxycinnamic acids (HCA) (Figure 8). The main role of phenolic acids from an enological point of view is to strengthen and stabilize color (anthocyanins) of red wine with the help of co-pigmentation of anthocyanins and phenolic acids (Roger 2001).

Stilbenes in grapes generally play a defensive response to environmental stresses, like microbial infection and UV-damage (Philippe et al. 1991). Because of their dietary benefit as antioxidants and their anti-carcinogenic capability, scientists have become more interested in these compounds for their human health benefits (Savouret and Quesne 2002). One of the most well-studied stilbenes is trans-resveratrol (resveratrol), which is mainly biosynthesized in the berry skin cells to respond to fungal diseases like botrytis and powdery mildew (Philippe et al. 1991, Ana et al. 2001). The biosynthesis of stilbenes is the same as the first steps of flavonoids

biosynthesis, and then progresses to a different branch, illustrated in Figure 3 (Gorham 1995). It has been found that resveratrol is impacted by different environmental factors, e.g., UV irradiation, microbial infection, botrytis, and powdery mildew, and there is an inverse correlation between sugar content and resveratrol level (Philippe et al. 1991). Generally, the concentration of trans-resveratrol in grape juice ranges from 0.09 to 0.18 mg/L (Stecher et al. 2001).

Flavor compounds: Impact of Environment and Viticultural Practices

It is worth noting that cluster sunlight exposure can also significantly affect the contents of flavor compounds in berries, e.g., vegetative and herbaceous flavor compounds. Methoxypyrazines and other related compounds in Bordeaux varieties were significantly reduced when sunlight exposure was induced to clusters before veraison (Koch et al. 2012, Ryona et al. 2008). Cluster light exposure can not only reduce the amount of negative flavor compounds, like methoxypyrazines, in berries, but increase the amount of positive flavor compounds, like terpenes, as well. Cluster sunlight exposure (more than 20% of full sun exposure) at veraison resulted in a significant increase of bound 1, 1, 6-trimethyl-1, 2-dihydronaphthalene (TDN) and 2, 2, 6, 8-tetramethyl-7, 11-dioxatricycloundec-4-ene (Riesling acetal) in grapes of Riesling (Gerdes et al. 2001).

The impact of temperature on the accumulation of flavor compounds was less investigated. As for the Riesling and Bordeaux varieties, cool climate usually led to a lower amount of TDN and a higher level of methoxypyrazines in berries at harvest (Gerdes et al. 2001, Boss et al. 2008). As for the Muscat varieties, naturally-shading did not affect the contents of free and bound terpenes in the berries as compared to the sun-exposed clusters (Bureau et al.

2000) and this result points to a higher berry temperature playing a more important role in determining terpene accumulation.

Methoxypyrazines (MPs)

Background

The class of compounds called methoxypyrazines (MPs) includes 3-isobutyl-2-methoxypyrazine (IBMP), 2-sec-butyl-3-methoxypyrazine (SBMP), 3-isopropyl-2-methoxypyrazine (IPMP) and 3-ethyl-2-methoxypyrazine (EMP) (Allen et al. 1991, Lacey et al. 1991, Sala et al. 2004, Augustyn et al. 1982) (Figure 9), and these compounds have been identified in grapes and wines. The family of methoxypyrazines has been identified throughout the plant and animal kingdoms. Buttery et al. (1969) first determined that IBMP was one of the compounds most responsible for the distinctive flavor of green bell pepper (Capsicum annuum), with SBMP being present as well. MPs have also been identified in potato (Murray, et al. 1970, 1975, Coleman et al. 1980), peas (Murray, et al. 1970, 1975), cheese (Suriyaphan et al. 2001), coffee (Czerny et al. 2000), parsley (Masanetz et al. 1998), chocolate (Counet et al. 2002), wood smoke (Maga et al. 1985), insects (Moore et al. 1990, Al Abassi et al. 1998) and bacteria (Gallois et al. 1985). They have been shown to differ slightly from each other in their perceived sensory description. IBMP has a distinct green bell pepper aroma (Buttery et al. 1969), IPMP is often described as having an aroma reminiscent of asparagus or sweet peas (Murray et al. 1970), and the aroma of SBMP is described as being similar to galbanum oil (Masuda et al. 1990) (Table 1). IBMP levels range from 4 to 30 ng/L (depending on variety, maturity, and growing conditions) in grape juice and concentration is typically at least 8 times higher than the other two pyrazines.

The high level of IBMP among MPs (>15 ng/L in white wines, >25 ng/L in red wines) makes IBMP the main factor contributing an undesirable herbaceous aroma to wines (Allen et al. 1999).

3-isobutyl-2-methoxypyrazine (IBMP) has been identified as the main compound responsible for the herbaceous, musty, and unripe aroma in wines characterized as "green bell pepper" (Buttery et al. 1969). It has an extremely low sensory threshold of 0.5 to 2 ng/L in water and 10 to 16 ng/L in red wine. The "green bell pepper" character associated with wine grapes (*Vitis vinifera*), especially the classic red Bordeaux varieties (i.e., Cabernet Franc, Cabernet Sauvignon and Merlot) are related to the high concentration of IBMP at harvest. Grapes grown in cool climate viticultural regions, like Michigan and New York in the U.S. or in New Zealand, tend to have higher levels of IBMP due to:

- the challenge of a short growing season and insufficient heat accumulation to fully ripen the fruit, and the level of IBMP is strongly correlated with fruit maturity (Koch et al. 2012, Ryona et al. 2008), and
- the cool growing conditions that favor the production of a higher amount of IBMP, including a higher peak value pre-veraison (Boss et al. 2008, Koch et al. 2012).

It is currently possible to detect the IBMP concentration in grapes using gas chromatography-mass spectrophotometry with selected ion monitoring, chemical ionization and improved extraction techniques (Harris et al. 1987; Lacy et al. 1991, and Peter et al. 2005).

Human Sensitivity Threshold for Methoxypyrazines

In addition to its widespread occurrence in nature, methoxypyrazines (MPs) are some of the most potent odorants known to man. Besides IBMP, the human recognition threshold for SBMP and IPMP has been reported to be in the range of 1 to 2.0 ng/L in water (Buttery et

al.1969, Seifert et al. 1970). The less frequently occurring compound EMP has a higher threshold of 425.0 ng/L in water (Seifert et al. 1970) and, although it is not typically found in grapes, 3,5-dimethyl-2-methoxypyrazine has recently been identified in wine corks and has a threshold of 2.1 ng/L in white wine (Simpson et al. 2004).

Levels of IBMP in Grapes and Wine

In grapes and wines, IBMP typically occurs at ten times the concentration of the other MPs (Allen et al. 1995, Lacey et al. 1991). Since they have similar recognition thresholds, most MPs studies have focused on IBMP. The distinct green bell pepper aroma can be detected in the range of 1.0 to 2.0 ng/L both in model wine (12% ethanol aqueous solution) (Maga 1989) and young white wines (Allen et al. 1991). In red wines, the recognition threshold has been reported to be 10.0 ng/L in Merlot (Kotseridas et al. 1998), 15.0 ng/L in Bordeaux and Loire wines (Roujou de Boubée et al. 2000) and 16.0 ng/L in other unspecified grape varieties (Maga1989). Chapman et al (2004) reported that an IBMP content from 0 to 10.0 ng/L in wine correlated with sensory perception of vegetative aroma and flavor. Thus, low levels of IBMP in a wine may have an impact on the perceived herbaciousness even though the wine lacks the distinct green bell pepper characteristic.

Distribution of IBMP in Grapes

IBMP is found in stems, skins, seeds, and pulp of the grape. Main proportion of IBMP is found in stems at harvest (53.4% of total amount of IBMP distributed in clusters) (Roujou de Boubee et al. 2002). As such, it is easy to understand why the green bell pepper character in a wine can be greatly influenced by an effective destemming process prior to pressing or

fermentation. Following the stems, the skin of the berry is another main source of IBMP (31%), while the seeds contribute a smaller amount (15%). The pulp contains very little IBMP (0.6%). From pre-veraison to harvest as the grapes mature, the relative proportion of IBMP changes: the proportion decreases in the stems (from 79.2 to 53%) and increases in the skins (from 14.8 to 44.9%). Accordingly, it was hypothesized that IBMP was translocated from stems to skins (Roujou de Boubee et al. 2002).

However, in exploring translocation via cluster grafting, it has been found that no IBMP was detected in the berries of Muscat blanc clusters grafted onto Cabernet Sauvignon vines when berries were sampled at fruit maturity. As a contrast, IBMP was detected in all of the berries of Cabernet Sauvignon clusters irrespective of the graft configuration. It seems assured that IBMP or its precursors originate in the berry and that its formation depends upon grape genotype and not on the translocation from the leaves to the fruit (Koch et al. 2010).

Impact of Light and Temperature on IBMP

IBMP is photo labile in aqueous solution (Heymann et al. 1986). Clear glass bottles of wine were exposed to fluorescent light for 120 h and IBMP decreased by 28% compared to the control. Similar results were reported with red wines in comparable experiments (Maga et al. 1990). Light was further suggested to reduce the IBMP concentration in the berries via photo degradation based on the work of Noble et al. (1995). Yet Blake et al. (2010) reported that there was no evidence that light can consistently influence the concentration of IBMP in the wines during storage.

Exploring the impact of viticultural practices on IBMP concentration, several field experiments have been done to manipulate cluster sunlight exposure via leaf removal and shoot

thinning. Results have shown that there is a significant decline in either IBMP concentrations (Allen et al. 1993) or vegetal flavors in wines due to more sunlight exposure to clusters (Morrison et al. 1990; Arnold et al. 1990). However, the level of IBMP in the berries was not investigated. Hashizume, et al. (1999) exposed the grapes to low light intensities and found that in ripe berries IBMP decreased compared to the controls in which berries were shaded, while in unripe berries IBMP increased. It indicated that the concentration of IBMP in the berry is largely influenced by the balance between the synthesis of that compound and its degradation during berry growth, as well as the level of grape maturity (Hashizume et al. 1999). These studies showed that the response of IBMP metabolism in berries to light is influenced by the stage of berry development. When considered together, the data indicates that there is an interaction between the metabolic responses of IBMP to light and the stage of berry development. However, the exact interaction between the IBMP response to light and developmental stage needs more studies.

Ryona, et al. (2008) has shown that the accumulation of IBMP reaches a peak 4 weeks prior to veraison after which the level of all MPs drops as the berry ripens. Five days post bloom is the first point at which quantifiable levels of IBMP (2 to 7 pg/g) can be detected (Ryona et al. 2008). Additionally, the research reported that exposure of the clusters to high intensity of light readily reduced the amount of IBMP peak by 21% to 44% before veraison compared to a shade treatment control (Figure 10). Pre-veraison cluster light exposure was more critical than post-veraison exposure in reducing IBMP concentration during berry growth (Hashizume et al. 1999; Roujou de Boubee et al. 2000; Koch et al. 2012, Scheiner et al. 2010). However, this result shows there is almost no influence on the IBMP level at harvest (Ryona et al. 2008).

A possible explanation of the fact of higher temperature and light intensity contributing to a less amount of IBMP might be due to the related gene expression during the pathway of biosynthetic (Boss et al. 2007). According to the hypothesized pathway for IBMP (Figure11), the gene *Vv*HPMT codes for the enzyme, *Vitis vinifera* O-methyltransferase (OMT) protein. This protein catalyzes the conversion of 2-hydroxy-3-alkylpyrazine into methoxypyrazine at the final step of biosynthesis in the presence of S-adenosyl-L-methionine (SAM). Because the *Vv*HPMT gene is heat restricted, and an excessive amount of heat resulted in a low level of expression of *Vv*HPMT. Ultimately, an elevated temperature in hot growing regions results in lower concentrations of MPs compared to cool growing areas. Conversely, higher levels of *Vv*HPMT expression have been detected in berries grown in cool conditions before and after veraison, which correlates well with the peak of IBMP accumulation (Boss et al. 2007; Falcao et al. 2007).

Impact of Crop Load and Water Stress on IBMP

Inherent vine vigor and related pruning methods also influence the change of IBMP in grape berries, e.g., by manipulating bud counts and, thereby, crop levels in Cabernet Sauvignon, IBMP concentration is found to be inversely related to yield (Chapman et al. 2004a, 2004b, Allen et al. 1990, and Arnold et al. 1990). Finally, vine water potential was believed to be another important environmental factor influencing IBMP changes (Chapman et al. 2005). Water stress might result in the reduction of IBMP concentration in grape berries, ultimately leading to fruitier and less vegetal flavor.

Biosynthesis of IBMP

Although several hypothesized pathways have been given, no biosynthetic pathways for IBMP in grapes have been conclusively demonstrated. Previously, Murray et al. (1970) proposed a biosynthetic pathway in vegetables for the synthesis of methoxypyrazines (Figure 11).

Although the pathway is reasonable chemically, enzymes required for amidation have not been found. In bacteria some clues for IBMP biosynthesis have been identified. Using radioactive labeling, Cheng et al. demonstrated that 2-methoxy-3-isopropylpyrazine (MIPP) is synthesized from endogenous valine, glycine and methionine by *Pseudomonas perol*, which indicated that the first step is initiated by the formation of a cyclic peptide through the condensation of two amino acids similarly to the formation of 2-isopropyl-3-methoxypyrazine from the initial condensation of the amino acids valine and glycine (Cheng et al. 1991). The most widely accepted biosynthetic pathway is similar to a previous laboratory synthesis of the compound (Murray et al. 1970), though it has not be fully demonstrated due to a lack of evidence regarding specific amidation enzymes. The final step of the pathway involves the methylation of the 2-hydroxy-3-alkylpyrazine (HP) intermediate to produce the methoxypyrazines product (Rizzi et al. 1990), and this activity has been detected in unripe wine grapes (Fig. 11). An enzyme with the ability to methylate HP precursors has been purified from grapes. The levels of HP precursors in grape berries were found to be between 2 and 20 times higher than the MP levels suggesting that methylation of HP might be a rate-limiting step in MP production (Hashizume et al. 2001). By sequencing N-terminus purified grape methyltransferase, researchers could obtain a grape cDNA to encode this enzyme (*Vv*HPMT) and then begin to understand the influence of its activity.

This final step of IBMP biosynthesis has been most studied as compared to other steps. It Fruit pectin containing methyl galacturonate units was investigated as a possible methyl source for the methylation in IBMP synthesis in grapes (Dunlevy et al. 2010), which would indicate MPs are mainly localized in the skins (in terms of the whole berry excluding the stem) or even pulp tissue containing the pectin. A recent study shows that O-methyltransferase (OMT) might catalyze the methylation, although the OMT activity observed in the crude extracts from the young shoot and berries was extremely low (Hashizume et al. 2001). Based on this work, two commonly regarded O-methyltransferases (*VvOMT 1* and *VvOMT 2*) were cloned and shown to be capable of forming MPs via O-methylation of HP precursor (Dunlevy et al. 2010). Unfortunately, the site and synthesis of MPs cannot be determined from any of the present information. In addition, the degradation pathway of IBMP in grape or in any other plants is not clearly established. As we know, during the cluster-ripening period there is an 80 to 90% decrease in IBMP level from pre-veraison to harvest (Scheiner et al. 2010). It would be interesting to confirm how IBMP changes and to learn its breakdown products.

Conclusion

Fruit components, like sugars, organic acids, polyphenols, and flavor compounds, significantly contribute to final fruit quality and integrated sensory characteristics. Subsequently, the degree to which these are judged to be *premium* outcomes ultimately decides the style and quality of wine. Environmental and viticultural conditions can have a significant effect on some of those fruit quality parameters and, by applying different vineyard management techniques, viticulturists and winemakers can have a higher probability of reaching their optimal fruit quality. Crop level is one of important parameters in determining the best fruit quality at harvest. Numerous studies and scientific papers have investigated the influence of environmental factors

and viticultural practices on final fruit composition. Among all the environmental factors, cluster sunlight exposure and temperature have received the most scrutiny. In cool climate growing regions increased cluster sunlight exposure has been achieved via basal leaf removal during different timings of the season to increase sugars, polyphenols, and positive aromas, like terpenes, or to reduce the malic acid contents and negative aromas, like methoxypyrazines. Temperature generally has a similar effect on fruit compositions in cool climate regions. Also, the different timings of sunlight exposure are critical to determining the fruit composition. Exposure of the cluster to sunlight occurring too early (in hot growing areas) or too late (post-veraison) was not suggested as a canopy management protocol due to the potential risk in influencing the fruit set, sun burn damage or insignificant effect on fruit composition along with the extra cost of labor. However, not enough information has been available on the effect of different timings of basal leaf removal on Cabernet Franc fruit composition in cool viticultural areas, like in Michigan.

Therefore, it will be necessary to study how to achieve optimal fruit quality by crop thinning and basal leaf removal of Cabernet Franc at different timings during the season. Our study focused on the interaction between different crop levels and the application of basal leaf removal at different timings of the season and the impact of these treatments upon fruit maturity and the desired fruit quality of Cabernet Franc grown under Michigan's cool climate conditions.

APPENDIX

Table 1. Odor descriptions of the methoxypyrazines found in grapes and wine.

Alkylpyrazine	Odor Description	Source
IBMP	green bell pepper	Buttery *et al*. 1969, Murray and Whitfield 1975, Seifert *et al*. 1970, Maga, 1989.
	herbaceous	Hashizume and Samuta 1997.
	musty	Maga 1989.
	earthy	Hashizume and Samuta 1997.
	leafy	Maga 1989.
IPMP	green bell pepper	Buttery *et al*. 1969, Suriyaphan *et al*. 2001, Maga 1989.
	musty	
	peas	Maga 1989.
	potato	Murray and Whitfield 1975.
	grassy	Buttery *et al*. 1969, Murray and Whitfield 1975.
	hazelnut	Hashizume and Samuta 1997.
	leafy	Counet *et al*. 2002.
		Maga 1989.
SBMP	green bell pepper	Maga 1989.
	musty	Maga 1989.
	peas	Murray and Whitfield 1975.
	potato	Murray and Whitfield 1975.
EMP	green bell pepper	Maga 1989.
	earthy	Maga 1989.

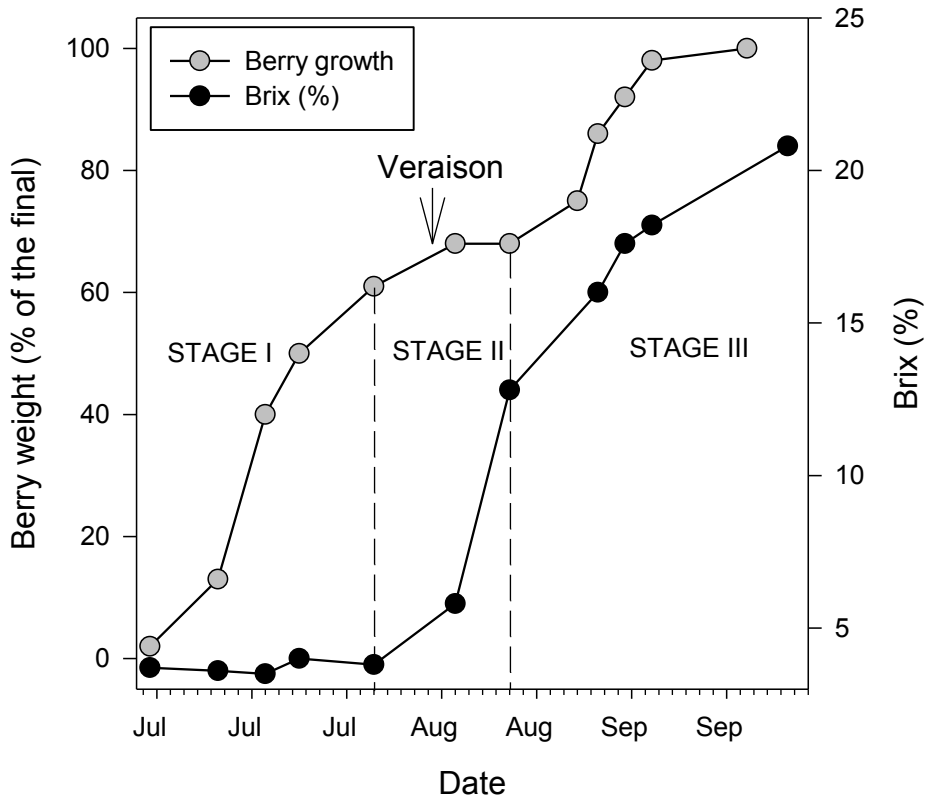


Figure 1. Berry growth (% fresh weight of final) and sugar accumulation (°Brix) in Cabernet Franc from fruit set to harvest. Three stages of double sigmoid curve are shown and sugar accumulation starts at the end of stage II. (Reproduced from Sabbatini, Dami and Howell 2012).

