

ABSTRACT

ELDER, KEVIN TURNER. Assessment of Wine Grape Cultivars in North Carolina and Impact of Leaf Removal on Grape Phenolic Content (Under the direction of Sara Spayd.)

The following two studies were performed in the piedmont region of North Carolina where there has been rapid growth of the wine industry in recent years. A trial of 10 red wine grape (*Vitis vinifera* L.) cultivar/rootstock combinations was assessed for viticultural performance in Dobson, North Carolina. The study was established in 2008 as a randomized complete block design. Vine yield, fruit composition, and vigor were measured from 2010 to 2014. Cold damage was assessed for the 2013/2014 winter. Average growing degree days for the site were 2167 (base 10°C) with an average of 1125 mm of precipitation. Cultivars varied in yield (0.90 to 4.59 kg/vine), yield/pruning weight (0.8 to 9.5), soluble solids concentration (17.8 to 21.9%), titratable acidity (3.8 to 7.2 g/L tartaric acid), and pH (3.56 to 4.09). The cultivars Carmenere and Nebbiolo had unacceptably low yields (< 1.3 tons per acre). Carmenere, Grignolino, Nebbiolo, Tinta Cao, and Touriga Nacional had the highest soluble solids concentration at harvest. Aglianico, Grignolino, and Nebbiolo had the most desirable titratable acidity and pH. Touriga Nacional and Tinta Cao were most affected by the cold and Lemberger was least affected. The best performing cultivars in this study based upon yield, soluble solids concentration, pH, and titratable acidity were Grignolino, Tinta Cao, and Touriga Nacional which performed as good as or better than the more commonly grown cultivars, Cabernet Sauvignon and Merlot. Lemberger and Aglianico also performed well but were slightly below the targeted soluble solids concentration. Results from this study can help inform cultivar selection for similar growing climates. The purpose of the second study was to determine the influence of three different leaf removal treatments on grape composition with a focus on phenolic compounds. The practice of leaf removal from the

fruiting zone of grapevines is common within vineyards and provides multiple important functions including increased light exposure, berry temperature, spray penetration, and airflow to reduce disease pressure. However, it can result in sunburning of berries. The study was established at a commercial vineyard in Lexington, North Carolina using *Vitis vinifera* cv. Cabernet Franc. In 2013 and 2014, three different leaf removal treatments (control, medium, high) were applied to the grapevines at beginning of bunch closure. Control vines had no leaf removal (2013) or basal leaf removal up to the first cluster on the east side only (2014). Medium leaf removal vines had basal leaves removed up to the first cluster on both sides of the canopy (2013) or up to the second cluster on both sides of the canopy (2014). High leaf removal vines had basal leaves removed up to the second cluster on both sides (2013) or to the second node above the second cluster on both sides (2014). Canopy density, light penetration, fruiting zone temperature, and internal berry temperature were measured throughout the growing season. Berry samples were assayed for: soluble solids concentration, pH, titratable acidity, berry color, and phenolic composition and concentration. Basal leaf removal increased sunlight exposure resulting in berry temperatures that were 1.4°C warmer after noon than berries from control vines. The concentration of total skin phenolics was higher in fruit from medium and high leaf removal vines in one out of two years. Total berry color was highest in fruit from high leaf removal vines in one out of two years. Content and concentration of tannin and individual anthocyanins and flavonols were unaffected by either leaf removal treatment. Exposure to elevated temperatures may be responsible for the lack of consistent differences in the phenolic concentration of more exposed fruit. The influence of leaf removal on grape composition in North Carolina may be less profound than that observed in cooler climates.

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Assessment of Wine Grape Cultivars in North Carolina and Impact of Leaf Removal on
Grape Phenolic Content

by
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DEDICATION

To Mom.

BIOGRAPHY

Kevin Elder was born July 5th 1991 to John and Adena Elder in Texas. When Kevin was five he moved to Colorado with his mother, father, and new baby brother Nicholas Elder. Kevin always enjoyed nature and observing animals and particularly enjoyed biology in school. At age 15 he moved to North Carolina where he met an amazing girl named Katie Romberger with whom he is still madly in love with. He went to Appalachian State University and received a degree in Cell/Molecular Biology. While at Appalachian he discovered an interest in fermentation and began making alcohol under his dorm room bed. In pursuit of turning his new hobby into a career he cultured and researched native yeast that could be used for fermentation and completed an internship at Jones vonDrehle Winery. He continued his education by researching wine grapes at North Carolina State University and was recently hired by a winery in North Carolina moving him closer to achieving his career goal of becoming a winemaker.