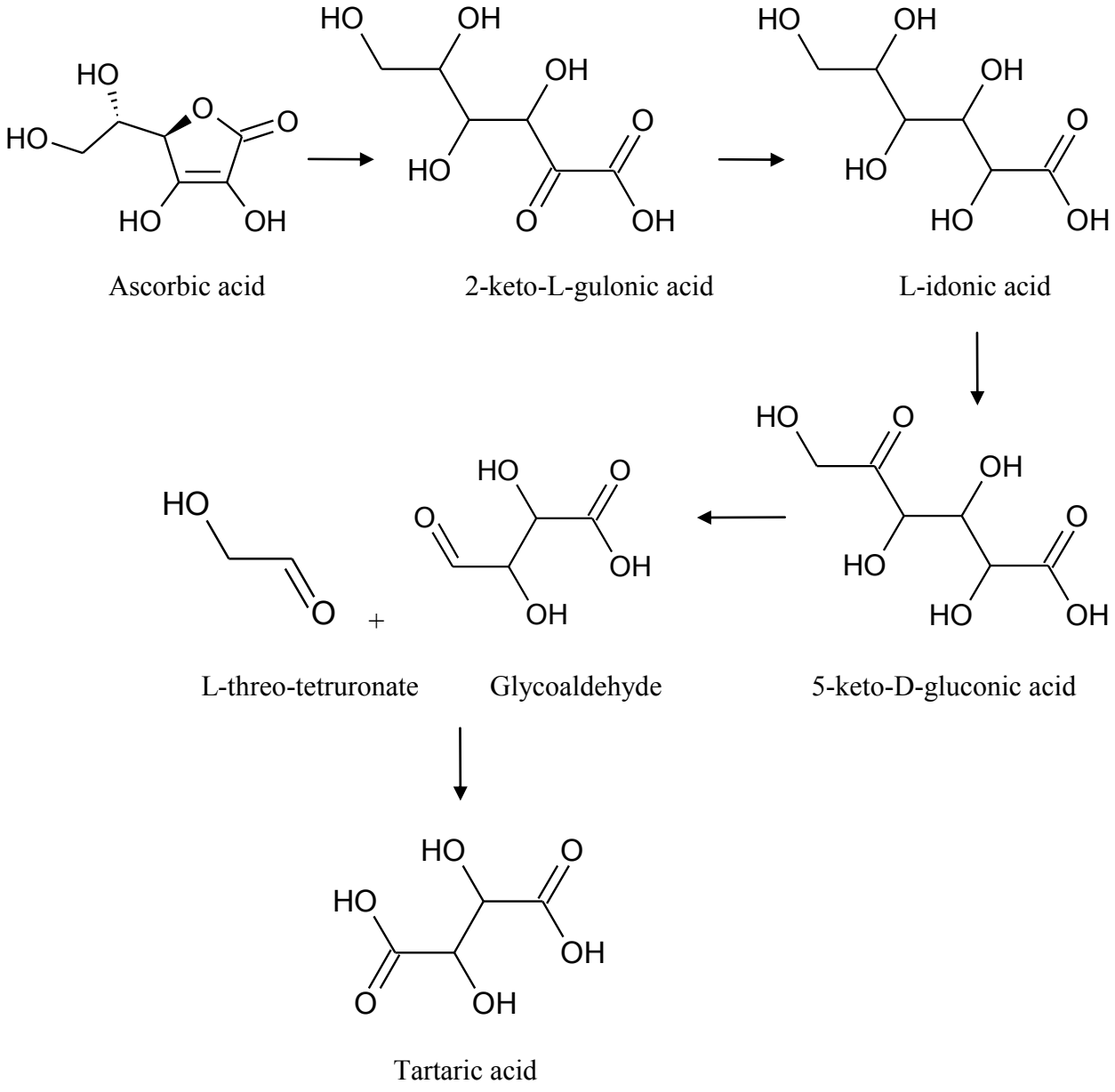
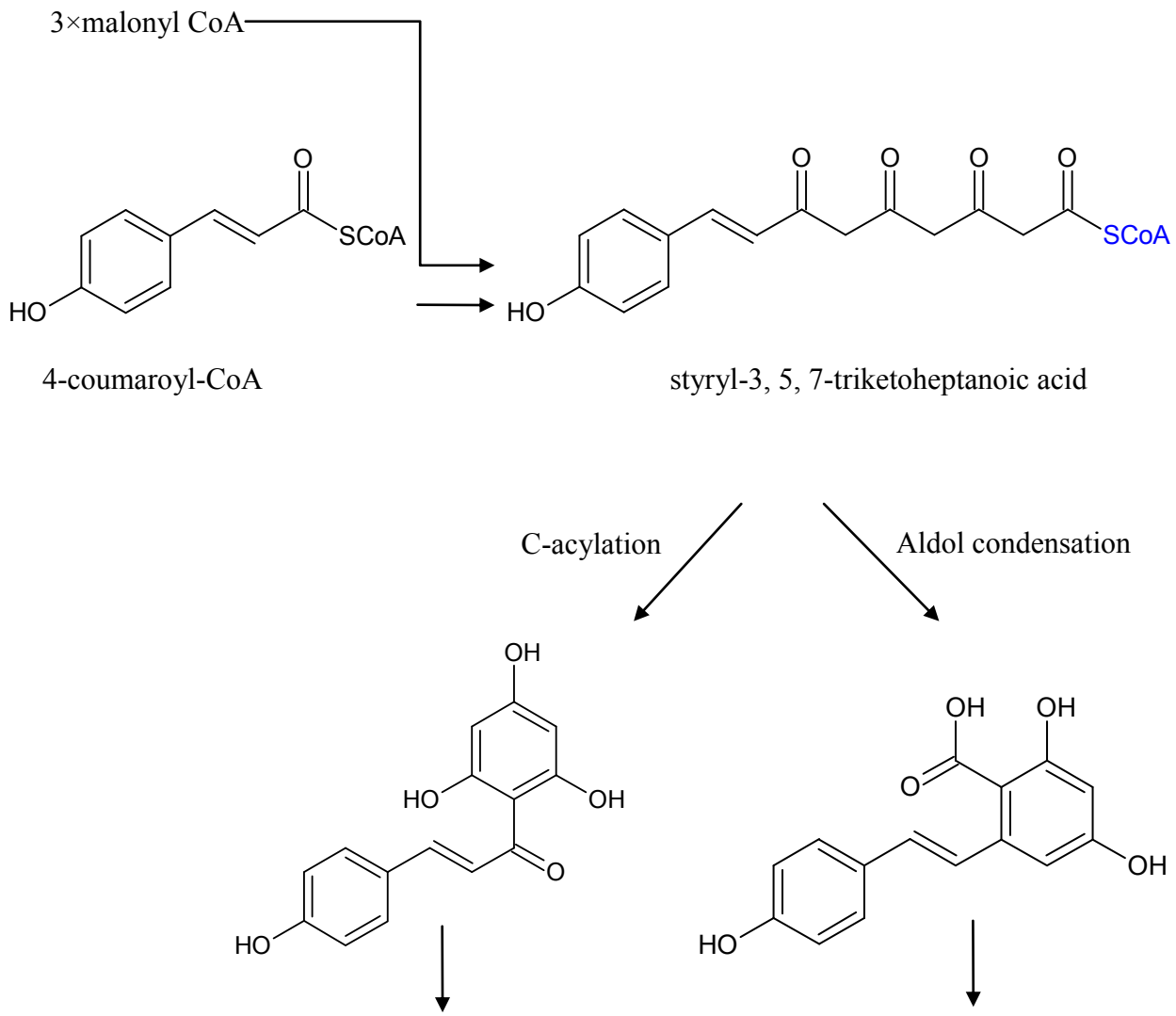
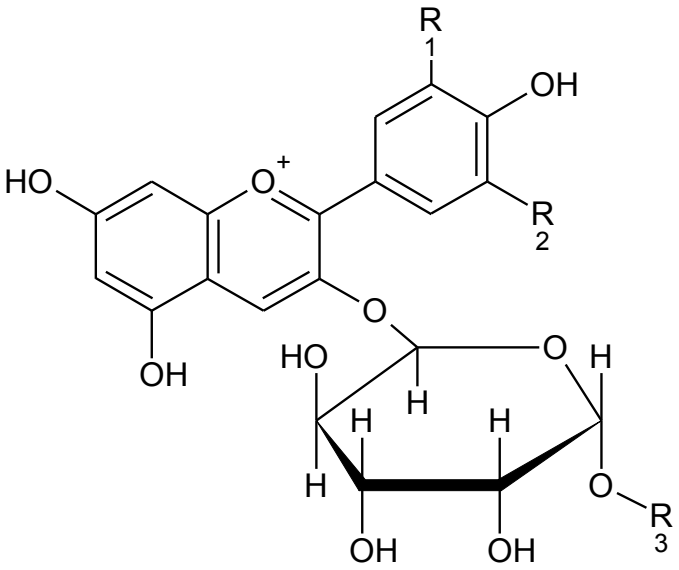


Figure 2. Proposed biosynthesis of tartaric acid in grapes started from Vitamin C (reproduced from DeBolt et al. 2006).

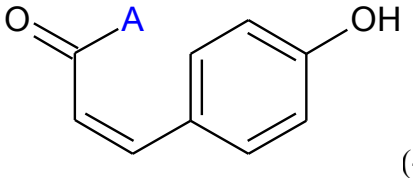


Flavonoids (anthocyanins, flavan-3-ols and flavonols) Stilbenoids (resveratrol)

Figure 3. The biosynthesis pathway for flavonoids and stilbenoids (reproduced from Michael Rentzsch, Andrea Wilkens, and Peter Winterhalter. 2009. Wine Chemistry and Biochemistry. Springer). For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

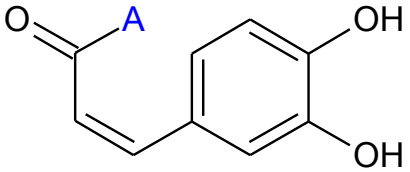


Anthocyanidin	R_1	R_2
Cyanidin	OH	H
Delphinidin	OH	OH
Peonidin	OCH_3	H
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3



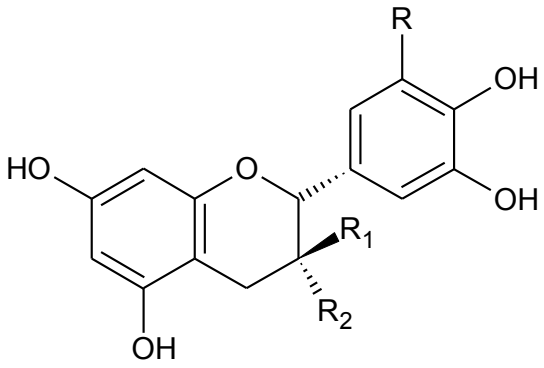
R_3 = -CO-CH_3 (-acetyl)

(-*p*-coumaroyl)



(-caffeoyl)

Figure 4. Chemical structures of anthocyanins (reproduced from María Monagas and Begona Bartolomé. 2009. Wine Chemistry and Biochemistry. Springer).



Flavan-3-ols	R	R_1	R_2
(+)-catechin	H	OH	H
(-)-epicatechin	H	H	OH
(-)-epicatechin 3-gallate	H	H	O-G
(+)-gallocatechin	OH	OH	H
(-)-epigallocatechin	OH	H	OH
(+)-gallocatechin 3-gallate	OH	H	O-G
(-)-epigallocatechin 3-gallte	OH	O-G	H

Figure 5. Chemical structures of flavan-3-ols (reproduced from Nancy Terrier, Céline Poncet-Legrand, and Véronique Cheynier. 2009. Wine Chemistry and Biochemistry. Springer)

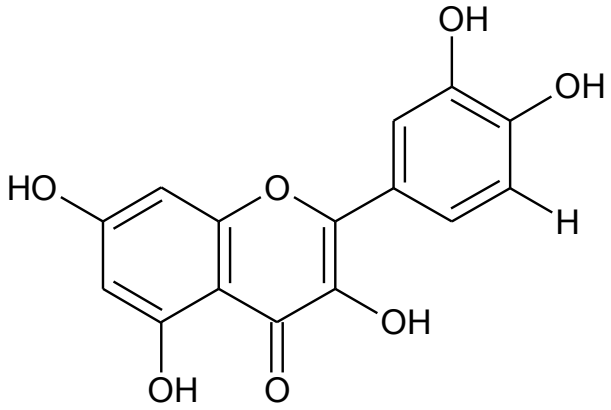
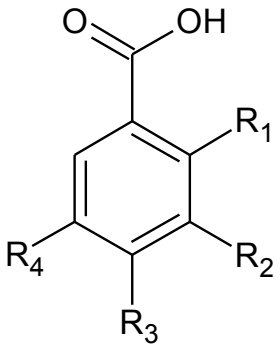
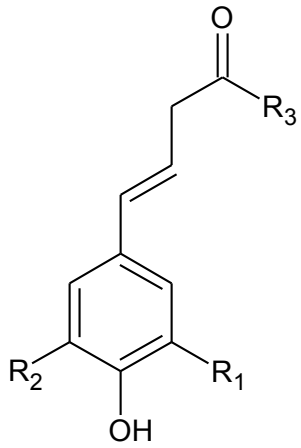


Figure 6. Chemical structure of quercetin, the main component of flavonols (reproduced from Nancy Terrier, Céline Poncet-Legrand, and Véronique Cheynier. 2009. Wine Chemistry and Biochemistry. Springer)



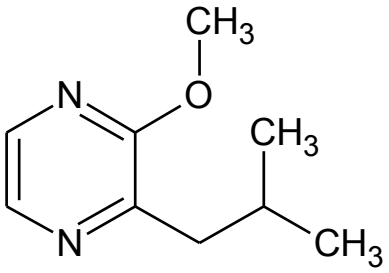
Hydroxybenzoic acids	R_1	R_2	R_3	R_4
Gallic acid	H	OH	OH	OH
Gentisic acid	OH	H	H	OH
P-hydroxybenzoic acid	H	H	OH	H
Protocatechuic acid	H	OH	OH	H
Salicylic acid	OH	H	H	H
Syringic acid	H	OCH_3	OH	OCH_3
Vanillic acid	H	OCH_3	OH	H

Figure 7. Chemical structures of hydroxybenzoic acids (HBA) (reproduced from Michael Rentzsch, Andrea Wilkens, and Peter Winterhalter. 2009. Wine Chemistry and Biochemistry. Springer).

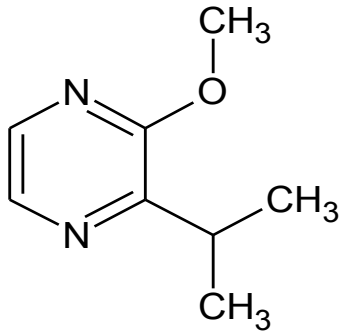


Hydroxycinnamic acids	R_1	R_2	R_3
Caffeic acid	OH	H	H
Caftaric acid	OH	H	Tartaric acid
p-Coumaric acid	H	H	H
p-Coutaric acid	H	H	Tartaric acid
Ferulic acid	OCH_3	H	H
Fertaric acid	OCH_3	H	Tartaric acid
Sinapic acid	OCH_3	OCH_3	H

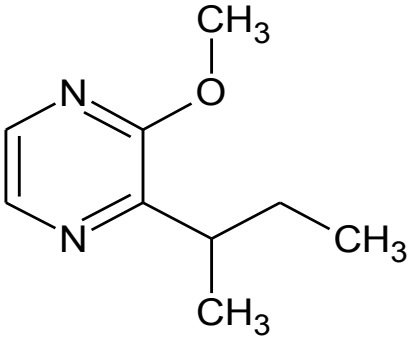
Figure 8. Chemical structures of hydroxycinnamic acids (HCA) (reproduced from Michael Rentzsch, Andrea Wilkens, and Peter Winterhalter. 2009. Wine Chemistry and Biochemistry. Springer).



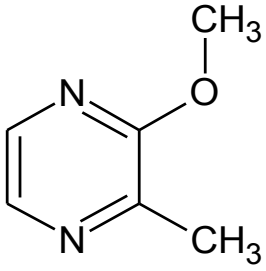
3-isobutyl-2-methoxypyrazine (IBMP)



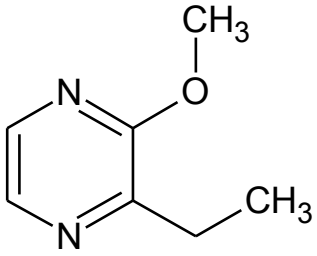
3-isopropyl-2-methoxypyrazine (IPMP)



3-secbutyl-2-methoxypyrazine (SBMP)



3, 5-dimethyl-2-methoxypyrazine



3-ethyl-2-methoxypyrazine (EMP)

Figure 9. Chemical structures of the alkylpyrazines found in grapes and wine.

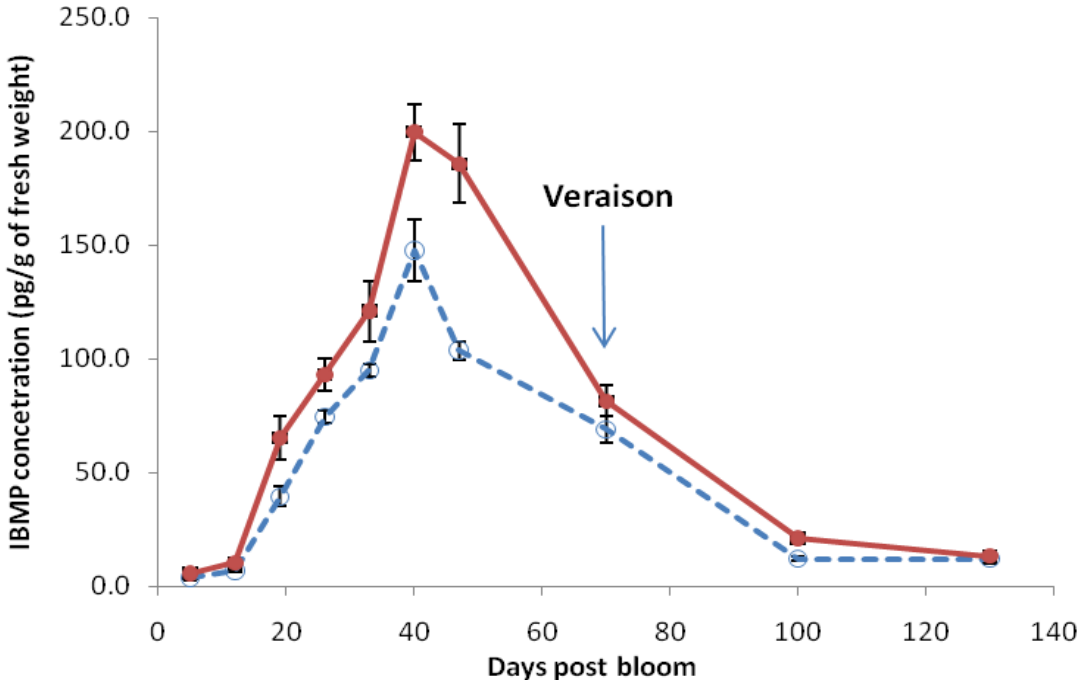


Figure 10. IBMP concentration of shaded (●—) and exposed berries (○- - -) during the growing season. The error bars reflect standard error for the replicates (Elaborated from Ryona et al. 2008).

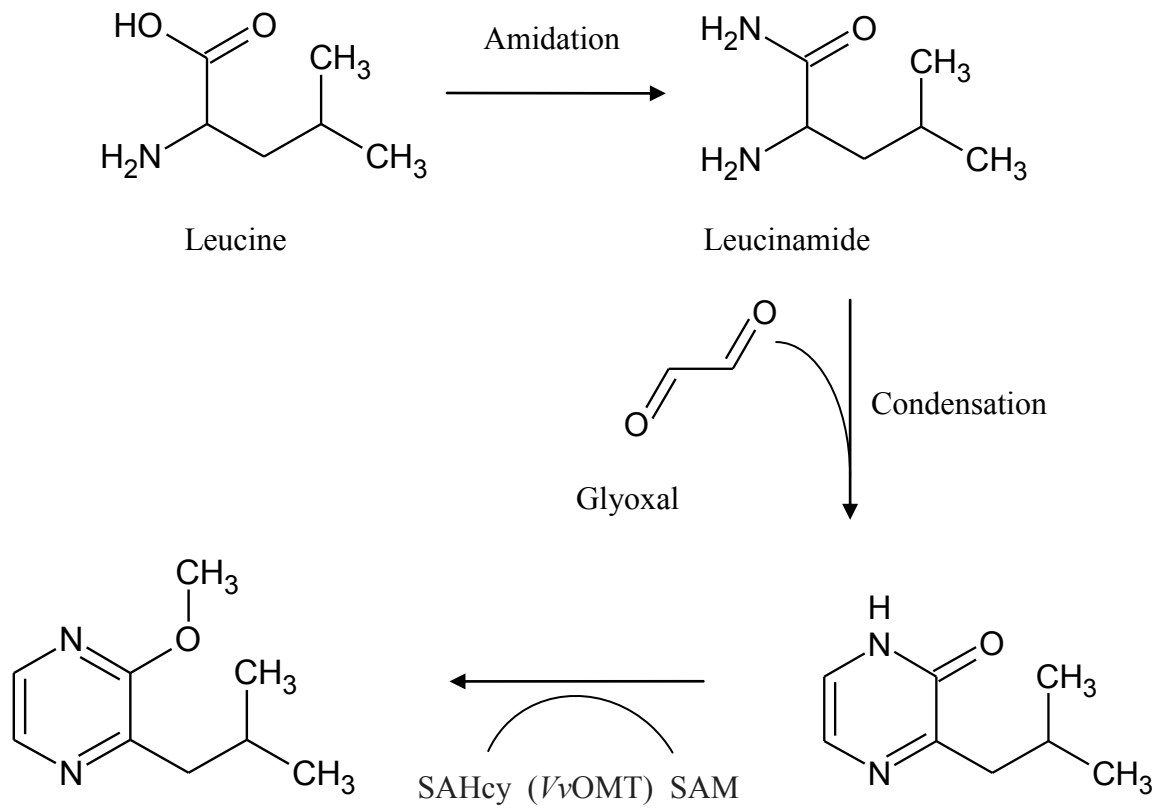


Figure 11. Hypothesized pathway formations of methoxypyrazines (Elaborated from Murray et al. 1970). Putative biosynthesis from IBHP to IBMP is mediated by *Vitis vinifera* O-methyltransferase protein (*Vv*OMT) in the presence of S-adenosyl-L-methionine (SAM) as a methyl group donor. As a product of this reaction, 3-alkyl-2-methoxypyrazine and S-adenosylhomocysteine (SAHcy) are generated (Elaborated from Hashizume et al. 2001).

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CHAPTER II
EVALUATION OF THE IMPACTS OF CROP THINNING AND LEAF REMOVAL UPON VEGETATIVE GROWTH AND FRUIT CHEMISTRY OF CABERNET FRANC IN MICHIGAN IN 2010

Introduction

Fruit quality in wine grape berries, as determined by the level of sugars (TSS), organic acids, polyphenols, and flavor compounds, becomes a critical contributor to final wine quality via fermentation. Irrespective of vintages, different fruit constituents have different responses to various environmental factors, like sunlight, temperature, humidity, water stress, and soil nutrition. In terms of achieving optimal fruit quality and full maturity under different climatic conditions, vineyard management is critical and many viticultural practices have been studied including canopy management (specifically basal leaf removal), irrigation, soil conditioning, and crop thinning. However, when it comes to the cool viticultural regions, like Michigan, the most important concepts to achieve the desired fruit compositions and maturity are vine balance (crop level) and canopy management (microclimate in terms of sunlight and temperature).

Canopy management, especially basal leaf removal, is a viticultural practice designed to provide more sunlight exposure and an increase of cluster temperature in cool growing areas. Higher amounts of polyphenols and a decrease of TA have both been found in cool climates as a result of cluster light exposure manipulation (Mark et al. 2006, Downey et al. 2004, Spayd et al. 2002, and Price et al. 1995). However, the loss of leaf area when the basal leaves are removed, leads to a decreased amount of photosynthetic leaf area to produce the carbohydrates supplying

the berries. Thus, the two levels of fruit exposure were applied in terms of management on basal leaves. First, leaf tucking was applied in which basal leaves were tucked to give opening to clusters which were exposed by sunlight; Second, as a comparison, leaf removal was applied in which basal leaves were simply pulled. The purpose of applying leaf tucking, rather than just leaf removal, is to give the benefit of cluster exposure on fruit composition without a loss of leaf area which could be the extra resources for photosynthesis.

Since the challenges of Cabernet Franc to be fully ripe and poor fruit quality occurred in Michigan, the objectives of our research are to investigate the impact of applying crop thinning, basal leaf removal, and leaf tucking at different timings of the growing season under cool climate on fruit compositions of Cabernet Franc. Different fruit parameters have been quantified, like TSS (Brix), pH, titratable acidity, anthocyanins and total phenolics, to investigate the effect of our treatments. This project will also help to define vine balance in Michigan's primary growing regions and to determine the optimal vineyard practices for improving fruit quality, especially the anthocyanins and phenolics.

Materials and Methods

Plant Materials

Vitis vinifera L. cv. Cabernet Franc vines (FPS 01), grafted on rootstock (3309 C), established in 1993 at the Michigan State University's Southwest Michigan Research and Extension Center (SWMREC), Benton Harbor, MI, were used for field experiments in 2010 and 2011. SWMREC is located 7 miles east of Lake Michigan and benefits from its significant lake effect on regional climate. The experimental vineyard consisted of 10 rows and 48 vines per row trained as VSP (vertical shoot positioning). The vines were planted in Spinks sandy loam soil

and spur pruned to about 48 nodes per vine during the winter pruning. Vine spacing was 2.4 m (8 ft) and row spacing was 3 m (10 ft). Vines were trained with multiple trunks to ensure their survival from winter damage. During the growing season, excessively vigorous shoots were hedged when the tips were one foot above the catch wire. Standard commercial pest-control practices were applied during the season based on scouting, experience, and weather conditions (Wise et al 2007). Drip irrigation was utilized if needed and scheduled based on the soil-water balance approach (Allen et al. 1998) using crop coefficient values (Williams et al. 2005). These were derived from the average leaf area values of the vines. Grapevine evapotranspiration (ET_c) is correlated with the use of crop coefficients (K_c) and reference evapotranspiration (ET_o). The equation: $ET_c = K_c \times ET_o$. Consequently, we can use grapevine evapotranspiration (ET_c) to estimate water usage in the field for efficient scheduling of irrigation.

Monthly rainfall and cumulative growing degree days (GDD) during the growing season were obtained from the Michigan Automated Weather Network (MAWN) station at SWMREC. Additional weather data details and parameters can be accessed at http://www.agweather.geo.msu.edu/mawn/station.asp.

Field Experimental Design and Treatments in 2010

The 2010 experimental design was a split-plot design with main- and sub-factors (Figure 12). The main factor is timing of application, while three levels of cluster thinning and two levels of cluster exposure are the sub-factors. Individual vines were organized in 8 blocks of 6 vines each for a total of 48 vines. Vine blocks were paired and then randomly assigned to one of the following periods, fruit set (two weeks after bloom, June 30th, 653 GDD), 3 weeks before

veraison (July 28th, 1046 GDD), veraison (August 18th, 1325 GDD), and 3 weeks post-veraison (September 8th, 1564 GDD). Each block consisted of a 2×3 factorial design.

Three levels of cluster thinning, 12, 24 and 48 clusters per vine, were integrated with two levels of cluster exposure. To modify cluster exposure, two levels of cluster exposure were created in the cluster-zone using two different techniques: basal leaf removal (LR) in which leaves from basal 6 nodes were simply pulled, including laterals if present, and as a comparison, basal leaf tucking (LT) in which basal leaves were tucked to expose the clusters (Figure 13). Apical clusters were removed first when the cluster thinning was performed manually due to their delay ripening compared to basal clusters. The number of clusters per vine was adjusted to 48 before fruit set. Since both fruit exposure and cluster thinning were applied at the same timing of four in our experiment, we used the term of timing to represent the timing in which both fruit exposure and cluster thinning were applied.

Canopy Growth Measurement in 2010

Total shoot number per vine was counted at bloom. In order to evaluate the impact of cluster thinning and leaf removal on vine vigor, Shoot length was monitored weekly from June 10th until hedging before veraison on July 21st. Five average representative model shoots per vine were selected and tagged based on vine vigor with average shoot growth. Excessively vigorous and week vines were excluded from the study. Shoot length was recorded by measuring tape. After hedging, shoot diameter, instead of shoot length, was measured for the same five shoots per vine. Total leaf area was estimated based on the regression between shoot length and shoot leaf area using a non-destructive method. First, the regression was built according to Mabrouk and Carbonneau (1996) (Figure 14). Weekly, twenty shoots, neither excessively

vigorous nor weak, were sampled randomly from non-experimental vines from June 10th to July 21st, stored in an ice cooler, and transported to campus. In the lab, shoot length was recorded and leaf area per shoot was measured using a leaf area meter (LI-3100 area meter, LI-COR, Lincoln, NE). This relationship between shoot length and leaf area was used to estimate the leaf area of the five tagged shoots from each experimental vine based on the weekly shoot length measurements. This average leaf area was multiplied by the shoot number to obtain the total leaf area per vine. Daily shoot growth rate was used to represent the canopy growth, and was calculated as the change in shoot length or diameter per day (cm/day or mm/day). Before hedging, the shoot growth rate was calculated; after hedging the rate of increase in shoot diameter per day (mm/day) was used as a measurement of shoot growth.

Canopy Density Assessment in 2010

Canopy density was assessed near veraison. A canopy density index was calculated using point quadrat analysis (PQA), per the practice developed by Smart and Robinson, (2008). Generally, a guiding board (2 m length) was horizontally fixed along the cluster-zone and a thin metal rod (1 m long and 2 mm diameter) was inserted into a canopy at regular intervals (5 cm) marked along the board. Fifty or more insertions were made for each vine. Sequential contacts with leaves and other vine parts from one side of the canopy to the other were recorded with L identifying leaf, C for cluster, and G for canopy gap; contact with the shoot was ignored. The calculation for canopy density based on PQA takes into account the percentage of gaps, the number of leaf layers (LLN), and the percentage of exterior leaves and clusters touched. The gap percentage is the total number of gaps (G) divided by the number of insertions, then multiplied by 100 to obtain a percentage. LLN is the total number of leaf contacts (L) divided by the

number of insertions. Both the percentage of exterior leaves and the percentage of exterior clusters are the count of the number of each touched by the rod, first and last on both canopy surfaces during each insertion, divided by the total number of leaves or clusters, respectively.

Photosynthesis measurement of basal leaves

At veraison (around 90 days after bloom), net photosynthesis (Pn), stomatal conductance (gs), transpiration (E) and intercellular CO_2 concentration (Ci) were determined simultaneously on tucked basal leaves from the LT treatment and basal leaves from untreated vines. Photosynthetic measurements were made using a CIRAS2 portable photosynthesis system (PP system, Amsbery, USA). This PP system was equipped with a leaf-clamped cuvette for 2.25 cm^2 leaf area and measurements were taken under field conditions (i.e., photosynthetically active radiation was $\geq$1450 μmol m^{-2} s^{-1}, inlet relative humidity was fixed around 26.7% and chamber temperature ranged from 26 to 31°C). The measurements were carried out once every 2 to 3 days, in total two times, between 1000 hr and 1600 hr at approximate veraison (August 18th, 1325 GDD). Three measurements per vine, on a total of three vines, were taken each time.

Canopy Microclimate for cluster temperature measurement in 2010

Canopy microclimate in terms of cluster temperature was monitored using the infrared thermometer (Raynger ST^{TM}, Raytek Corporation, CA). For all the experimental vines, temperature of basal clusters from five tagged shoots per vine was recorded weekly from 1200 to 1500 hr from July 1st to September 9th. Ambient temperature during the season was obtained

from the weather station at SWMREC (Michigan Automated Weather Network [MAWN] at SWMREC).

Sampling Procedures and Harvest Data Collection

From fruit set through harvest, thirty berries from each vine were randomly collected on a biweekly basis, immediately frozen in liquid nitrogen, transported to campus, and stored a -60°C for future analysis. At the beginning of veraison, an additional twenty berries from each vine were randomly sampled from the clusters of non-tagged shoots to track fruit maturation until harvest. At harvest, total crop weight and total cluster number were recorded. Clusters from the tagged shoots from each vine were harvested and immediately put in the ice coolers, transported to campus, and stored at -20°C. Each cluster was then weighed to estimate the average cluster weight. Total berry numbers from each cluster were counted and total berry weight was obtained to calculate the average berry weight. Brix, pH, TA, anthocyanins, and total phenolics were measured as described below from the clusters that were collected from the tagged shoots at harvest. Pruning weight per vine was collected in the following winter pruning to ascertain the weight of pruned one-year old wood.

Basic Fruit Chemistry Measurements in 2010

Harvested frozen grapes in collection bags were thawed under the room temperature before analysis. After that, berries were crushed with a manual press, and free-run juice was decanted into 50 mL tubes. Grape juice soluble solids were measured using a digital Brix refractometer (ATA-3810 PAL-1 Pulse Inc. Van Nuys, CA). Titratable acidity and pH were measured in using a 370 Thermo Orion pH meter (Thermo Fisher Scientific Inc. Logan, UT).

Titratable acidity was measured using Multi-T 2.2 digital titrator (Laboratory Synergy Inc. Goshen, NY). Ten mL clear juice per sample diluted with distilled water to 100 mL was titrated with 0.1M sodium hydroxide (NaOH) to a pH of 8.2 using an equation to yield the TA (g/L), according to Iland et al. (2004).

TA (g/L as tartaric acid) = 75 × 0.1 (molarity of NaOH) × Titre value (mL)/10 (volume of juice)

Anthocyanin and total phenolics content were measured by the total phenol assay, using the Folin-Ciocalteu reagent (Singleton et al. 1999). One hundred berries stored at -30°C were partially thawed prior to grinding in a Brinkmann Homogenizer (Brinkmann Instruments, Westbury, NY) at a speed of four on the manufacturer's scale for about 1 min. The process was conducted under cool conditions to minimize oxidation. The homogeneous mixture 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 mL 50% v/v aqueous ethanol acidified to pH 2 (~1 mL 12.1 M HCL) was added to the 1 g sample, and then it was mixed once per 5 min manually for 1 hour. After that, the sample was centrifuged at 5000 rpm for 20 min. One mL of extract (supernatant liquid) was pipetted into a 15 mL centrifuge tube. Ten mL of 1 M HCL was added and the mixture was equilibrated for 3 hours (Anthocyanin compounds contain acidic protons which change their characteristic absorbance wavelength when removed. Acidifying the solution is very important to ensure that acidic protons remain bonded and the correct absorbance values are attained), which is necessary because acidifying the solution is critical to ensure that acidic protons remain bonded with anthocyanins and the correct absorbance values are attained. Eventually, the absorbance values were obtained using a UV-Vis spectrophotometer (Model UV-1800, Shimadzu Corporation, Kyoto, Japan) at 280, 520, and 700 nm. The absorbance values

obtained at 280 nm indicated the total phenolics of the sample, at 520 nm the anthocyanin content, and at 700nm a turbidity check.

Statistical Analysis

Analyses of variance (ANOVA) for basic fruit chemistry and yield components in 2010 were performed using the PROC MIXED procedure of SAS for Windows, version 9.2 (Cary, NC). Split block design and pair-wise comparisons were performed by LSD test at a significance level of 0.05 and 0.10.

Results

2010: Climatic conditions

As for growing degree days (GDD), 2010 was a good year with 1805 GDD versus the past 10 years' average value of 1687 GDD, which indicated more than 100 GDD higher in terms of heat accumulation in 2010 compared to the average data from last 10 years. GDD and weather data were shown in Figure 15 and phenological stages were also shown in Table 2. Maximum air temperature in 2010 reached at 35 °C during July and August, and the excessive amount of heat caused some sun burn damage on the fruit, especially when the clusters were exposed to sun light. In 2010, precipitation allocates in every month from May to October, and the majority of amount was concentrated in June and July, during which more than 50 mm of weekly precipitation occurred. When the season was approaching the harvest, like September and October, the rain was usually undesirable in terms of reducing the sugar contents and causing the

rot and fungal diseases, however, in 2010 20% of annual precipitation was concentrated in these two months, and this always resulted in delay of harvest in Michigan.

2010: Seasonal canopy vegetative growth

None of our treatments affected shoot growth, either the rate of shoot growth or increasing rate of shoot diameter. Also, there were no interactions. Thus, data of shoot and diameter growth rates were pooled from each treatment. The earlier the cluster thinning and leaf removal have been performed, the more significant effect of our treatments on shoot growth rate will be. Therefore, the vines for which cluster thinning and fruit exposure were performed at fruit set were selected to represent the results.

There was no difference of shoot growth rate (expressed as shoot length or diameter) during the season due to the impact of three levels of cluster thinning (Figure 16 and 18). Fruit exposure did not affect rate of shoot growth (expressed as shoot length or diameter) (Figure17 and 19).

In comparison with basal leaf tucking, basal leaf removal reduced leaf layers numbers by more than 50% (Table 3), and increased the percentage of canopy gaps by 400% in the cluster-zone. Most of the clusters in the basal leaf removal treatment were categorized to be exterior clusters (more than 80%); they received more sun exposure during the season independent of the crop level. Leaf tucking resulted in a similar percentage of exterior clusters as basal leaf removal, but resulted in a higher number of leaf layers and tighter canopies in the cluster-zone.

2010: Cluster temperature during the season

Temperature of exposed clusters, either from basal leaf removal or leaf tucking, increased by from 0.2 to 1.8 °C during the season as compared to clusters without any treatment (Figure 20 and 21).

2010: Yield components and basic fruit chemistry parameters at harvest

There was a three-way interaction between timing, cluster thinning and fruit exposure. We sliced the three-way interaction into two groups of leaf tucking and leaf removal.

For the leaf tucking, the severe cluster thinning (12 clusters retained per vine) at fruit set reduced the yield significantly (Table 4 and 5); however, there was no difference on yield between treatments of 24 clusters per vine and 48 clusters per vine, when the number of clusters has been doubled. Similarly, when the cluster thinning was applied in pre-veraison, veraison and post-veraison, no significant difference has been found in terms of yield between 12 clusters per vine and 24 clusters per vine, but a significant increase of yield was found in 48 clusters per vine.

For the leaf removal (Table 6 and 7), severe cluster thinning (12 clusters retained per vine) at fruit set significantly reduced the yield compared to 48 clusters per vine; however, yield did not differ between 12 clusters per vine and 24 clusters per vine. Similar results were found when cluster thinning was applied pre-veraison and post-veraison. However, when applied at veraison, yield differed for all three levels of cluster thinning.

As for the cluster weight, three-way interaction has been found significant as well. Similar to the yield, we sliced the three-way interaction of cluster weight to two groups of leaf tucking and leaf removal. The data is shown in Table 8, 9, 10, and 11.

For the leaf tucking (Table 8 and 9), heavier clusters were found in both the 12 clusters per vine and 24 clusters per vine sets when the cluster thinning treatment was applied at fruit set.

However, cluster weight was significant increased only in the 12 clusters per vine set when cluster thinning was applied at pre-veraison and veraison. There was no difference in cluster weight when cluster thinning was applied post-veraison. Interestingly, when comparing the cluster weight at different times using cluster thinning (12, 24 and 48 clusters per vine), we did not find any significant difference.

For the leaf removal (Table 10 and 11), it was a little complicated. Similar to leaf tucking, heavier clusters were found in the 12 clusters per vine set when cluster thinning was applied at pre-veraison. However, we have also observed heavier clusters in both the 12 and 48 clusters per vine sets. There was no difference in cluster weight in any of the three levels of cluster thinning when it was applied at veraison and post-veraison.

Severe cluster thinning resulted in a higher pruning weight at fruit set and veraison. However, leaf tucking and leaf removal did not affect the pruning weight (Table 14, 15 and 16).

The Ravaz Index was affected by three different levels of cluster thinning irrespective of timing, but, not by timing of treatment or fruit exposure. Ravaz Index values ranged from 0.85 to 2.64 in three levels of cluster thinning (Table 14 and 15).

There was no difference in accumulation rate (°Brix/day) or in duration of ripening from the three levels of cluster thinning (Figure 22).

There was a two-way interaction of timing and fruit exposure for the total soluble solids (°Brix). Fruit exposure (leaf tucking and leaf removal) had no effect on °Brix when fruit exposure was applied at fruit set and pre-veraison (Table 12 and 13). There was an effect of fruit exposure on °Brix when the fruit exposure was applied later (veraison and post-veraison). However, we found a significant decrease in °Brix when we applied the fruit exposure earlier (fruit set or pre-veraison), either by leaf tucking or leaf removal.

There were no interaction for the rest of fruit compositions (pH, TA, anthocyanins and phenolics), we, therefore, pooled the data for each treatment; there was no impact of fruit exposure and cluster thinning timings, cluster thinning, and fruit exposure.

Discussion

The lack of an effect of timing, cluster thinning levels, or degrees of fruit exposure on shoot growth was complicated. The inherent vine vigor was not affected by yield manipulation in this study, when the vines were not heavily overcropped. Also, more than 50 mm of monthly precipitation in 2010 from May to October made vines in Michigan even harder to observe any impact on shoot growth as compared to other hot and dry areas (Figure 15). However, larger vine size (heavier pruning weight) from excessively cluster thinning when applied at fruit set and pre-veraison could be due to the fact of more vigorous vines because of a lower amount of yield manipulated at earlier of the season.

In terms of yield, we found a compensation effect for 12 and 24 clusters per vine, but not for the 48 clusters per vine treatment, with the exception when cluster thinning was applied at fruit set along with leaf tucking. Cluster weight also exhibited compensation. Cluster weight increased following severe cluster thinning (12 clusters retained per vine) when applied at fruit set, pre-veraison and veraison, but not at post-veraison. In other words, compensation in cluster weight due to the cluster thinning occurred during the early portion of the season. There was no or little compensation for cluster weight after veraison. However, the results were not consistent for leaf tucking and leaf removal. Since berry number was fixed at fruit set (two weeks after bloom), the compensation in cluster weight was primarily due to an increase in berry weight. The Ravaz Index in this study ranged from 0.8 to 2.6, which is representative of severely under-

cropped vines. Sun burn damage has also been observed in vines which early leaf removal has been applied. This factor could result in the negative impact of early leaf removal on °Brix. Surprisingly, impact of the timing of leaf removal on other fruit compositions (pH, titratable acidity, anthocyanins and total phenolics) has not been found in 2010. The lack of an effect of the timing of leaf removal on other fruit compositions could be complicated. Elevated cluster temperature from leaf removal is beneficial for decreasing the malic acid content in berries (Keller et al. 2005, Alan and Kliewer 1975, 1977). Also, temperature and light have been regarded as important factors in influencing the contents of anthocyanins and phenolics (Downey et al. 2004, Spayd et al. 2002, Price et al. 1995). In terms of sunlight and temperature on clusters, early leaf removal in cool climate has been regarded beneficial for the fruit compositions, e.g. reducing the titratable acidity and increasing the anthocyanins and phenolics. However, the vines in the experiment have been severely undercropped for all the three levels of cluster thinning. This means vine vigor (or leaf area) was not limiting factor to ripen the amount of fruit in achieving the desired fruit composition, e.g. °Brix, at harvest (Kliewer and Dokoozlian 2005). Therefore, it could explain the reason why no significance has been found in fruit compositions due to the timing of leaf removal in our experiment.

We anticipated that leaf tucking would improve carbon assimilation by the fruit because of less reduction in basal leaf photosynthesis compared to leaf removal. However, the tucked basal leaves in the leaf tucking treatment were photosynthetic at very low levels during veraison according to the results of single leaf photosynthesis measurement (Table 17). Consequently, two groups of fruit exposure had no effect on fruit compositions. Shading was the cause for the reduction of photosynthetic activities of tucked leaves. Tucked leaves were always shaded with each other at the cluster zone (Figure 13) and the shaded environment led to accelerating the

aging process of leaves (Cartechini et al. 1995 and Porro et al. 2001). Senescence and a low photosynthetic rate were observed in tucked leaves during veraison in our experiment.

Yield impacted fruit compositions, especially sugar (°Brix). Overcropped vines have difficulty ripening the fruit to desired quality parameters (Bravdo et al. 1984, 1985). The lack of an effect of cluster thinning on fruit compositions, e.g. °Brix, in our experiment was due to the fact of an excessively lower Ravaz Index (less than 3) from all three levels of cluster thinning. Under the range of crop load (5 to 10) or leaf area/fruit weight ratio (8 to 12 cm^2/g), higher yield did not affect the final sugar accumulation, but delayed the date of arrival of targeted °Brix values (Bravdo et al. 1984, 1985, Petrie and Clingeleffer 2006). No significance in fruit compositions from cluster thinning treatments could be explained by undercropping rather than overcropping in our cluster thinning treatments.

The literature suggests that organic acids in berries are more influenced by environmental conditions like cluster temperature and light intensity (Keller et al. 2005, Lakso and Kliewer 1975, 1977). Higher cluster temperatures (especially above 30°C), due to climatic conditions like ambient temperature or viticultural practices like basal leaf removal, increased the metabolization of the malic acid through respiration by enhancement of malate enzyme activity during the ripening stage and, ultimately, reduction of a significant amount of TA at harvest (Lakso and Kliewer 1975, 1977). However, in our experiment for the two groups of fruit exposure, clusters were well exposed at the same level. This could explain why we did not find any significance in pH or TA in leaf tucking and leaf removal treatments.

Similar to organic acids, the levels of anthocyanins and phenolics in berries are affected by temperature, and also by the sunlight (Downey et al. 2004, Spayd et al. 2002, and Price et al. 1995). Specifically, Anthocyanins are more influenced by temperature, rather than sunlight

(Spayd et al. 2002). For phenolics, it is complicated and could be due to different environmental and viticultural factors. However, as for favonols (proportion of phenolics), it is mainly affected by sunlight rather than temperature (Downey et al. 2004). Since there was no difference of sunlight exposure on clusters from two groups of fruit exposure in our experiment, this could explain why no significance of anthocyanins and phenolics has been found in the results.

APPENDICES

APPENDIX A: TABLES AND FIGURES

Crop Levels per Vine: 12, 24, and 48 clusters

Canopy Management: Leaf removal and Leaf tucking

Fruit set	Pre-veraison	Veraison	Post-veraison
1 block	1 block	1 block	1 block
1 block	1 block	1 block	1 block

Figure 12. 2010's field experimental design; each block consists of 6 vines; each vine was designated for one treatment.

Figure 13. Basal leaf tucking (top) and basal leaf removal (bottom) at cluster zone. For both treatments basal clusters were well exposed with the difference in leaf area from the first 6 basal nodes per shoot.

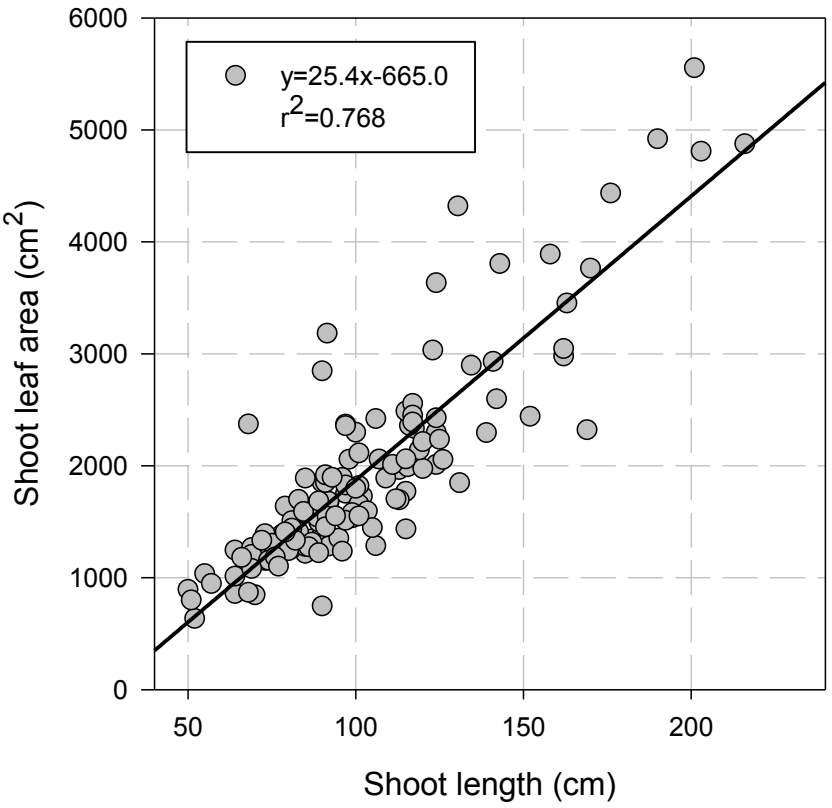

Figure 14. Linear regressions between shoot length and shoot leaf area in 2010.

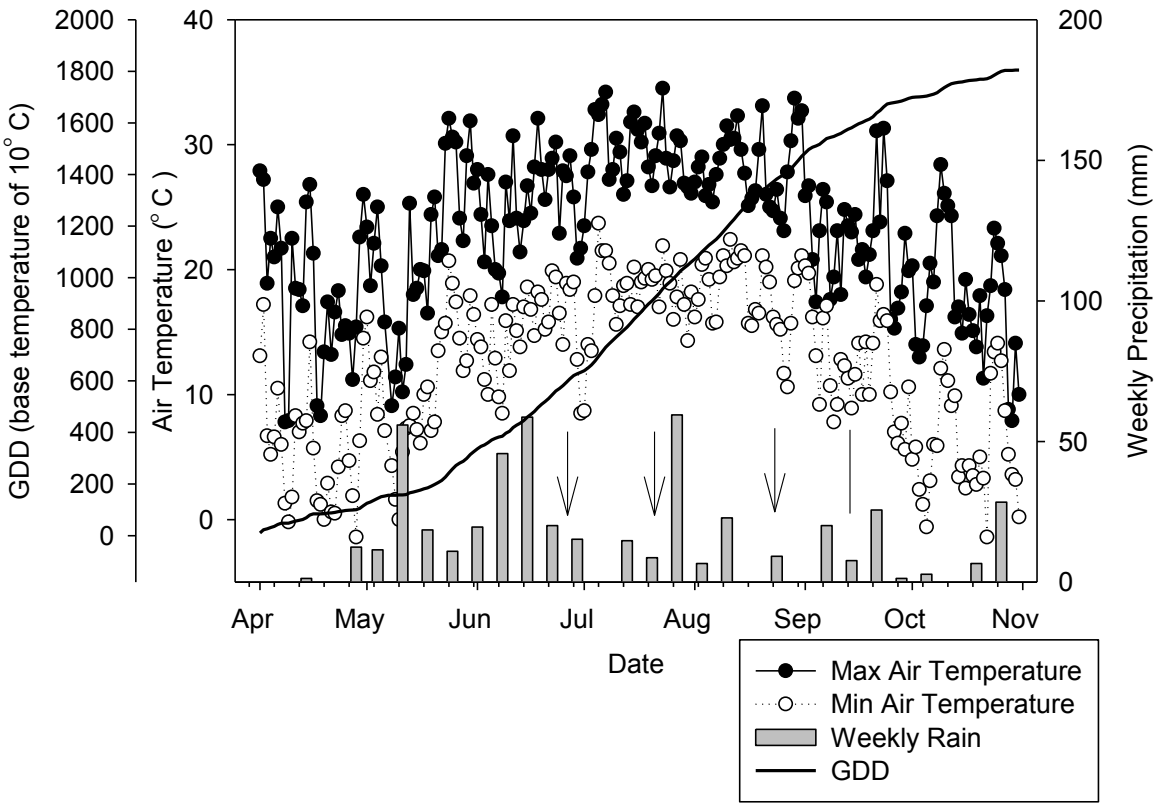

Figure 15. Growing degree days (GDD) and weekly precipitation for 2010 at the Southwest Michigan Research and Extension Center near Benton Harbor. GDD was calculated at a base temperature of 10°C from April 1 through October 31. Daily maximum and minimum air temperature were also obtained. Arrows indicated the four different timings of our treatments during the season.

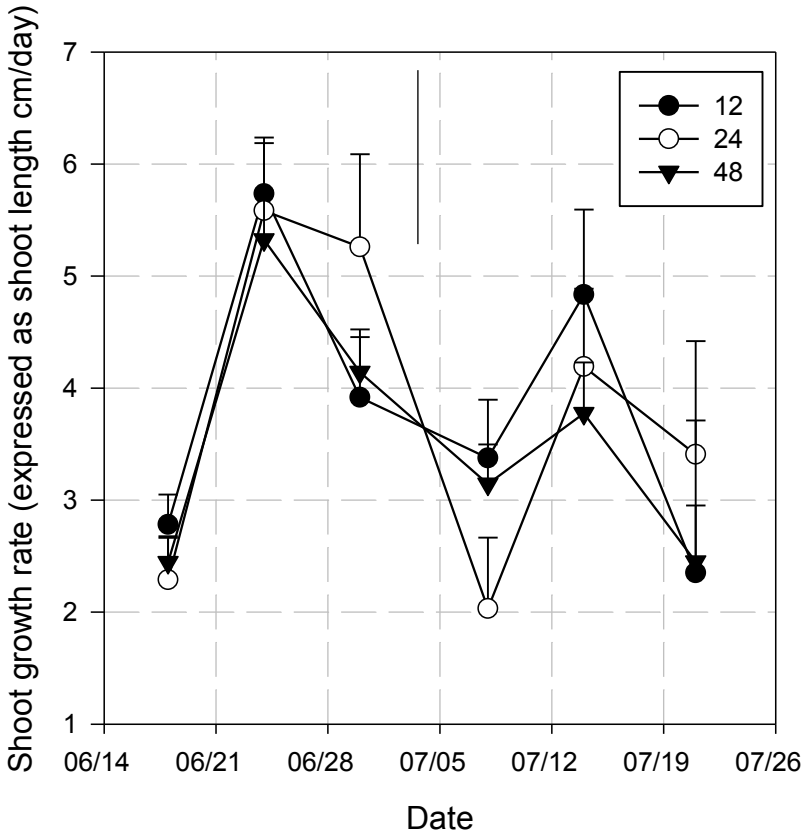

Figure 16. Shoot growth rate (expressed as shoot length cm/day) from the beginning of the season to late middle of July, the arrow indicated our first treatment of cluster thinning and fruit exposure at fruit set. In the legend 12, 24 and 48 were the abbreviation of 12, 24 and 48 clusters per vine. Each color of bars represented one of three levels of cluster thinning with pooling of the data from two levels of fruit exposure. Data presented in this figure were selected from the vines to which were applied the cluster thinning and fruit exposure treatments at fruit set.

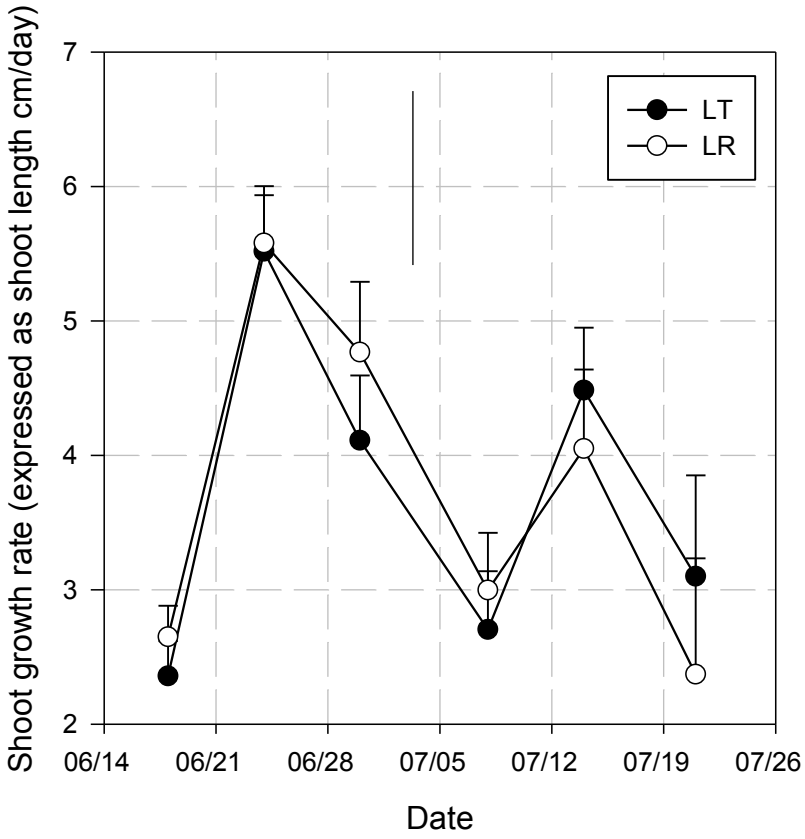

Figure 17. Shoot growth rate (expressed as shoot length cm/day) from the beginning of the season to late middle of July, the arrow indicated our first treatment of cluster thinning and fruit exposure at fruit set. LT and LR were the abbreviation of leaf tucking and leaf removal. Each color of bars represented one of two levels of fruit exposure with pooling of the data from three levels of cluster thinning. Data presented in this figure were selected from the vines to which were applied the cluster thinning and fruit exposure treatments at fruit set.

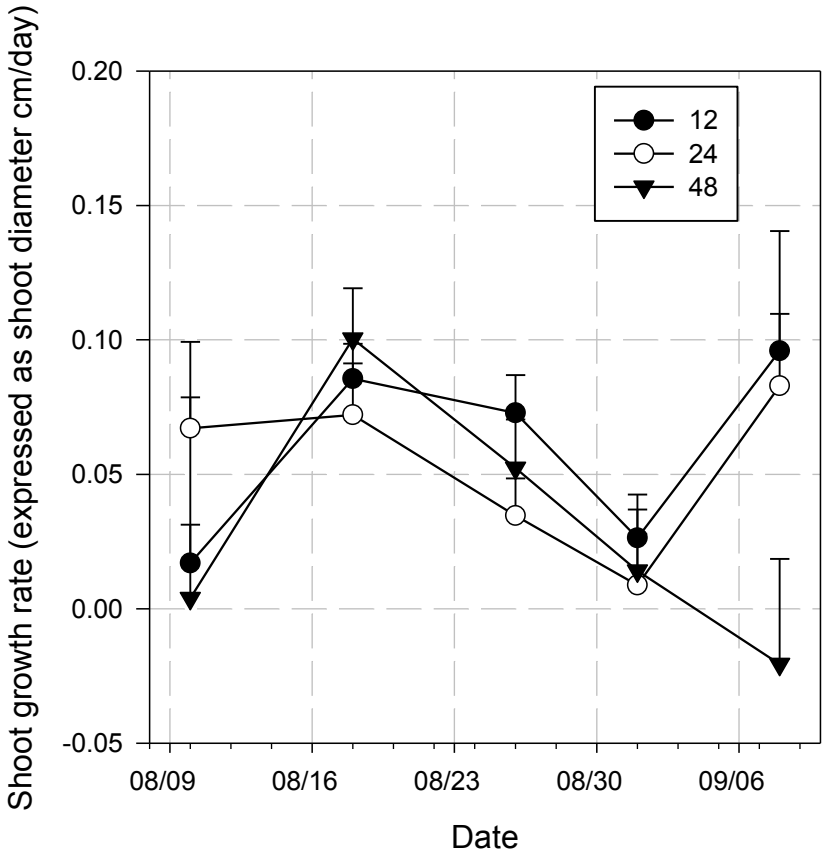

Figure 18. Shoot growth rate (expressed as shoot diameter cm/day) from the beginning of August to the end of September; in the legend 12, 24 and 48 were the abbreviation of 12, 24 and 48 clusters per vine. Each color of bars represented one of three levels of cluster thinning with pooling of the data from two levels of fruit exposure. Data presented in this figure were selected from the vines to which were applied the cluster thinning and fruit exposure treatments at fruit set.

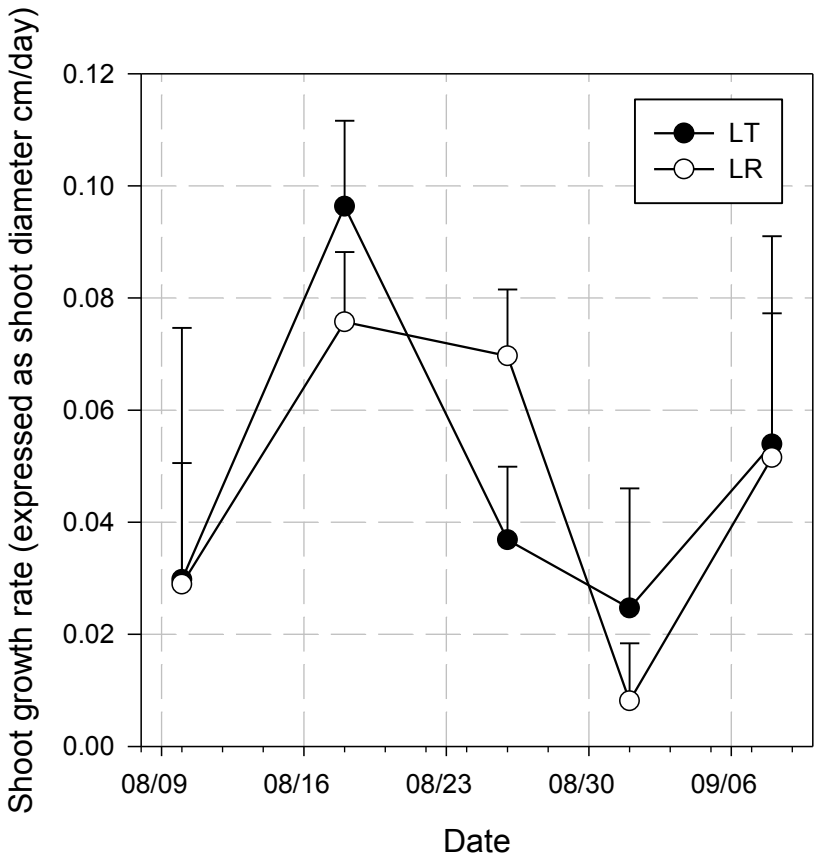

Figure 19. Shoot growth rate (expressed as shoot diameter cm/day) from the beginning of August to the end of September; LT and LR were the abbreviation of leaf tucking and leaf removal. Each color of bars represented one of two levels of fruit exposure with pooling of the data from three levels of cluster thinning. Data presented in this figure were selected from the vines to which were applied the cluster thinning and fruit exposure treatments at fruit set.

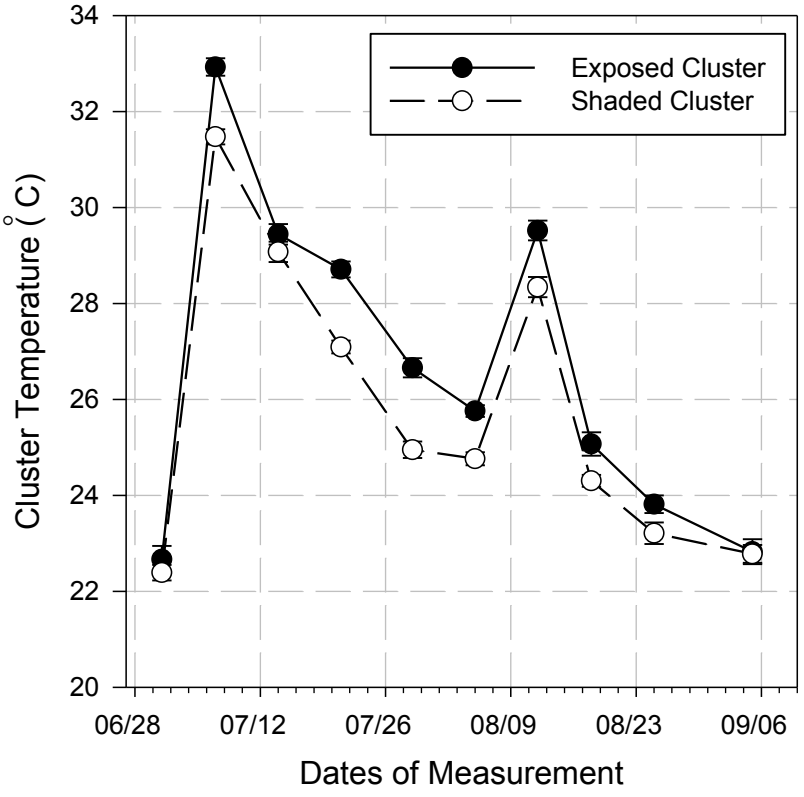

Figure 20. Weekly measurement of temperature for exposed and shaded clusters during the season. Error bars represent the standard error of the mean (SEM).

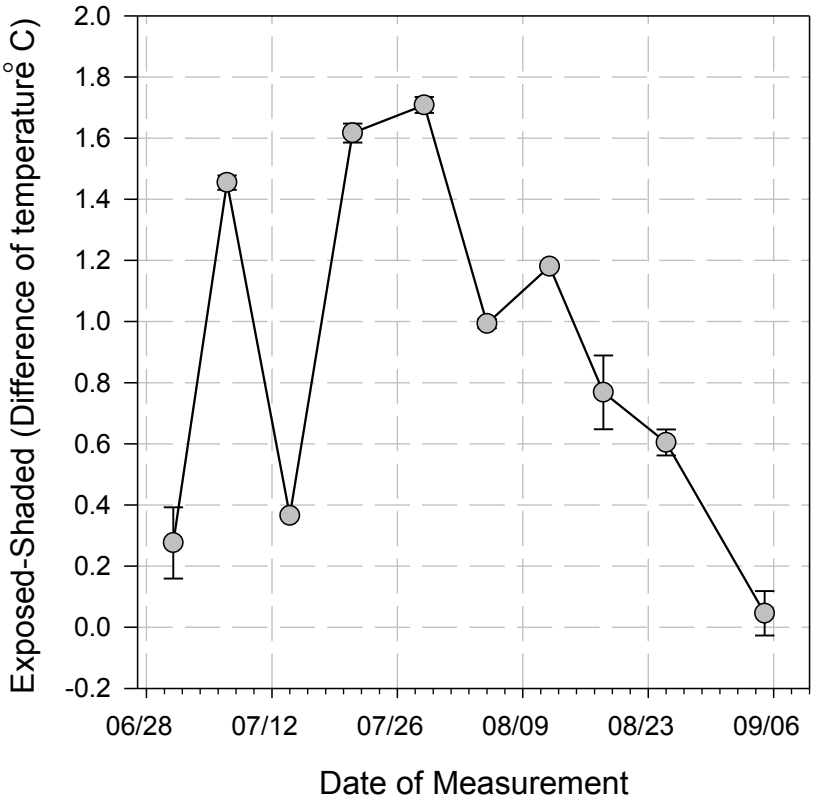

Figure 21. Temperature difference between exposed and shaded clusters during the season; weekly measurements were taken. Error bars represent the standard error of the mean (SEM).

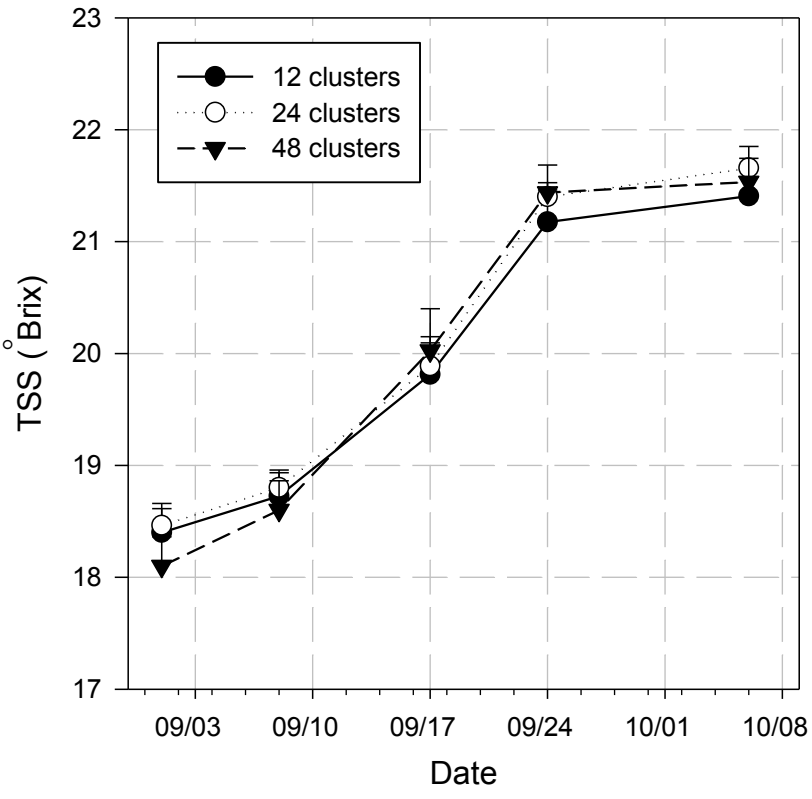

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Figure 22. Ripening curve (indexed as °Brix) during the season from the three levels of cluster thinning. Treatments from fruit exposure and four different times were pooled.

Table 2. Dates of anthesis, fruit set, veraison, and harvest and corresponding growing degree days (GDD). DOY is given in parentheses following each calendar date.

Year	Anthesis	Fruit set	Veraison	Harvest
2010	June 12 (163)	June 30 (181)	August 18 (230)	October 6 (279)
2010	428 GDD	633 GDD	1306 GDD	1708 GDD

Table 3. Differences in canopy density due to basal leaf removal and leaf tucking treatment using the PQA analysis; LLN was the abbreviation of leaf layer number.

	Leaf removal			Leaf tucking		
Crop level	LLN	% canopy gaps	% exterior clusters	LLN	% canopy gaps	% exterior clusters
12 clusters	0.54	48%	100%	2.6	2%	91%
24 clusters	0.09	51%	91%	2.3	6%	62%
48 clusters	0.8	20%	83%	1.9	5%	78%

Table 4. The effect of timing of cluster thinning on yield when the basal leaf tucking was applied.

Leaf tucking	Yield (kg/vine)			
Treatment	Fruit-set	Pre-veraison	Veraison	Post-veraison
12 clusters	1.25 a[x]	1.35 a	1.65 a	1.65 a
24 clusters	2.9 b	1.75 a	2.3 a	2.15 a
48 clusters	2.6 b	3.3 b	3.65 b	4.95 b

[x]Means within columns not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 5. The effect of timing of cluster thinning on yield when the basal leaf tucking was applied.

Leaf tucking	Yield (kg/vine)			
Treatment	Fruit-set	Pre-veraison	Veraison	Post-veraison
12 clusters	1.25 a[x]	1.35 a	1.65 a	1.65 a
24 clusters	2.9 a	1.75 a	2.3 a	2.15 a
48 clusters	2.6 a	3.3 a	3.65 ab	4.95 b

[x]Means within rows not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 6. The effect of timing of cluster thinning on yield when the basal leaf removal was applied.

Leaf removal	Yield (kg/vine)			
Treatment	Fruit-set	Pre-veraison	Veraison	Post-veraison
12 clusters	1.4 a[x]	1.6 a	1.3 a	1.3 a
24 clusters	1.65 a	2.1 ab	3.2 b	2.05 a
48 clusters	4.3 b	3.2 b	5.1 c	3.4 b

[x]Means within columns not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 7. The effect of timing of cluster thinning on yield when the basal leaf removal was applied.

Leaf removal	Yield (kg/vine)			
Treatment	Fruit set	Pre-veraison	Veraison	Post-veraison
12 clusters	1.4 a[x]	1.6 a	1.3 a	1.3 a
24 clusters	1.65 a	2.1 a	3.2 a	2.05 a
48 clusters	4.3 ab	3.2 a	5.1 b	3.4 a

[x]Means within rows not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 8. The effect of timing of cluster thinning on cluster weight when basal leaf tucking was applied.

Leaf tucking	Cluster weight (g)			
Treatment	Fruit-set	Pre-veraison	Veraison	Post-veraison
12 clusters	93 a[x]	104 a	132 a	109 a
24 clusters	116 a	61 b	96 ab	80 a
48 clusters	56 b	70 b	78 b	86 a

[x]Means within columns not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 9. The effect of timing of cluster thinning on cluster weight when basal leaf tucking was applied.

Leaf tucking	Cluster weight (g)			
Treatment	Fruit set	Pre-veraison	Veraison	Post-veraison
12 clusters	93 a[x]	104 a	132 a	109 a
24 clusters	116 a	61 b	96 ab	80 ab
48 clusters	56 a	70 a	78 a	86 a

[x]Means within rows not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 10. The effect of timing of cluster thinning on cluster weight when basal leaf removal was applied.

Leaf removal	Cluster weight (g)			
Treatment	Fruit set	Pre-veraison	Veraison	Post-veraison
12 clusters	106 a[x]	111 a	105 a	104 a
24 clusters	63 b	71 b	113 ab	88 a
48 clusters	99 a	67 b	103 b	70 a

[x]Means within columns not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 11. The effect of timing of cluster thinning on cluster weight when basal leaf removal was applied.

Leaf removal	Cluster weight (g)			
Treatment	Fruit set	Pre-veraison	Veraison	Post-veraison
12 clusters	106 a[x]	111 a	105 a	104 a
24 clusters	63 b	71 b	113 a	88 a
48 clusters	99 a	67 a	103 a	70 a

[x]Means within rows not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 12. The effect of timing of basal leaf removal and leaf tucking on °Brix.

Treatment	TSS (°Brix)			
	Fruit set	Pre-veraison	Veraison	Post-veraison
Leaf tucking	20.7 a[x]	21.8 a	22.1 a	21.3 a
Leaf removal	21.3 a	21.5 a	21.2 a	22.3 b

[x]Means within columns not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 13. The effect of timing of basal leaf removal and leaf tucking on °Brix.

Treatment	TSS (°Brix)			
	Fruit set	Pre-veraison	Veraison	Post-veraison
Leaf tucking	20.7 b[x]	21.8 ab	22.1 a	21.3 ab
Leaf removal	21.3 a	21.5 ab	21.2 a	22.3 b

[x]Means within rows not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 14. Summary of yield components and fruit chemistry in 2010 harvest based on timing. Data were pooled from three levels of cluster thinning, also pooled from basal leaf tucking and leaf removal.

Yield components and fruit chemistry	Timing treatment			
	Fruit set	Pre-veraison	Veraison	Post-veraison
Yield (kg/vine)	2.35 a[x]	2.22 a	2.87 a	2.58 a
Pruning weight (kg)	1.78 ab	1.43 bc	1.84 a	1.32 c
Ravaz Index	1.58 a	1.58 a	1.76 a	2.20 a
Cluster weight (g)	89 a	81 a	104 a	89 a
TSS (°Brix)	21.0 a	21.7 a	21.7 a	21.8 a
pH	3.8 a	3.8 a	3.9 a	3.8 a
TA (g/L)	4.9 a	5.2 a	4.7 a	5.4 a
Anthocyanins (mg/g)	0.72 a	0.66 a	0.62 a	0.70 a
Phenolics (au/g)	1.06 a	0.90 a	0.89 a	0.96 a

[x]Means within rows not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 15. Summary of yield components and fruit chemistry in 2010 harvest based on cluster thinning. Data were pooled from four different timings when the treatments were applied. Also, data were pooled from the basal leaf tucking and leaf removal.

Yield components and fruit chemistry	Cluster thinning treatment		
	12 clusters	24 clusters	48 clusters
Yield/vine (kg)	1.44 a[x]	2.26 b	3.81 c
Pruning weight (kg)	1.81 a	1.43 b	1.54 b
Ravaz Index	0.85 a	1.85 b	2.64 c
Cluster weight (g)	108 a	86 b	79 b
TSS (°Brix)	21.4 a	21.7 a	21.5 a
pH	3.8 a	3.8 a	3.8 a
TA (g/L)	4.9 a	5.2 a	5.1 a
Anthocyanins (mg/g)	0.68 a	0.65 a	0.69 a
Phenolics (au/g)	0.97 a	0.93 a	0.96 a

[x]Means within rows not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 16. Summary of yield components and fruit chemistry in 2010 harvest based on leaf removal and leaf tucking. Data were pooled from four different timings when the treatments were applied. Also, data were pooled from different levels of cluster thinning.

Yield components and fruit chemistry	Fruit exposure treatment	
	Leaf removal	Leaf tucking
Yield (kg/vine)	2.55 a[x]	2.46 a
Pruning weight (kg/vine)	1.52 a	1.67 a
Ravaz Index	1.81 a	1.75 a
Cluster weight (g)	92 a	90 a
TSS (°Brix)	21.6 a	21.5 a
pH	3.8 a	3.8 a
TA (g/L)	5.1 a	5.1 a
Anthocyanins (mg/g)	0.67 a	0.67 a
Phenolics (au/g)	0.96 a	0.95 a

[x]Means within rows not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 17. Photosynthetic parameters at veraison. Photosynthesis measurements were taken on basal leaves from non-treated vines, apical and tucked basal leaves from treated vines.

	Assimilation ($\mu molCO_2 \cdot m^{-2} \cdot s^{-1}$)	SD	Gs ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	SD	Ci ($\mu molCO_2 \cdot m^{-2} \cdot s^{-1}$)	SD
Tucked basal leaves	1.9	0.26	196.81	50.8	332.15	85.8
Basal Leaves	7.75	0.59	166.07	42.9	259.73	67.1
Apical Leaves	7.46	0.49	157.6	40.7	273.73	70.7

APPENDIX B: TYPE 3 TABLES OF ANOVA

Table 18. Analyses of variance (ANOVA) of yield in 2010.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	3	3	0.8	0.5722
crop	2	20	58.62	<.0001
time*crop	6	20	0.94	0.49
leaf	1	20	0.25	0.6197
time*leaf	3	20	2.33	0.1055
leaf*crop	2	20	0.61	0.5515
time*leaf*crop	6	20	2.89	0.034

Table 19. Analyses of variance (ANOVA) of pruning weight in 2010.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	3	13.3	4.72	0.0188
crop	2	16.1	3.32	0.0623
time*crop	6	13.3	1.6	0.2224
leaf	1	16.1	1.5	0.2384
time*leaf	3	13.3	2.39	0.1148
leaf*crop	2	16.1	0.87	0.4383
time*leaf*crop	6	13.3	1.41	0.281

Table 20. Analyses of variance (ANOVA) of Ravaz Index in 2010.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	3	3	0.82	0.5615
crop	2	20	36.49	<.0001
time*crop	6	20	1.08	0.4095
leaf	1	20	0.2	0.656
time*leaf	3	20	2.18	0.1223
leaf*crop	2	20	1.14	0.3396
time*leaf*crop	6	20	1.24	0.3295

Table 21. Analyses of variance (ANOVA) of cluster weight in 2010.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	3	3	1.37	0.4012
crop	2	20	11.39	0.0005
time*crop	6	20	0.6	0.73
leaf	1	20	0.09	0.7618
time*leaf	3	20	0.19	0.8997
crop*leaf	2	20	1.05	0.3669
time*crop*leaf	6	20	3.11	0.0256

Table 22. Analyses of variance (ANOVA) of ˚Brix in 2010 fruit chemistry.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	3	3	1.7	0.3364
crop	2	20	0.38	0.6919
time*crop	6	20	0.94	0.4856
leaf	1	20	0.17	0.6886
time*leaf	3	20	3.51	0.0343
leaf*crop	2	20	0.41	0.6666
time*leaf*crop	6	20	0.18	0.9791

Table 23. Analyses of variance (ANOVA) of pH in 2010 fruit chemistry.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	3	3	1.34	0.4085
crop	2	20	1.54	0.2391
time*crop	6	20	1.16	0.3676
leaf	1	20	1.44	0.2444
time*leaf	3	20	0.38	0.765
leaf*crop	2	20	0.43	0.6549
time*leaf*crop	6	20	1.92	0.1277

Table 24. Analyses of variance (ANOVA) of TA (TA) in 2010 fruit chemistry.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	3	3	0.51	0.7028
crop	2	20	0.89	0.4258
time*crop	6	20	0.76	0.6098
leaf	1	20	0.01	0.9431
time*leaf	3	20	0.69	0.5715
leaf*crop	2	20	2.31	0.1247
time*leaf*crop	6	20	1.92	0.1267

Table 25. Analyses of variance (ANOVA) of anthocyanins in 2010 fruit chemistry.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	3	3	1.98	0.2938
crop	2	20	0.29	0.7525
time*crop	6	20	0.88	0.5244
leaf	1	20	0	0.9466
time*leaf	3	20	1.68	0.2023
leaf*crop	2	20	0.53	0.5953
time*leaf*crop	6	20	0.32	0.921

Table 26. Analyses of variance (ANOVA) of phenolics in 2010 fruit chemistry.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	3	3	2.13	0.2747
crop	2	20	0.67	0.521
time*crop	6	20	1.12	0.3871
leaf	1	20	0.09	0.7679
time*leaf	3	20	1.05	0.3929
leaf*crop	2	20	0.87	0.4354
time*leaf*crop	6	20	0.19	0.9752

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LITERATURE CITED

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CHAPTER III
EVALUATION OF THE IMPACTS OF CROP THINNING AND LEAF REMOVAL UPON VEGETATIVE GROWTH AND FRUIT CHEMISTRY OF CABERNET FRANC IN MICHIGAN IN 2011

Introduction

In 2010, we had four different timings set for treatment (fruit set, pre-veraison, veraison and post-veraison) during the season when we applied three levels of cluster thinning and two levels of fruit exposure. However, according to our results from 2010 and the literature (Keller et al. 2005, Bravdo et al. 1984, 1985), cluster thinning and fruit exposure after veraison does not have any significant influence on sugar accumulation or other components of fruit composition. In 2011, we chose to delete the cluster thinning and fruit exposure treatment after veraison. Thus, our design was reduced to only three different timings during the season.

Vine balance is a critical concept in determining the yield in order to reach the desired fruit quality at harvest. In 2010, we proposed to adjust the number of clusters per vine to increase the yield by increasing the number of clusters retained on vines. Three levels of cluster thinning per vine (12, 24, and 48 clusters retained per vine, respectively) gave us a crop load (fruit weight/pruning weight ratio) range from 0 to 3, which was much lower than the range of 5 to 10, generally considered to mark a balanced vine (Kliewer and Dokoozlian 2005, Bravdo et al. 1984, 1985). Our vines in 2010 were severely “undercropped”, which resulted in an excessively low fruit tonnage at harvest without improving fruit quality. Therefore, we moved to two levels of cluster thinning in 2011. The cluster number retained on vine in 2011 increased from 12 clusters

to 40 clusters per vine for the low crop level, and from 48 to 80 clusters per vine for the high crop level, respectively. In 2011, the crop levels of vines were adjusted to achieve the crop load ratio of 5 to 10, or leaf area/fruit weight ratio of 8 to 12 cm^2/g for Cabernet Franc grown in Michigan according to Kliewer and Dokoozlian (2005). This concept of crop load and leaf area/fruit weight ratio will help us to have a better understanding of the relationship between vine balance and fruit quality in the future, especially for a cool climate growing region.

Also, considering the time-consuming and labor-intensive work, we elected to omit basal leaf tucking from our experiment in 2011. Instead, we have basal leaf removal plus control (no basal leaf removal) along with cluster thinning treatments on the vines.

In addition, we wanted to make a more detailed evaluation of fruit flavor chemistry in 2011, focusing on the formation/accumulation of the important wine flavor compound 3-isobutyl-2-methoxypyrazine (IBMP). IBMP, one of a family of methoxypyrazines, has been found in grapes and wines, and is a powerful flavor compound typically described as vegetal, herbaceous and "bell pepper".

The research objectives in 2011 were to understand the impact of basal leaf removal and crop load on fruit composition and flavor profile development in Cabernet Franc. We evaluated whether: 1) different timings of cluster thinning and leaf removal during the season can influence fruit composition (Brix°, TA, anthocyanins and phenolics) and IBMP level, 2) basal leaf removal could affect the fruit composition and IBMP level, and 3) yield manipulation, through cluster thinning, during the season could impact the fruit composition and flavor IBMP level at harvest.

Materials and Methods

Plant Material

Vitis vinifera L. cv. Cabernet Franc vines (FPS 01), grafted on rootstock (3309 C), established in 1993 at the Michigan State University's Southwest Michigan Research and Extension Center (SWMREC), Benton Harbor, MI, were used for field experiments in 2010 and 2011. SWMREC is located about 7 miles east of Lake Michigan. The experimental vineyard consisted of 10 rows and 48 vines per row trained onto a vertical shoot positioned (VSP) trellis. The vines were planted in Spinks sandy loam soil, and spur pruned to about 48 nodes per vine during winter pruning. Vine spacing was 2.4 m (8 ft) and row spacing was 3 m (10 ft). Vines were trained with multiple trunks to ensure survival of winter injury. During the growing season, excessively growing shoots were hedged when the tips were one foot above the catch wire. Standard commercial pest-control practices were applied during the season based on scouting, experience and weather conditions (Wise et al 2007). Drip irrigation was utilized if needed and scheduled based on the soil-water balance approach (Allen et al. 1998) in using the crop coefficient values (Williams et al. 2005), these were derived from the average leaf area values of the vines. Grapevine evapotranspiration (ET_c) was used to estimate the water usage in the field for scheduling effective irrigation according to Williams et al. (2005). Monthly rainfall and cumulative growing degree days (GDD) during the growing season were obtained from the Michigan Automated Weather Network at SWMREC. Additional weather data details and parameters can be accessed at http://www.agweather.geo.msu.edu/mawn/station.asp.

Field Experimental Design and Treatments

The experimental design was a split-plot design with main and sub-factors (Figure 23). Individual vines were organized in 12 blocks of 4 vines each for a total of 48 vines. The main factor was the timing of cluster thinning and leaf removal. Each four blocks of vines were randomly designated to one of the following timings, fruit set (two weeks after bloom, June 30th, 482 GDD), three weeks before veraison (August 4th, 961 GDD) and veraison (August 25th, 1183 GDD). The different timings have been chosen to evaluate the optimum timing of cluster thinning and leaf removal to improve the fruit compositions, especially decreasing the IBMP. Each block consisted of a 2×2 factorial design. Sub-factors are two levels of cluster thinning (40 and 80 clusters retained per vine) and two levels of cluster exposure (basal leaf removal and no leaf removal). All the vines were uniformly trimmed to approximately 90 clusters per vine before fruit set. Apical clusters were removed first when cluster thinning was performed manually due to the inconsistency of maturity of apical clusters. Two levels of sunlight exposure to clusters at the cluster-zone were provided by basal leaf removal (LR) from 6 basal nodes and control, in which no leaf removal was applied (Figure 24).

Canopy Growth Measurement

Total shoot number per vine was counted at bloom. Shoot length was monitored weekly from June 3rd until hedging (before veraison, July 20th). Five average representative model shoots per vine were selected and tagged based on vine vigor with average shoot growth, neither too vigorous nor too weak. Shoot lengths were recorded. After hedging shoot diameters, instead of shoot lengths, were measured for the same five model shoots. Total leaf area was estimated

based on the regression between shoot length and shoot leaf area using no-destructive method. First, the regression was built according to Mabrouk and Carbonneau (1996). Twenty shoots (neither excessively vigorous nor weak), were weekly sampled randomly from non-experimental vines from June 3rd to July 20th (before hedging), stored in an ice cooler, and transported to campus. In the lab, 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 leaf area and shoot length data taken from June 10th to July 21st, was used to describe the relationship between these variables (Figure 25), and estimate leaf area for the five tagged shoots from each experimental vine using the weekly shoot length measurement. Average leaf area from five tagged shoots was multiplied by the shoot number to obtain total leaf area per vine during the season. At three timings of basal leaf removal treatment during the season, we removed the six basal leaves from each shoot, but collected the six basal leaves from each tagged shoot, placed them in sampling bags, stored them appropriately, and measured the area of those leaves using a leaf area meter in order to estimate the percentage of removed leaf area (Table 27). Daily shoot growth rate was calculated and used to represent the canopy growth. Before the hedging, the increased shoot length per day (cm/day) was calculated, and after hedging the increased shoot diameter per day (mm/day) was calculated, rather than shoot length, to represent the shoot growth.

Canopy Density Assessment in 2011

Canopy density was assessed at veraison. Canopy density index was assessed using point quadrat analysis (PQA) according to Smart and Robinson (Smart and Robinson, 2008). Generally, a guiding board (2 m length) was horizontally fixed along the cluster-zone, and a thin

metal rod (1 m long and 2 mm diameter) is inserted into the canopy at regular intervals (5 cm) marked along the board. Sequential contacts with leaves and other vine parts from one side of the canopy to the other are recorded. L is used for leaf, C is used for cluster, and G is used for canopy gap. The contact with the shoot is normally ignored. Fifty or more insertions are made for each canopy. The calculation for canopy density based on PQA is percentage of gaps, number of leaf layers (LLN), and percentage of exterior leaves and clusters. Percentage of gaps is the total number of gaps G divided by number of insertions, then multiplied by 100 to obtain a percentage. LLN is the total number of leaf contacts L divided by the number of insertions. Percent exterior leaves and percent of exterior clusters are the leaves and clusters, which the rod touches first and last during each insertion (either surface of the canopy), divided by the total number of leaves and clusters.

Daily Cluster Temperature and Radiation Measurement in 2011 Year's Experiment

Light intensity on both east and west sides of the canopy was measured using photosynthetically active radiation (PAR) sensors (model SQ-110; Apogee Instruments, Logan, UT) positioned at fruit-zone level inside the canopy. For monitoring the cluster temperature and light intensity, three represented vines in basal leaf removal and control groups were chosen. For cluster diurnal light intensity, two PAR sensors were positioned horizontally close to the cluster-zone on both east and west sides of the canopy for the chosen vines. Daily cluster temperature was measured based on the skin temperature of selected berries. Fine-wire (American Wire Gauge [AWG]) thermocouples (Type T [copper-constantan]) were attached to the skin of the selected berries to measure the skin temperature. Those berries were selected from near the cluster shoulder and midway along its vertical axis. One berry of each individual cluster was

chosen to estimate the average cluster temperature. Two represented clusters were measured on both east and west sides of the canopy. The same three represented vines, which were chosen for monitoring the cluster light intensity, were selected. 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, Logan, UT). Ambient air temperature at cluster level was also monitored by shielded, aspirated, fine-wire thermocouples (AWG; type T) placed at fruit-zone right close to the canopy. All signals were scanned at 20-sec intervals and the data was recorded once every 20 min. All the data was collected continuously from July 26th to harvest. Mean diurnal PAR (0500 to 2100 hr) and temperature (00 to 24 hr) pattern were calculated based on average 20 min value from the data of the whole season.

Sampling Procedures and Harvest Data Collection

From fruit set through harvest, thirty berries from the sample pool of all experimental vines were randomly collected on a biweekly basis, immediately frozen in liquid nitrogen, transported to campus, and stored at -60°C for the future volatile analysis. At the beginning of veraison an additional twenty berries from each vine were sampled randomly from clusters on non-tagged shoots to track fruit maturation until harvest. At harvest, total crop weight and total cluster number were recorded. Clusters from the tagged shoots from each vine were harvested and immediately put in the ice coolers, transported to campus, and stored in -20°C. Then each cluster was weighed in the lab to get the single cluster weight. Total berry numbers from each cluster were counted and total berry weight was obtained to calculate the average berry weight. °Brix, pH, TA, anthocyanin, and total phenolics were measured from the clusters collected from

the tagged shoots. Pruning weight per vine was collected in the following winter to get the weight of pruned one-year old canes per vine.

Fruit Chemistry Measurements

Harvested frozen grapes were thawed at the room temperature before the analysis. After that, berries in the collection bag were crushed with a manual press, and free-run juice was decanted into 50 mL tubes. Juice soluble solids were measured using a digital Brix refractometer (ATA-3810 PAL-1 Pulse Inc. Van Nuys, CA). Titratable acidity and pH were measured in using a 370 Thermo Orion pH meter (Thermo Fisher Scientific Inc. Logan, UT). Titratable acidity (TA) was measured using Multi-T 2.2 digital titrator (Laboratory Synergy Inc. Goshen, NY). 10 mL clear juice per sample diluted with distilled water to 100 mL was titrated with 0.1M sodium hydroxide (NaOH) to a pH of 8.2 using an equation to yield the TA (g/L), according to Iland, et al. (2004).

TA (g/L as tartaric acid) = 75 × 0.1 (molarity of NaOH) × Titre volume (mL)/10 (volume of juice)

Anthocyanin and total phenolics were measured by the total phenol assay, using the Folin-Ciocalteu reagent (Singleton et al. 1999). One hundred berries stored at -30°C were partially thawed prior to grinding in a tissue homogenizer (Brinkmann Instruments, Westbury, NY) at a speed of four on the manufacturer's scale, for about 1 min. The process was conducted under cool conditions 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. 10 mL 50% v/v aqueous ethanol acidified to pH 2 (~1 mL 12.1M HCL) was added to the 1 g sample, and then it was mixed once per 5 min manually for 1 hour.

The sample was then centrifuged at 5000 rpm for 20 min. One mL of extract (supernatant liquid) was pipetted into a 15 mL centrifuge tube. Ten mL 1M HCL was added and the mixture was clear equilibrated for 3 hours, which is necessary because acidifying the solution is critical to ensure that acidic protons remain bonded with anthocyanins, and the correct absorbance values are attained. Eventually, the absorbance values were obtained using a UV-Vis spectrophotometer (Model UV-1800, Shimadzu Corporation, Kyoto, Japan) at 280, 520, and 700 nm. The absorbance values collected included 280 nm (total phenolics), 520 nm (anthocyanin content), and 700 nm (turbidity check).

Analysis of IBMP in berries

After fruit set, thirty berries per vine were randomly collected on a biweekly basis. Berries were frozen in liquid nitrogen immediately after harvest, and transported to campus, stored at -60°C. IBMP quantification in berries was according to the method of standard addition as described by Koch et al. 2010. Thirty-two grams of frozen whole berries were homogenized on ice which took approximately 1 min at a speed of four on the manufacturer's scale (Brinkmann Homogenizer; Brinkmann Instruments, Westbury, NY) with 10 mL of an aqueous 2 mM NaF solution containing 200 ng/L^{-1} of internal standard, 2-($^{2}D_3$)-methoxy-3-isobutylpyrazine (deuterated IBMP [dIBMP]; CDN Isotopes, Pointe-Claire, Quebec, Canada, 98% atom %D) in a 50 mL plastic centrifuge tube. The homogenate was then centrifuged (Sorvall Legend X1R Centrifuge, Thermo Scientific) at 5000 rpm for 5 min at 4°C. At least five replicates of 32 g each were prepared for each analysis.

Following centrifugation, an aliquot (10 mL) of the supernatant was transferred to each of two separate 25 mL amber glass vials, each with screwcap headspace, (Supelco, Bellefonte, PA)

containing 3.0 g of NaCl and fitted with a 20 mm mininert valve (Supelco, Bellefonte, PA). The vial was then closed tightly and allowed to equilibrate for at least 1 hr at 20°C before headspace solid-phase microextraction (SPME) coupled with gas chromatography (6890 series, Hewlett Packard Corporate, CA) -mass spectrometry (Pegassus III, LECO, MI) (GC-MS) analysis. Headspace extractions were performed using a 23 ga, 2cm divinylbenzene/Carboxen^{TM}/polydimethylsiloxane (DVB/CARB/PDMS) SPME fiber, which was conditioned and cleaned according to manufacturer's specifications. The SPME fiber was exposed to the headspace of each sample vial and the sample extracted for 30 min at 40°C with continuous agitation. The SPME fiber was then removed from the vial and placed into the GC-MS inlet (0.7 mm straight glass liner), held in the inlet at 260°C in splitless mode for 5 min for the analytes to desorb from the fiber, and finally the inlet flow was switched on at 50 mL min^{-1} with the fiber in the inlet for an additional 5 min to insure no carryover from previous sample.

An Omegawax^{TM} 250 fused silica capillary column (30 m length × 0.25 mm ID; 0.25 μm film thickness; Supelco) was used for separation. The oven temperature was maintained at a constant temperature of 40°C for 5 min, then increased 120°C min^{-1} to 80°C, 5°C min^{-1} to 110°C, and 25°C min^{-1} to 230°C before holding steady for 5 min. The MSD interface was held at 280°C and the carrier gas was He at a constant pressure of 4.77 psi with a nominal initial flow of 0.8mLmin^{-1}. Selected ions at mass channels of m/z = 94 and 124 for IBMP and m/z =127 and 154 for dIBMP were monitored. Peak areas of the ions m/z 124 and 127 were used for quantification and ions m/z 94 and 154 were used for identification.

External standard calibration and quantification by standard addition

Standard IBMP calibration samples were prepared in water to give concentrations of 0, 5, 10, 20, 40, 100, and 200 ng L^{-1}. The internal standard (IS), dIBMP (a concentration of 200 ng L^{-1}), was also added to each standard solution. A 10.0 mL aliquot of each standard was transferred into 25 mL amber headspace vial that contained NaCl (3 g). At least three aliquots of each standard were analyzed by headspace SPME-GC-MS as described above. Peak area ratios of IBMP and dIBMP were used to create linear calibration curves. The average regression equation for IBMP was: IBMP (ng L^{-1}) = 130.02 × (A/AIS)-4.6145 (R^2=0.9908) (A: area of IBMP peak and AIS: area of internal standard dIBMP peak)

Statistical Analysis

Analyses of variance (ANOVA) in 2011 were performed using the PROC MIXED procedure of SAS for Windows, version 9.2 (Cary, NC). Split block design and pair-wise comparisons were performed by LSD test at a significance level of 0.05 and 0.10.

Results

Climatic conditions

In terms of growing degree days (GDD), 2011 was an average year with 1700 GDD for the whole season compared to the past 10 years' average value of 1687 GDD (Figure 26). Due to a very late and cold spring, all the phenological stages (bloom, fruit set and veraison) have been delayed approximately a week (Table 28). Maximum air temperature increased to 35 °C during

June and July, but no severe sunburn damages of berries was observed. The minimum seasonal temperature reached 0 °C at the October 2nd, just prior to harvest.

PAR and temperature in canopy cluster-zone area

Compared to the control group in which no basal leaves were removed, basal leaf removal treatment increased the recorded temperatures of the cluster-zones by 2 to 3°C from 900 hr to 1400 hr on both east side and from 1400 hr to 1800 hr on west side (Figure 27). In the morning, the east side of the canopy for vines with basal leaf removal, indicated average 25°C as compared to 22°C for vines with no leaf removal. For the west side of the canopy in terms of vines with basal leaf removal, in the afternoon, the average temperature was 26°C as compared to 22.5°C for vines with no leaf removal. Consequently, the difference of temperature peak of cluster zone is due to the orientation of canopy to the solar light.

Basal leaf removal increased the PAR in the east side of the canopy to 750 μmol s^{-1} m^{-2} compared to below 50 μmol s^{-1} m^{-2} in the shaded canopy during the morning (Figure 28). During the afternoon, cluster light exposure increased to 750 μmol s^{-1} m^{-2} on the west side of the canopy with leaf removal compared to less than 50 μmol s^{-1} m^{-2} in the vines with no leaf removal.

Seasonal canopy vegetative growth

There was no difference in rate of shoot growth (expressed as shoot length cm/day) between cluster thinning (Figure 29) and leaf removal treatments (Figure 30). Similarly, cluster

thinning and basal leaf removal did not influence rate of shoot growth (expressed as shoot diameter mm/day) (Figure 31 and 32).

Using the linear regression between shoot length and shoot leaf area (Figure 25), we calculated the percentage in which the area of six removed basal leaves accounted for total shoot leaf area of each tagged shoot. From the result of Table 28 approximately 28% to 35% of the total leaf area was removed with each basal leaf removal treatment. The timing of basal leaf removal has no effect on the percentage of removed leaf area. The total leaf area of Cabernet Franc vine in Michigan, at fruit set, had already reached 80% of the total leaf area before the vines were hedged. A reasonable interpretation of this result could be that the canopy of Cabernet Franc vines in Michigan grew quickly at the start of the season; a phenomenon also seen in other cool climate growing regions, likely due to the vine response to the short growing season.

There was a large difference between the basal leaf removal treatment and the control in terms of the number of leaf layers (LLN), percentage of gaps, and, more importantly, percentage of exterior clusters (Table 29). The percentage of exterior clusters increased up to 94% for vines with basal leaf removal; Vines with no basal leaf removal had only 26% exterior clusters. This increase of exterior clusters was beneficial to improve cluster-zone microclimate, especially in cool climate. As shown in Figure 27 and 28, exposed clusters had higher cluster temperature and light interception, although the increase depended upon the orientation of canopy. As discussed earlier, basal leaf removal clearly influences cluster microclimate.

Yield components and basic fruit composition at harvest

No effect of the timing of cluster thinning and basal leaf removal on yield components has been found (Tables 34).

None of the yield components and fruit compositions was significantly impacted by the two levels of cluster thinning (Table 35). No interaction between cluster thinning and time on yield components was observed either. However, it is noteworthy that the Ravaz Index (crop load ratio) values ranged from 5 to 11 in the 2011 results meaning that vines with two levels of cluster thinning were still generally in balance, a desirable outcome. This indicated even a double amount of crop on vine (from 40 clusters to 80 clusters) can still have no difficulties in reaching the desired fruit quality at harvest when the crop load or leaf area/fruit weight ratio is under the optimum range.

Basal leaf removal had no effect on yield components (Table 36), nor did it affect most parameters of fruit composition, however, a significant reduction of TA was found in leaf removal treatment in comparison with no leaf removal. However, no effect due to the interaction between cluster thinning and time of leaf removal was found.

Also, the timing of cluster thinning and leaf removal had no measurable effect on yield components or fruit composition (Table 34). The sole interaction effect found was between berry weight (Table 30 and 31) and anthocyanins (Tables 32 and 33). A heavier berry has been found when basal leaf removal was applied at veraison. As for the interaction effect of cluster thinning and leaf removal on anthocyanins, both contributed to an increase of the total amount of anthocyanins in the berries, as well as a higher efficiency of accumulation or the ratio of anthocyanins divided by sugars (Figure 33).

Sugar (°Brix) accumulation was delayed by less severe cluster thinning (80 clusters per vine) compared to severe cluster thinning (40 clusters per vine). However, it is noticeable that no difference was found in final sugar contents at harvest (Figure 34).

Correlations between sugar (°Brix) and anthocyanins, as well as total phenolics were found in 2011 (Figure 35). Higher sugar accumulation of °Brix occurred simultaneously with an increase of both anthocyanins and total phenolics.

Uniformity of fruit compositions

Besides measuring the average values of basic fruit composition, we were also interested in assessing the variation in fruit compositions due to the treatments. Since we measured basic fruit chemistry using the basal clusters from five tagged shoots per vine, we could calculate the variation in fruit chemistry based on each vine to test the difference of variation among treatments.

Severe cluster thinning (40 clusters per vine) at different times during the season did not change the coefficient of variation (CV) compared to less severe cluster thinning (80 clusters per vine) (Table 37). However, in comparison with the CV of sugar (around 4), the anthocyanins and total phenolics tended to have higher coefficients of variation (from 15 to 20). This indicates that, even if the fruit had very uniform sugar content, it still did not necessarily mean anthocyanins and total phenolics would follow suit. When considering CV based on leaf removal at different seasonal times (Table 38), we saw there was a significant difference in the variation of both sugars and anthocyanins. Generally, leaf removal at fruit set was beneficial to producing a lower variation compared to no leaf removal for sugar and anthocyanin contents, but not TA or total phenolics. Regression was generated with the CVs of sugar and anthocyanins plotted

against the GDD at different timings when leaf removal treatment was applied during the season (Figure 36). The earlier the basal leaf removal was applied, the more uniform was the result for fruit composition at harvest.

IBMP contents at harvest

Basal leaf removal caused differences in IBMP content (Table 39). Leaf removal reduced the IBMP by 35% compared to fruit from vines without the leaf removal treatment. However, the time of leaf removal did not impact the IBMP concentration at harvest (Table 40) and, furthermore, no interaction was seen between leaf removal and cluster thinning at harvest either.

Differences in quantity of IBMP contents were found (Table 41), based on severity of cluster thinning treatment. Generally, severe cluster thinning decreased IBMP concentration by 17% in comparison with less severe cluster thinning. However, there was no significant effect of the time at which cluster thinning was applied on IBMP at harvest (Table 42) and, again, no interaction was found.

Discussion

The data for sugar accumulation of °Brix demonstrated that high-cropped vines (double amount of yield) are capable of adjusting carbon allocation to permit attaining the same level of sugar as the fruit of vines with a lower yield. Similarly, the lack of an effect of cluster thinning on other parameters of fruit composition (pH, titratable acidity, anthocyanins and phenolics) could be explained by the same fact of the capability of higher-cropped vines to reach the maturity of fruit at harvest. Previous studies in hot climate have indicated that yield did not affect the sugar accumulation of °Brix and other parameters of fruit composition when the crop load or

leaf area/fruit weight ratio was among the optimum range (crop load ratio of 5 to 10 and leaf area/fruit weight ratio of 8 to 12 cm^2/g) (Bravdo et al. 1984, 1985, Petrie and Clingeleffer 2006). In additions, the optimum ratios of crop load and leaf area/fruit weight could also explain the lack of an effect of cluster thinning on shoot growth rate and vine size (pruning weight).

Although basal leaf removal at different timings of the season did not influence canopy growth, basal leaf removal did decrease the canopy density by 500% and increased the percent of exterior clusters by 260% (Table 29). These results are likely related to the improvement of cluster-zone microclimate with higher light interception and increased cluster temperature at the fruit-zone (Figure 27 and 28). The impact of leaf removal on fruit composition, specifically on the basic fruit chemistry, was not significant with one exception: leaf removal resulted in lower fruit titratable acidity (TA). Increased cluster temperature caused by leaf removal around the fruit-zone likely accelerated the degradation of malic acid through an enhancement in respiration; however, it is known that elevated temperature does not affect the tartaric acid and citric acid levels (Lakso and Kliewer 1975, 1977). In fact, malate and tartrate are main two organic acids in grapes which account for 70% to 90% of total organic acids (Jackson 2008, Kliewer 1966, and Ruffner 1982), and compared to tartaric acid, the respiration of malic acid caused the significant decline of titratable acidity in berries after veraison. Additionally, Smith et al. (1988) observed that basal leaf removal is associated with a reduction in potassium uptake.

Higher cluster temperatures, induced by leaf removal, have proved more effective than sunlight to the biosynthesis of anthocyanins (Downey and Dokoozlian et al. 2006). Increased temperature will increase the rate of metabolic processes in the fruit and this will lead to an associated increase in the primary metabolism. In grapevines, 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 (Downey and Dokoozlian et al. 2006). Generally, leaf removal exposed the cluster to significantly increase the cluster temperature in comparison with cluster under the shade. Moreover, the effect of cluster thinning on the accumulation of anthocyanins might be due to the reducing the source : sink ratio in flavor of production of anthocyanins and other secondary metabolites.

The content of sugar, anthocyanins and total phenolics was positively correlated with (Figure 35), although the R-squared values (0.54 for anthocyanins and 0.65 for total phenolics) in both correlations are relatively low. In the grape and wine industry, sugar (°Brix) is used as a standard index to evaluate the maturity of fruit at harvest, so it is imperative to know that the measured sugar level is accurate enough to reliably evaluate the overall maturity of the fruit. Our data suggests that a higher sugar accumulation indicates more mature fruit based on higher amounts of anthocyanins and total phenolics. However, anthocyanins and total phenolics concentration can be impacted by many environmental conditions, e.g. sunlight, temperature, or water stress (Downey and Dokoozlian, et al. 2006). The impact on anthocyanins and total phenolics contents is more complicated than just a linear relationship with sugar.

A higher efficiency of anthocyanins accumulation (a higher ratio of anthocyanins/sugar) has been found in vines treated with basal leaf removal and cluster thinning which might be explained by reducing the source : sink ratio and increasing cluster temperature. Previous studies have also indicated not excessively high temperature (between 30 °C and 35 °C, and depends on varieties) promoted the anthocyanins accumulation in berries in hot climate (Spayd et al. 2002, Price et al. 1995). However, except the investigations on individual compound, not a lot of studies have focused on the correlation of these two and the efficiency of anthocyanins accumulation as sugar increased. Also, all these experiments have been done in a hot climate,

and not a lot of information in cool climate. Recently, Sadras and Moran (2012) has reported that elevated temperature (usually above 35 °C) decoupled the anthocyanins and sugars which resulted in a lower ratio of anthocyanins/sugar (mg/g °Brix) in hot climate, and no consistent effect of changing source : sink ratio (cluster thinning) on the ratio of anthocyanins/sugar has been found. In our study, in comparison with the cool temperature in hot climate, a higher temperature (maximum temperature less than 30 °C) (Figure 27) caused by basal leaf removal was actually beneficial for the accumulation of anthocyanins, and an increased ratio of anthocyanins/sugar could be due to the increased cluster temperature when basal leaf removal was applied. In additions, no consistent effects of reducing source : sink ratio (cluster thinning) on the accumulation of anthocyanins and ratio of anthocyanins/sugar in hot climate might be due to a less limitation of photosynthetic productivity of leaf area (Guidoni et al. 2008). However, in cool climate, carbon partitioning from relative deficiency of photosynthetic productivity of leaf area had a more significant impact on the accumulation of anthothocynins. Therefore, that could be explained that in our study reducing source : sink ratio (cluster thinning) did not enhanced the effect on the accumulation of anthocyanins, but on the ratio of anthocyanins/sugar as well.

Uniformity of fruit composition was also improved when basal leaf removal was implemented early in the season. Specifically, the coefficient of variation for sugar and anthocyanins was strongly correlated with the time of leaf removal. As a result, we can conclude two things: firstly, as uniformity of cluster microclimate, in terms of sunlight, increases (measured as percent of exterior clusters in Table 29), so does the uniformity of fruit composition in the vineyard and, secondly, the earlier the basal leaf treatment is applied, the more uniform the fruit composition will be at harvest (Figure 36).

Our research results have shown that basal leaf removal did, in fact, reduce the IBMP concentration by 35% compared to no leaf removal. However, we did not find any significant difference related to the timing of cluster thinning or basal leaf removal on the final IBMP content. Ryona et al. (2008) and Koch et al. (2011) have reported that IBMP accumulation reaches a peak two weeks before veraison, and then declines throughout development, even during berry maturation and ripening. It has been found that exposure to light after veraison did not impact the rate of IBMP degradation. Increasing the light exposure at any point prior to the peak (2 weeks before veraison) is likely to advance the arrival date of the peak value and also reduce the peak value resulting in a lower level of IBMP at harvest (Koch et al., 2011). We applied the leaf removal treatment to the vines at fruit set, at 3 weeks before veraison, and at veraison, and the lack of an effect of timing of basal leaf removal on IBMP content might be due to the different cultivars and climate.

Chapman et al. (2004) have reported that IBMP concentrations were significantly negatively correlated with bud count per vine when winter pruning was implemented. A lower number of buds resulted in higher concentration of IBMP in the fruit, and a higher number of buds led to a lower amount of IBMP in the fruit. This result was believed to be related to vine vigor. A lower number of buds per vine results in less fruit per vine and, in return, more vigorous shoot growth. Therefore, winter pruning that leaves a higher number of buds produces more fruit and less vigor, which ultimately produces a lower amount of IBMP. In our experiment, we manipulated the crop level by dropping clusters at different times of the season. No difference in canopy size was found, nor in amount of IBMP.

Consequently, regarding fruit quality at harvest, our results indicate that leaf removal, rather than cluster thinning and timing, had more significant impact on fruit composition. We

also observed that the earlier that leaf removal was applied, fruit composition (sugar and anthocyanins) was increasingly uniform. Moreover, leaf removal reduced significant amounts of IBMP, while cluster thinning was less effective. In our study, IBMP content in harvest grapes generally from all the treatments ranged from 6 to 15 pg/g of FW, which was below or near the sensory threshold of IBMP in wine (Buttery et al. 1969).

APPENDICES

APPENDIX A: TABLES AND FIGURES

Crop Levels per Vine: 40 and 80 clusters per vine

Canopy management: Leaf removal and no leaf removal

Fruit set	Pre-veraison	Veraison
1 block	1 block	1 block
1 block	1 block	1 block
1 block	1 block	1 block
1 block	1 block	1 block

Figure 23. 2011's field experimental design; each block consist of 4 vines; each vine was designated for one treatment.

Figure 24. Basal leaf removal (top) and no leaf removal (bottom) in 2011. As for basal leaf removal vines, the first six leaves from the basal node of each shoot were defoliated to expose the clusters.

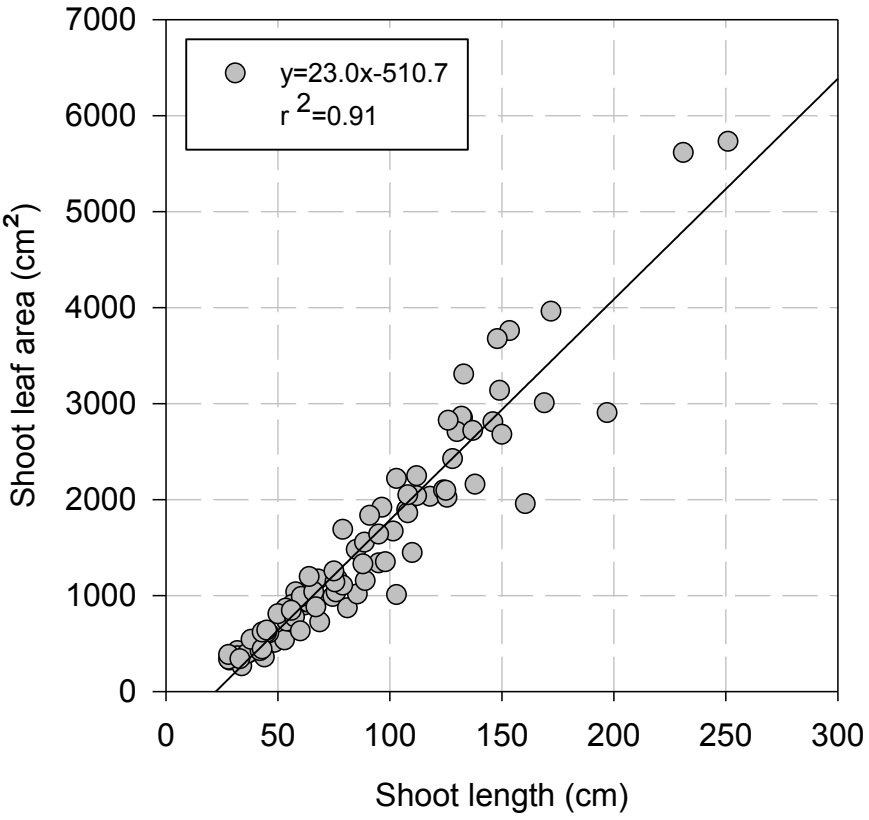

Figure 25. Linear regressions between shoot length and shoot leaf area in 2011.

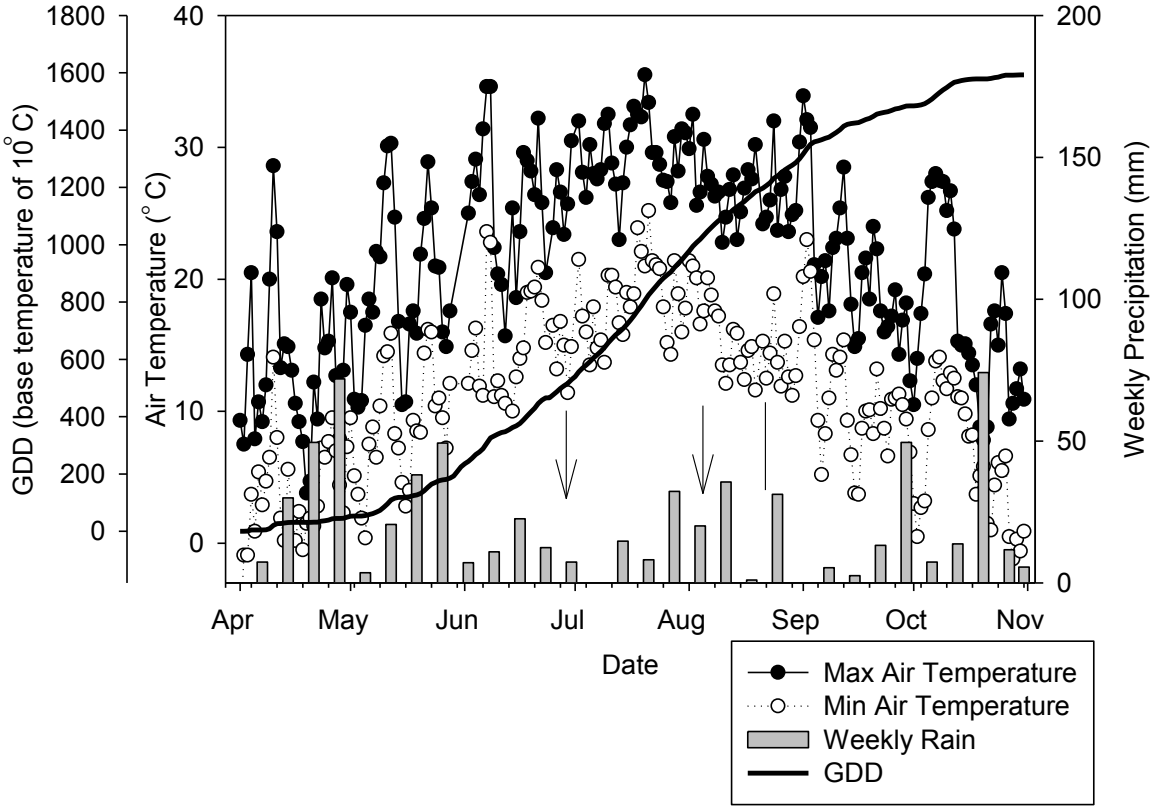

Figure 26. Growing degree days (GDD) and weekly precipitation for 2011 at the Southwest Michigan Research and Extension Center near Benton Harbor (SWMREC, MI). GDD was calculated at a base temperature of 10°C from April 1 through October 31. Daily maximum and minimum temperature of air was also obtained. Arrows indicated the four different timings of our treatments during the season.

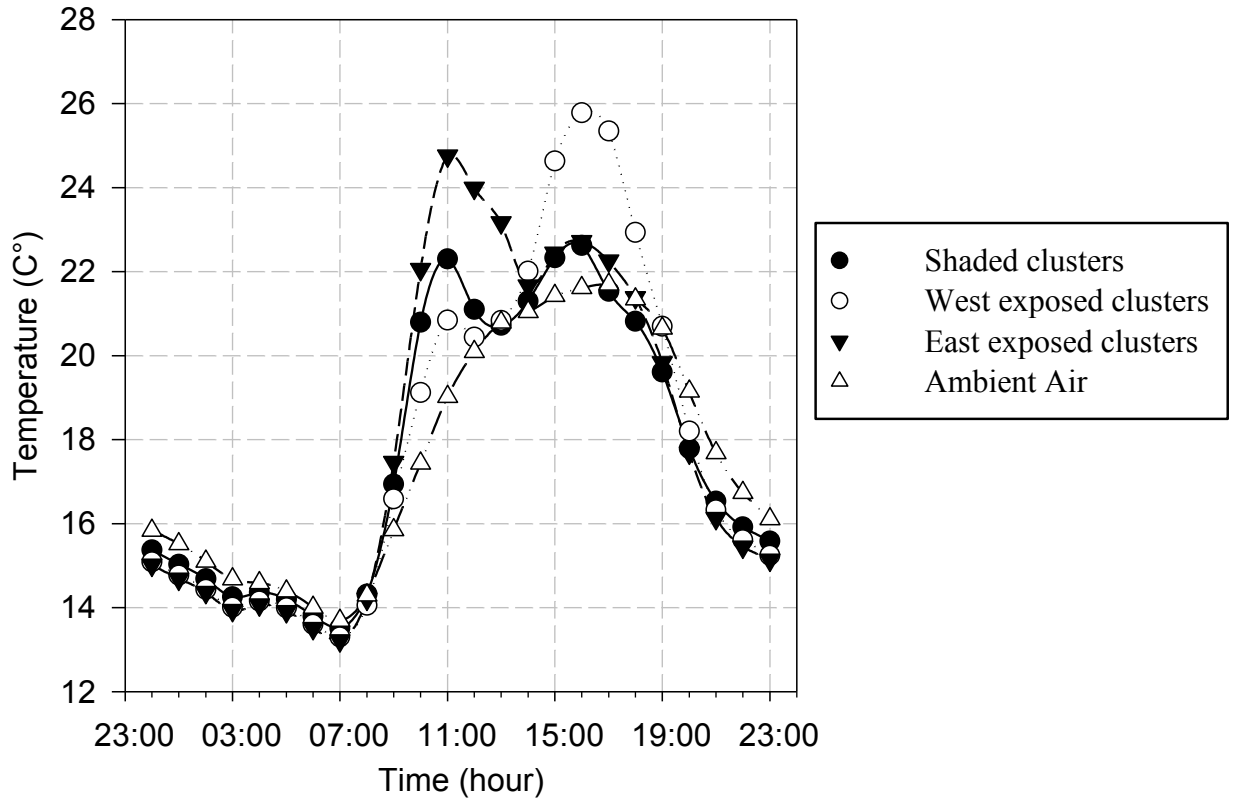

Figure 27. Daily average temperatures for different sides of the canopy based on basal leaf removal and no leaf removal from beginning of July to harvest.

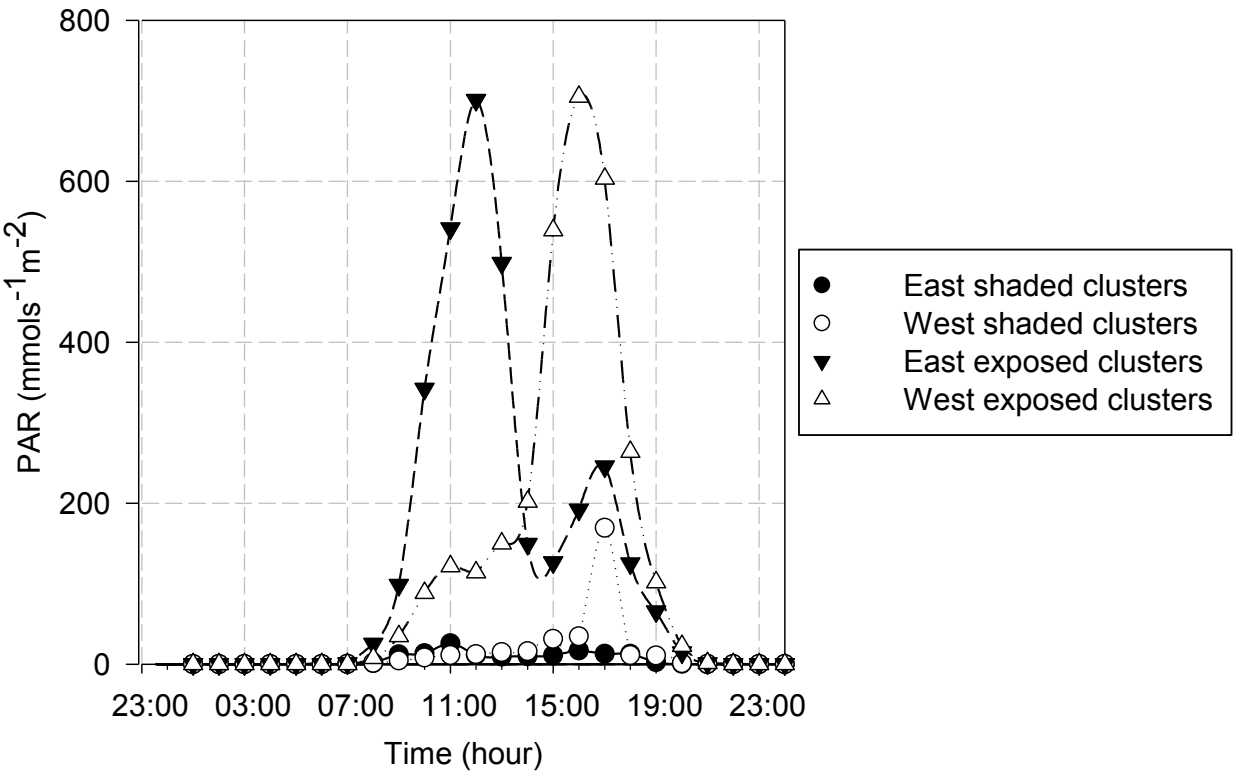

Figure 28. Daily average PAR for opposing sides of the canopy based on basal leaf removal and no leaf removal treatments from the beginning of July to harvest.

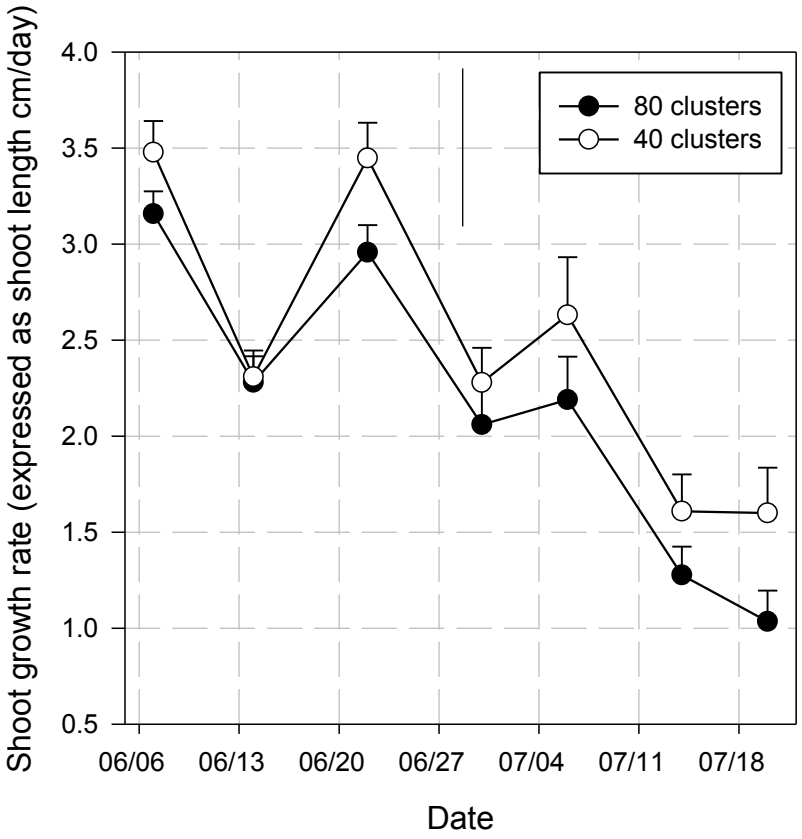

Figure 29. Shoot growth rate (expressed as shoot length cm/day) from the beginning of the season to late middle of July, the arrow indicated our first treatment of cluster thinning and leaf removal at fruit set. Each color of bars represented one of two levels of cluster thinning (40 and 80 clusters) with pooling of the data from leaf removal and control. Data presented in this figure were selected from the vines to which were applied the cluster thinning and leaf removal treatments at fruit set.

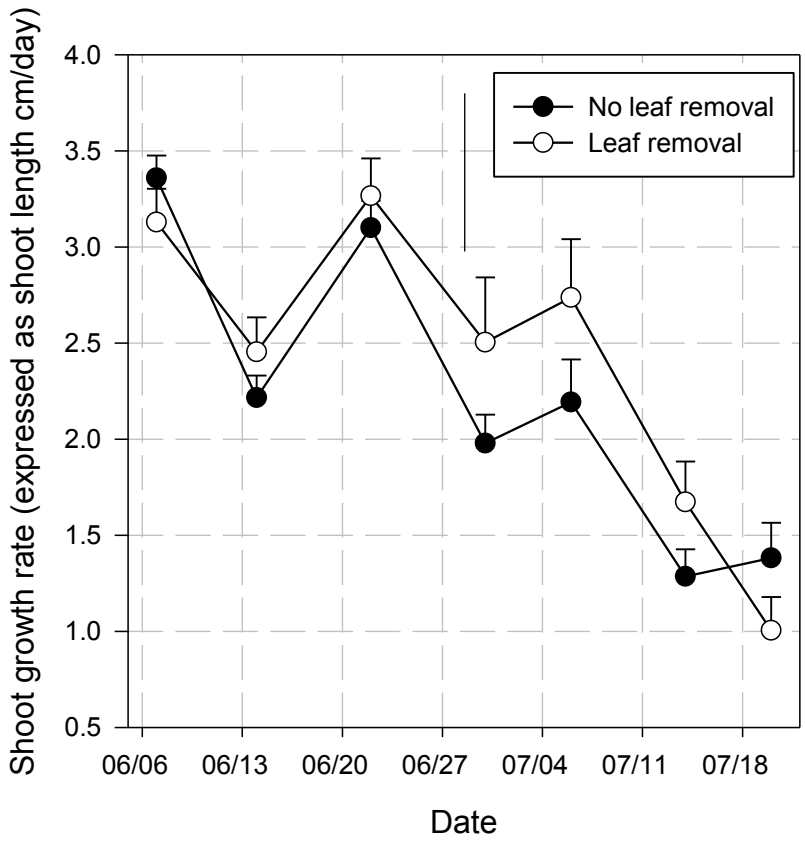

Figure 30. Shoot growth rate (expressed as shoot length cm/day) from the beginning of the season to late middle of July, the arrow indicated our first treatment of cluster thinning and leaf removal at fruit set. Each color of bars represented basal leaf removal or control with pooling of the data from two levels of cluster thinning. Data presented in this figure were selected from the vines to which were applied the cluster thinning and leaf removal treatments at fruit set.

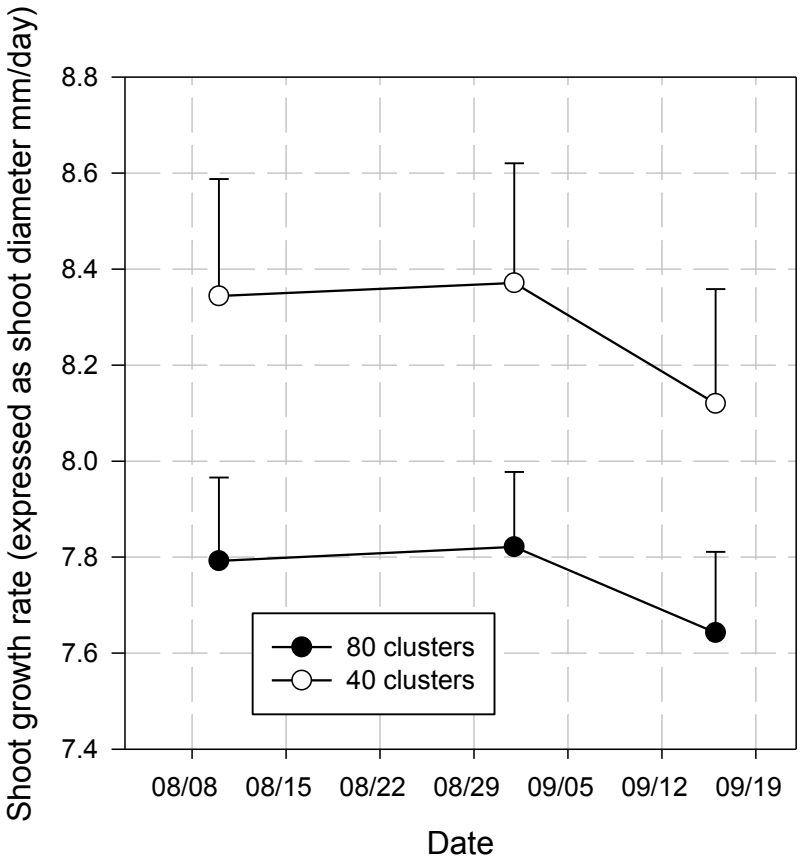

Figure 31. Shoot growth rate (expressed as shoot diameter mm/day) from beginning of August to end of September. Each color of bars represented one of two levels of cluster thinning (40 and 80 clusters per vine) with pooling of the data from leaf removal and no leaf removal. Data presented in this figure were selected from treatments done at fruit set.

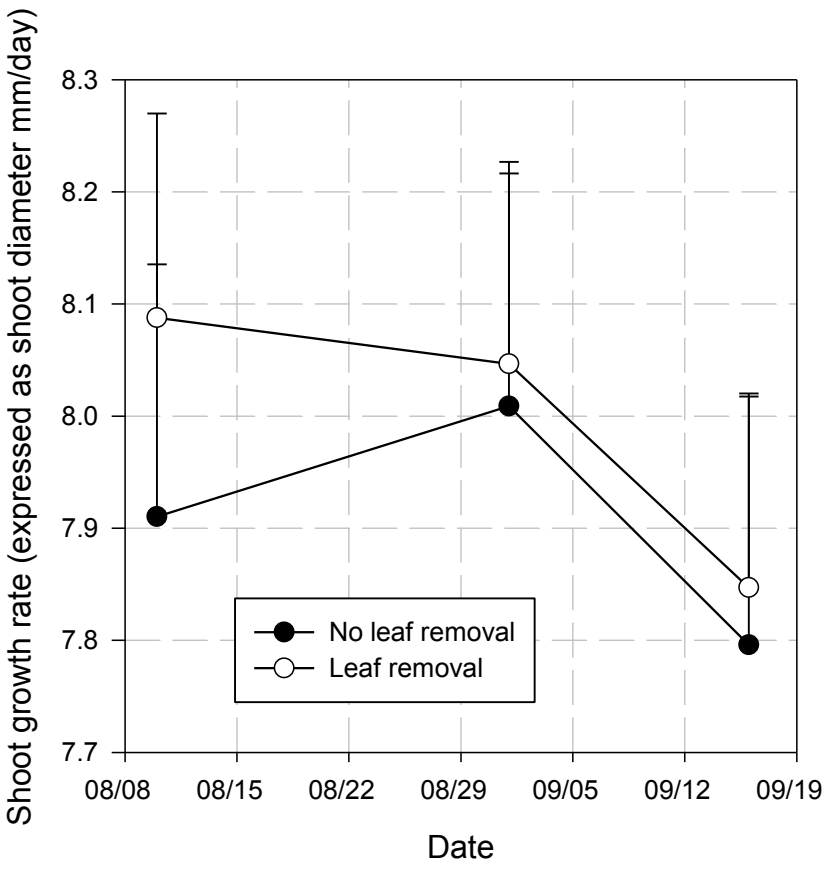

Figure 32. Shoot growth rate (expressed as shoot diameter mm/day) from beginning of August to end of September. Each color of bars represented one of leaf removal and no leaf removal with pooling of the data from leaf removal and control. Data presented in this figure were selected from treatments done at fruit set.

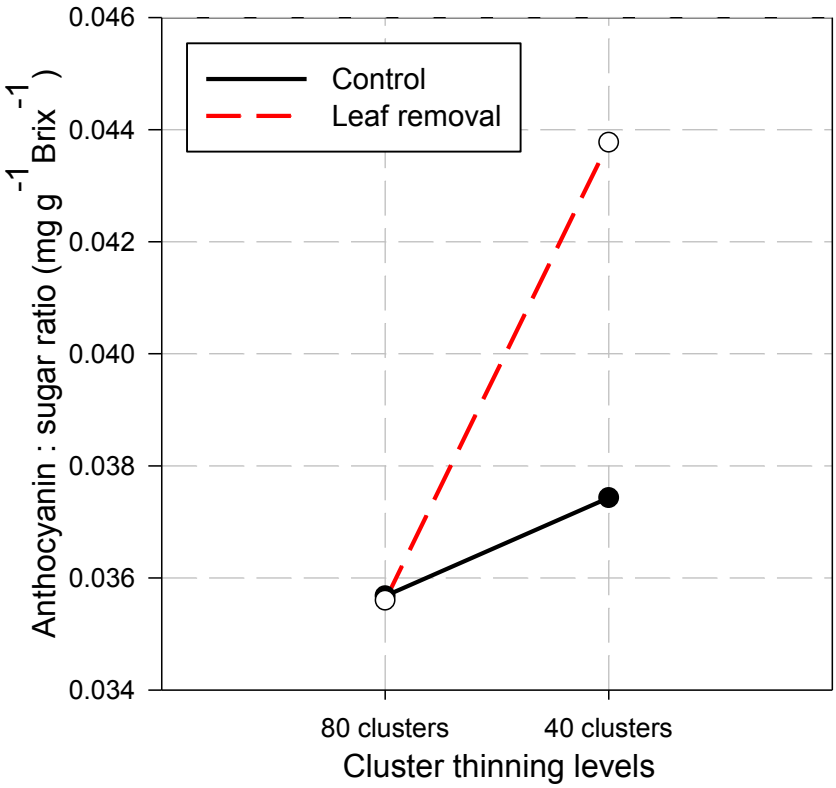

Figure 33. Cluster thinning and basal leaf removal increased the anthocyanins to sugar ratio in 2011 harvest data.

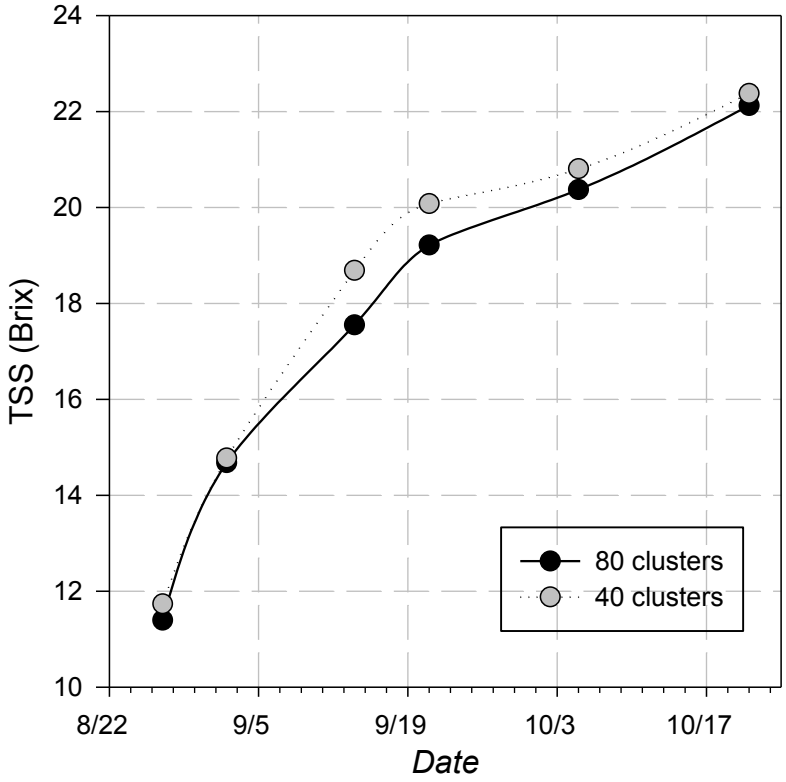

Figure 34. Ripening curve of weekly sugar accumulation (indexed as °Brix) during the season from two levels of cluster thinning. Data from basal leaf removal and three different times were pooled.

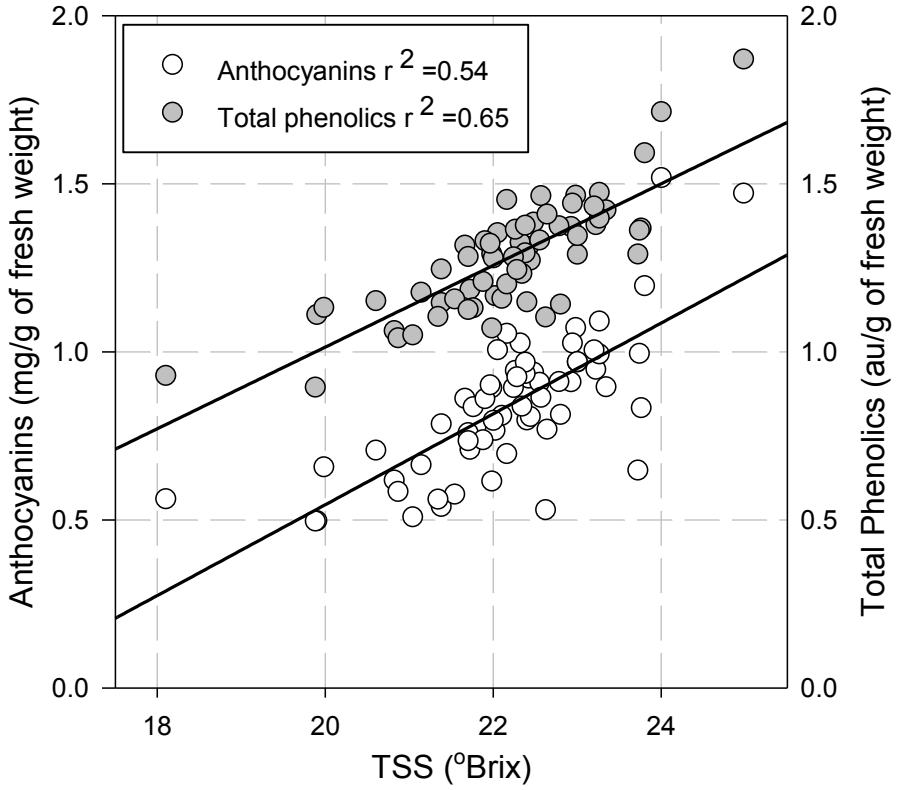

Figure 35. Correlation between anthocyanins, total phenolics and sugar (°Brix) in 2011 harvest data.

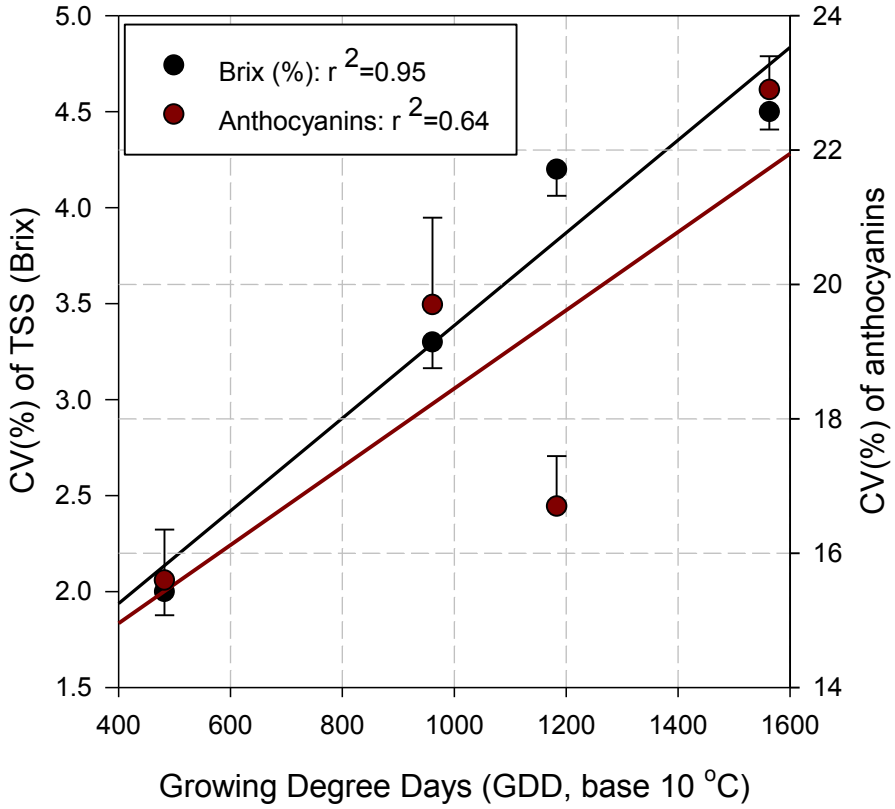

Figure 36. Coefficient of variation of sugar and anthocyanins in leaf removal treatment versus the timing of basal leaf removal

Table 27. Removed leaf area at different timings during the season as part of basal leaf removal treatment in 2011.

Time	GDD	Removed shoot leaf area (cm^2)	Sd	Total shoot leaf area (cm^2)	Sd	Percent of removed shoot leaf area
Fruit set	482	781	69	2134	102	35%
Pre-veraison	961	640	35	2573	146	28%
Veraison	1183	797	49	2576	150	33%

Table 28. Dates of anthesis, fruit set, veraison, and harvest and corresponding growing degree days (GDD). DOY is given in parentheses following each calendar date.

Year	Anthesis	Fruit set	Veraison	Harvest
2011	June 18 (169)	June 30 (181)	August 25 (237)	October 21 (294)
2011	403 GDD	529 GDD	1241 GDD	1579 GDD

Table 29. Differences of canopy density as result of basal leaf removal and no leaf removal using the PQA analysis, LLN was the abbreviation of leaf layer number.

	Basal leaf removal			No leaf removal		
Treatment	LLN	% canopy gaps	% exterior clusters	LLN	% canopy gaps	% exterior clusters
80 clusters	0.48	21%	94%	2.87	0	26%
40 clusters	0.46	19%	96%	2.76	2%	26%

`

Table 30. The effect of timing of basal leaf removal on berry weight.

Treatment	Berry weight (g)		
	Fruit set	Pre-veraison	Veraison
No leaf removal	1.36 a[x]	1.28 a	1.24 a
Leaf removal	1.34 a	1.26 a	1.38 b

[x]Means within columns not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 31. The effect of timing of basal leaf removal on berry weight.

Treatment	Berry weight (g)		
	Fruit set	Pre-veraison	Veraison
No leaf removal	1.36 a[x]	1.28 a	1.24 a
Leaf removal	1.34 a	1.26 a	1.38 a

[x]Means within rows not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 32. The effect of cluster thinning and basal leaf removal on the accumulation of anthocyanins.

Treatment	Anthocyanins (mg/g of FW)	
	80 clusters	40 clusters
No leaf removal	0.89 a[x]	0.84 a
Leaf removal	0.80 a	0.97 a

[x]Means within columns not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 33. The effect of cluster thinning and basal leaf removal on the accumulation of anthocyanins.

Treatment	Anthocyanins (mg/g of FW)	
	80 clusters	40 clusters
No leaf removal	0.89 a[x]	0.84 a
Leaf removal	0.80 a	0.97 b

[x]Means within rows not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 34. Summary of yield components and fruit chemistry in 2011 harvest based on timing. Data were pooled from different levels of cluster thinning, also pooled from the basal leaf removal and no leaf removal.

Yield components and fruit chemistry	Timing treatment		
	Fruit set	Pre-veraison	Veraison
Yield (kg/vine)	14.7 a[x]	12.4 a	11.5 a
Pruning weight (kg)	1.6 a	1.6 a	1.3 a
Ravaz Index	9.2 a	9.7 a	10.7 a
Cluster weight (g)	117 a	105 a	113 a
Berry number	81.3 a	77.3 a	80.6 a
Berry weight (g)	1.35 a	1.27 a	1.31 a
TSS (°Brix)	22.1 a	22.5 a	22.5 a
pH	3.6 a	3.6 a	3.5 a
Titratable acidity (g/L)	6.0 a	6.2 a	6.1 a
Anthocyanins (mg/g)	0.85 a	0.85 a	0.93 a
Phenolics (au/g)	1.27 a	1.31 a	1.35 a

[x]Means within rows not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 35. Summary of yield components and fruit chemistry in 2011 harvest based on cluster thinning. Data were pooled from three different timings when the treatments were applied. Also, data were pooled from the basal leaf removal and control.

Yield components and fruit chemistry	Cluster thinning treatment	
	80 clusters	40 clusters
Yield (kg/vine)	16.5 a[x]	9.1 b
Pruning weight (kg)	1.6 a	1.5 a
Ravaz Index	11.6 a	8.0 a
Cluster weight (g)	114 a	107 a
Berry number	81.0 a	77.6 a
Berry weight (g)	1.32 a	1.30 a
TSS (°Brix)	22.3 a	22.4 a
pH	3.5 a	3.6 a
Titratable acidity (g/L)	6.1 a	6.0 a
Anthocyanins (mg/g)	0.85 a	0.90 a
Phenolics (au/g)	1.28 a	1.33 a

[x]Means within rows not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 36. Summary of yield components and fruit chemistry in 2011 harvest based on leaf removal. Data were pooled from three different times when the treatments were applied. Also, data were pooled from two levels of cluster thinning.

Yield components and fruit chemistry	Leaf removal treatment	
	No leaf removal	Leaf removal
Yield (kg/vine)	12.9 a[x]	12.8 a
Pruning weight (kg)	1.6 a	1.4 a
Ravaz Index	8.6 a	9.6 a
Cluster weight (g)	111 a	111 a
Berry number	80.2 a	79.2 a
Berry weight (g)	1.29 a	1.33 a
TSS (°Brix)	22.4 a	22.3 a
pH	3.6 a	3.6 a
Titratable acidity (g/L)	6.4 a	5.8 b
Anthocyanins (mg/g)	0.87 a	0.88 a
Phenolics (au/g)	1.29 a	1.32 a

[x]Means within rows not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 37. Coefficient of variation (CV%) from two levels of cluster thinning at different timings during the season. Data were pooled from leaf removal and no leaf removal.

CV%	40 clusters/vine			80 cluster/vine
Fruit chemistry	Fruit set	Pre-veraison	Veraison	
TSS (°Brix)	4.2 a[x]	3.9 a	4.2 a	3.5 a
TA (g/L)	9.7 a	11.6 a	10.6 a	11.6 a
Anthocyanins (mg/g)	19.5 a	22.8 a	20.8 a	19.9 a
Phenolics (au/g)	10.3 a	11.5 a	14.3 a	12.9 a

[x]Means within rows not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 38. Coefficient of variation (CV%) from leaf removal at different times during the season. Data were pooled from two levels of cluster thinning.

CV%	Leaf removal			No leaf removal
Fruit chemistry	Fruit-set	Pre-veraison	Veraison	
TSS (°Brix)	2.0 a[x]	3.3 ab	4.2 ab	4.5 b
TA (g/L)	9.7 a	12.1 a	11.7 a	11.1 a
Anthocyanins (mg/g)	15.6 a	19.7 ab	16.7 ab	22.9 a
Phenolics (au/g)	12.6 a	11.6 a	11.0 a	13.6 a

[x]Means within rows not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 39. IBMP (pg/g of FW) concentration from leaf removal treatment at harvest in 2011. Data collected at three different timings (fruit set, pre-veraison, and veraison) showed no significant difference. Consequently, data from the three different timings were pooled. Also, data from two levels of cluster thinning were pooled.

	Treatment	
	Leaf removal	No leaf removal
IBMP (pg/g of FW)	8.7 a[x]	11.8 b

[x]Means within rows not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 40. IBMP (pg/g of FW) concentration at harvest based on timing of leaf pulling in 2011. Data collected from two levels of cluster thinning (40 and 80 clusters per vine) showed no significant difference. Consequently, data from two levels of cluster thinning were pooled.

Treatment	IBMP (pg/g of FW)		
	Fruit set	Pre-veraison	Veraison
No leaf removal	12.6 a[x]	11.9 a	10.9 a
Leaf removal	7.8 a	9.6 a	8.7 a

[x]Means within rows not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

Table 41. IBMP (pg/g of FW) concentration from crop thinning treatment at harvest in 2011. Data collected from two levels of cluster thinning (40 and 80 clusters per vine) showed no significant difference. Consequently, data from three different timings were pooled. Also, data from leaf removal and no leaf removal were pooled.

	Treatment	
	40 clusters/vine	80 clusters/vine
IBMP (pg/g of FW)	9.3 a[x]	10.9 b

[x]Means within rows not followed by the same letter are significantly different at $P \leq 0.10$ by LSD's pairwise comparison.

Table 42. IBMP (pg/g of FW) concentration at harvest based on timing of crop thinning in 2011. Data from leaf removal and no leaf removal were pooled.

Treatment	IBMP (pg/g of FW)		
	Fruit set	Pre-veraison	Veraison
80 clusters/vine	10.8 a[x]	10.7 a	11.2 a
40 clusters/vine	9.2 a	10.7 a	8.1 a

[x]Means within rows not followed by the same letter are significantly different at $P \leq 0.05$ by LSD's pairwise comparison.

APPENDIX B: TYPE 3 TABLES OF ANOVA

Table 43. Analyses of variance (ANOVA) of yield in 2011.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	2	7.05	2	0.2046
crop	1	25.7	28.1	<.0001
time*crop	2	25.8	1.19	0.3204
leaf	1	25.9	0	0.9612
time*leaf	2	25.7	0.48	0.6213
leaf*crop	1	25.5	0.75	0.3941
time*leaf*crop	2	25.4	0.09	0.9162

Table 44. Analyses of variance (ANOVA) of pruning weight in 2011.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	2	11.7	2.01	0.1774
crop	1	18.1	0.39	0.54
time*crop	2	18.1	1.76	0.2008
leaf	1	18.1	2.24	0.1514
time*leaf	2	18.1	2.92	0.0795
leaf*crop	1	17.9	2.35	0.1425
time*leaf*crop	2	17.9	0.55	0.5837

Table 45. Analyses of variance (ANOVA) of cluster weight in 2011.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	2	4.47	0.2	0.8231
crop	1	18.3	0.01	0.9336
time*crop	2	21.5	0.49	0.6209
leaf	1	23.8	0.01	0.9341
time*leaf	2	20.9	1.9	0.1748
leaf*crop	1	21.7	0.2	0.6562
time*leaf*crop	2	22.2	0.36	0.705

Table 46. Analyses of variance (ANOVA) of berry number in 2011.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	2	33.5	0.02	0.9755
crop	1	33.5	0	0.9971
time*crop	2	33.5	0.99	0.3831
leaf	1	33.5	0.01	0.9234
time*leaf	2	33.5	2.77	0.0769
leaf*crop	1	33.5	0.24	0.6271
time*leaf*crop	2	33.5	0.22	0.8052

Table 47. Analyses of variance (ANOVA) of berry weight in 2011.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	2	6.17	1.21	0.3596
crop	1	25.2	1.59	0.2191
time*crop	2	25.5	0.82	0.4523
leaf	1	25.9	0.32	0.5786
time*leaf	2	25.4	6.23	0.0063
leaf*crop	1	25.2	0.81	0.3781
time*leaf*crop	2	25.3	0.41	0.6686

Table 48. Analyses of variance (ANOVA) of TSS (°Brix) in 2011.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	2	6.62	0.48	0.6376
crop	1	24.3	2.46	0.1299
time*crop	2	24.4	1.78	0.1896
leaf	1	24.4	0.92	0.3464
time*leaf	2	24.2	0.94	0.403
leaf*crop	1	24	3.09	0.0916
time*leaf*crop	2	24.1	1.41	0.2647

Table 49. Analyses of variance (ANOVA) of pH in 2011.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	2	6.17	2.79	0.1368
crop	1	25.5	3.64	0.0676
time*crop	2	25.5	1.2	0.3179
leaf	1	25.6	1.98	0.1715
time*leaf	2	25.5	1.8	0.1862
leaf*crop	1	25.2	0.98	0.3324
time*leaf*crop	2	25.3	0.98	0.3908

Table 50. Analyses of variance (ANOVA) of TA in 2011.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	2	5.12	0.49	0.6362
crop	1	23.1	1.29	0.2674
time*crop	2	23.1	1.11	0.3464
leaf	1	23.1	21.92	0.0001
time*leaf	2	22.9	1.04	0.3704
leaf*crop	1	22.6	1.59	0.2199
time*leaf*crop	2	22.7	2.32	0.1215

Table 51. Analyses of variance (ANOVA) of anthocyanins in 2011.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	2	8.57	0.3	0.7507
crop	1	24.4	2.21	0.15
time*crop	2	23.5	2.13	0.1412
leaf	1	24.3	0.04	0.8494
time*leaf	2	23.4	0.27	0.766
leaf*crop	1	24.1	5.3	0.0303
time*leaf*crop	2	23.2	0.43	0.6564

Table 52. Analyses of variance (ANOVA) of total phenolics in 2011.

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
time	2	5.72	0.45	0.6559
crop	1	24.2	2.01	0.1694
time*crop	2	24.3	0.24	0.7872
leaf	1	24.3	0.42	0.525
time*leaf	2	24.2	0.04	0.9647
leaf*crop	1	23.9	3.06	0.0929
time*leaf*crop	2	23.9	1.17	0.3285

LITERATURE CITED

LITERATURE CITED

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CHAPTER IV

SUMMARY OF RESULTS IN 2010 AND 2011

Conclusion

We conducted similar experiments in both 2010 and 2011 with a focus on the effect of crop load and fruit exposure on yield components and fruit composition of Cabernet Franc in Michigan's principal cool climate growing regions. In 2010, we had three levels of cluster thinning and two levels of fruit exposure. Since the crop load ratio in 2010 was less than 3, a severely low Ravaz Index value well below the desired 5 to 10, (Kliewer and Dokoozlian 2005, Bravdo et al. 1984, 1985), we decreased the severity of cluster thinning in 2011. Eighty clusters were maintained on each vine in 2011 compared to 48 clusters per vine in 2010, and 40 clusters were maintained on vine compared to 12 clusters per vine, respectively. Two levels of crop (40 and 80 clusters retained per vine) were manipulated in 2011 as compared to three levels (12, 24 and 48 clusters retained per vine) in 2010. Also, we changed from the leaf tucking treatment in 2010 to no leaf removal in 2011 due to its insignificant impact. Generally, both years delivered similar results with regard to basic fruit chemistry, except for TA and anthocyanins. There was also a difference in yield components based on treatments in the two years of the study.

Climatic condition and phenology data are shown in Figure 37 and Table 53. As mentioned before, 2010 was a better than average year in terms of heat accumulation and precipitation, while 2011 was average. There was a difference of 300 GDD between the two years largely due to 2011 getting a slow start due to a very late spring. As a result, it can be seen that the phenological stages of vines in 2011 were delayed one week in comparison with 2010 (Table 53). In addition, in 2011 more precipitation fell, particularly concentrated in September

and October, resulting in delaying the harvest date. As a consequence, two different years materialized in which to conduct similar experiments.

A summary of results in 2010 and 2011 is drawn in Table 54. Significant compensation of cluster weight and pruning weight was found in 2010, but not in 2011. In 2010, early cluster thinning (12 clusters per vine before veraison) increased the cluster weight and pruning weight. However, in 2011 we did not find a similar result after adjusting the severity of cluster thinning. In considering the crop load ratio, we could explain that in 2010 vines applied by three levels of cluster thinning were undercropped based on vine balance. From 12 clusters to 48 clusters per vine the crop load ratio was from one to three, which was much lower than the balanced range from 5 to 10. In comparison with 2010, we had balanced vines in 2011, and the vines were less impacted by cluster thinning in terms of cluster weight and pruning weight. For both two years, we did not find any significant effect on shoot growth during the season from either cluster thinning or leaf removal.

Similar results in basic fruit chemistry were found in both years, except that the TA and anthocyanins showed some difference. TA was reduced by leaf removal as the increased cluster temperature from sunlight exposure resulted in more respiration of malic acid in the berries. Anthocyanins were affected by the interaction between cluster thinning and leaf removal. As we discussed before, this could have resulted from the increase in the sink to source ratio stimulated by both cluster thinning and the increased cluster temperature from using leaf removal to open the canopy. However, TSS (°Brix), pH, and phenolics were not significantly impacted by any of our treatments. Basically, berry sugar accumulation was relatively stable for a given variety, which was less influenced by environmental conditions and viticultural practices, when it was in comparison with organic acids (Keller et al. 2005) and anthocyanins (Keller et al. 1998, Sadras et

al. 2007). Inconsistent results were found based on different viticultural practices and environmental conditions in terms of berry sugar accumulation. Increase of TSS (°Brix) by cluster thinning or pruning techniques was found in some studies (Chorti et al. 2010, Guidoni et al. 2002, Ollat and Gaudillere 1998, Petrie and Clingeleffer 2006, Petrie et al. 2000, Reynolds et al. 1994). However, no or little effect of crop load ratio or leaf removal on TSS (°Brix) was also found in other studies (Keller et al. 2005, Nuzzo and Matthews 2006, Tardaguila et al. 2010). Specifically, we did not found any effect on harvest sugar contents in either year. Although the effect on final sugar contents was still not clear, high crop level delayed the sugar accumulation during the ripening stage. The possible explanation was that vines under balance were capable of reaching the targeted sugar level at harvest with a difference only in arrival date (Petrie and Clingeleffer 2006; Bravdo et al. 1984, 1985). Vines with a higher crop load ratio reached the same level of sugar later compared to the vines with a lower crop load ratio.

Another important result related to basic fruit chemistry was the variation of population. In Table 54 we applied coefficient of variation (CV) to address the skewness of different populations relative to basic fruit chemistry. As we discussed in average values of basic fruit chemistry, the effects of viticultural practices and environmental conditions on basic fruit compositions were inconsistent, and from our data, generally, these parameters were hardly impacted. However, not a lot of studies have investigated the effect on variation. Early leaf removal decreased CV, resulting in more uniform fruit in terms of sugar and anthocyanins. A similar result was found in shoot thinning and leaf removal studies (Wolpert and Howell 1984, Wolpert et al. 1983). However, we did not find the same effect on CV by cluster thinning as studies indicating more uniform sugar contents by cluster thinning (Petrie and Clingeleffer 2006).

In addition, we found there were correlations of sugar and anthocyanins, sugar and phenolics in 2011. As the sugar accumulated, anthocyanins and total phenolics increased as well. After onset of veraison, berries started to accumulate sugar and other compounds (anthocyanins, phenolics and flavor compounds) to get maturity. Higher amount of sugar accumulation was always related to more ripe fruit, and TSS was always used as an index to evaluate the fruit maturity. Correlations of sugar and anthocyanins, sugar and phenolics were also found in other studies (Keller et al. 2005). However, in 2010 we did not find the same correlation as it was in 2011. The possible reason could be that in 2011 we had more balanced vines and in 2010 vines were severely "undercropped". The undercropped effect in 2010 could overwrite correlations of sugar and anthocyanins, sugar and phenolics. It resulted in almost same levels of anthocyanins or phenolilcs with the increase of sugar accumulation.

As for the IBMP levels in 2011, leaf removal reduced the IBMP concentration significantly at harvest when we applied the leaf removal at three different timings during the season. The latest time when we removed the basal leaves was veraison, which was still influential in reducing the amount of IBMP in comparison with no leaf removal. Sunlight exposure to clusters was regarded as the most powerful tool in decreasing the IBMP levels in berries, and studies have been done to investigate the critical time when the sunlight was imposed. Pre-veraison cluster light exposure was believed to be more critical than post-veraison exposure in reducing IBMP concentration during berry growth (Hashizume et al. 1999; Roujou de Boubee et al. 2000; Koch et al. 2012, Scheiner et al. 2010). In our studies, we applied the leaf removal at fruit set, pre-veraison and veraison, and we did not find any significant difference of IBMP between three timings. IBMP levels were reduced regardless of three timings when we applied leaf removal during the season.

Finally, from 2010 and 2011 two years' experiments, we can see that for balanced vines, e.g. in 2011, cluster thinning was less effective on yield components and fruit compositions. For the "undercropped" vines, e.g. in 2010, tended to have higher vigor and bigger cluster when the cluster thinning was applied early in the season and it is usually undesirable. Cluster thinning might be more effective in terms of "overcropped" vines, which needs to be further studied. In the contrast, leaf removal is more influential in terms of fruit compositions. Significant reduction of TA was found when the leaf removal treatment was applied before veraison. Less acidic contents of fruits are regarded as an improvement of fruit quality in a cool climate. As for Cabernet Franc, IBMP was regarded as dominant flavor compound in determining the herbaceous flavors. Leaf removal applied before veraison significantly reduced the IBMP levels in berries. Furthermore, earlier leaf removal increased the uniformity of fruit in terms of fruit compositions. Therefore, under Michigan cool climate earlier basal leaf removal is suggested to improve the fruit quality of Cabernet Franc. However, cluster thinning is complicated. Vine balance (crop load ratio) is suggested to look at first and for balanced vines it is not necessary to thin the crop. Finally, vine balance (crop load ratio) and earlier basal leaf removal are crucial in improving the fruit quality of Cabernet Franc in Michigan.

APPENDIX

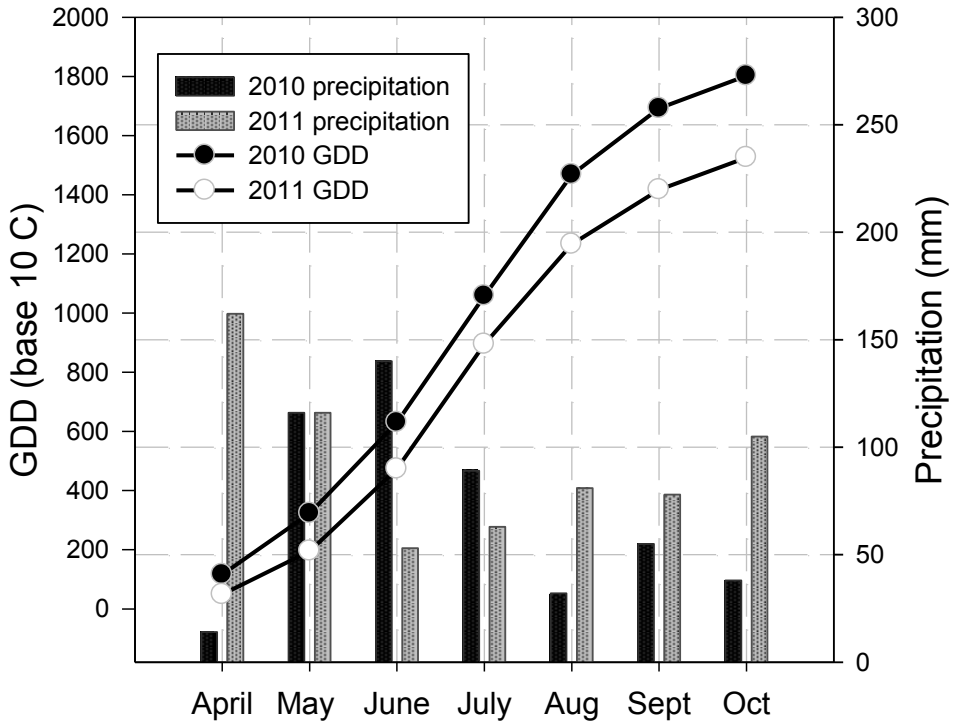

Figure 37. Growing degree days and monthly precipitation for 2010 and 2011 at the Southwest Michigan Research and Extension Center near Benton Harbor. GDD based on 10°C from April 1 through October 31.

Table 53. Dates of anthesis, fruit set, veraison, and harvest and corresponding growing degree days (GDD) in 2010 and 2011. DOY is given in parentheses following each calendar date.

Year	Anthesis	Fruit set	Veraison	Harvest
2010	June 12 (163)	June 30 (181)	August 18 (230)	October 6 (279)
2011	June 18 (169)	June 30 (181)	August 25 (237)	October 21 (294)
2010	428 GDD	633 GDD	1306 GDD	1708 GDD
2011	403 GDD	529 GDD	1241 GDD	1579 GDD

Table 54. Summary results of effect on yield components, fruit compositions and uniformity of vines due to cluster thinning and leaf removal in 2010 and 2011.

Parameters	Year	
	2010	2011
Shoot growth	-	-
Pruning weight (kg)	Cluster thinning before veraison	-
Ravaz Index	Cluster thinning	-
Cluster weight (g)	Cluster thinning at fruit set and pre-veraison	-
Berry number	N/A	-
Berry weight (g)	N/A	Leaf removal at veraison
TSS (°Brix)	-	-
pH	-	-
TA (g/L)	-	Leaf removal
Anthocyanins (mg/g)	-	Cluster thinning and leaf removal
Phenolics (au/g)	-	-
IBMP (pg/g)	N/A	Cluster thinning and leaf removal
CV of TSS	N/A	Leaf removal at fruit set
CV of TA	N/A	-
CV of anthocyanins	N/A	Leaf removal at fruit set
CV of phenolics	N/A	-

N/A represents measurements were not available in 2010.
- represents no effects were found based on cluster thinning and leaf removal.

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