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CHAPTER 1

Assessment of Wine Grape Cultivars in North Carolina

(In the format appropriate for submission to the American Journal of Enology and Viticulture)

Abstract

A trial of 10 red wine grape (*Vitis vinifera* L.) cultivars/rootstock combinations was assessed for viticultural performance in Dobson, North Carolina. The plot was established in 2008 as a randomized complete block design. Vine yield, fruit composition, and vigor were measured from 2010 to 2014. Cold damage was assessed for the 2013/2014 winter. Average growing degree days (base 10° C) for the site were 2184 with an average of 1125 mm of precipitation. Cultivars varied in yield (0.90 to 4.59 kg/vine), yield/pruning weight (0.8 to 9.5), soluble solids concentration (17.8 to 21.9%), titratable acidity (3.8 to 7.2 g/L tartaric acid), and pH (3.56 to 4.09). The cultivars Carmenere and Nebbiolo had unacceptably low yields (< 1.3 tons per acre). Carmenere, Grignolino, Nebbiolo, Tinta Cao, and Touriga Nacional had the highest soluble solids concentration at harvest. Aglianico, Grignolino, and Nebbiolo had the most desirable titratable acidity and pH. Grignolino, Touriga Nacional, and Tinta Cao were most affected by the cold and Lemberger and Merlot were least affected. Results from this study can help growers make decisions for cultivar selection in similar growing climates.

Introduction

The North Carolina wine industry has grown rapidly with an increase in winery number from 25 in 2000 to 156 in 2015 (W. Winslow, personal communication, 2015).

Traditional wine grapes (*Vitis vinifera* L.) are predominantly grown in the northern piedmont and mountain regions of North Carolina due to Pierce's disease which is more prevalent in the more southern and eastern parts of the state (Poling and Spayd, 2015). Most growing sites have more than 1944 growing degree days (base 10°C) and are located in region IV or V on the Winkler scale (Winkler et al. 1974). Another consideration for the region is the risk of cold injury. Wolf (2003) found that -22°C is the point where significant cold injury occurs in dormant *V. vinifera* cultivars in northern Virginia. However, susceptibility to cold injury depends on cultivar and weather conditions prior to as well as during a cold event (Ferguson et al. 2014). Cultivar trials have played an important role in the development of the wine industry in California (Amerine and Winkler 1963) and Washington (Clare et al. 1972, Clare et al. 1976) and should be helpful in advancing the industry in North Carolina. In this region the average daily high temperature in July is 31°C and average daily humidity throughout the year is 74% (CRONOS, Raven Knob, lat. 36°48'N; long. 80°86'W, <https://www.nc-climate.ncsu.edu/cronos/?station=NRAV>). Finding cultivars that are suited to the warm, humid growing conditions of the region may be helpful to North Carolina vineyards and wineries.

The purpose of the present study was to compare the viticultural performance of alternative red-skinned cultivars to that of the more commonly grown Cabernet Sauvignon and Merlot. This project is part of the NE-1020 regional project in which sentinel cultivars were used to compare the performance of the cultivars across multiple sites (http://nimss.umd.edu/lgu_v2/homepages/home.cfm?trackID=4034). The yield, basic fruit composition, phenology, vigor, and cold hardiness of the vines were assessed.

Materials and Methods

The trial was established in 2008 and data was collected from 2010 to 2014 at Surry Community College in Dobson, North Carolina (lat. 36°38'N; long. 80°72'W) as a part of the USDA project: “NE-1020 Multi-state Evaluation of Winegrape Cultivars and Clones” (http://nimss.umd.edu/lgu_v2/homepages/home.cfm?trackID=4034). The soil was a Clifford sandy clay loam (Fine, kaolinitic, mesic Typic Kanhapludults) (Soil Survey Staff, 2014). Growing-degree days (GDD) were calculated as the sum of the average of daily temperature using a base temperature of 10°C with no upper limit from 1 Apr to 31 Oct. For the period 1971 to 2000 mean growing degree days (GDD) for the site are 2184 GDD (Poling and Spayd 2015). Within the NE-1020 project, the site was classified as having a hot growing season (>1944 GDD) and a mild dormant season (0-4.4°C average minimum temperature of the coldest month). Therefore, Cabernet Sauvignon cl. 8 and Merlot cl. 3 grafted to 101-14 Mgt rootstock were the so-called ‘sentinel cultivars’ used for comparison of cultivars across sites within the NE-1020 project. The cultivars planted were: Aglianico cl. FPS01, Carmenere cl. VCR702, Cabernet Sauvignon cl. FPS08, Grignolino cl. FPS03, Lemberger cl. 1, Merlot cl. FPS03, Nebbiolo cl. FPS03, Tinta Cao cl. FPS03, and Touriga Nacional cl. FPS01 all grafted to 101-14 Mgt rootstock. Additionally, Cabernet Sauvignon cl. 8 was evaluated using *V. riparia* × *V. rupestris* 3309 Couderc (3309C) rootstock.

The vines were planted in 2008 in a randomized complete plot design replicated six times with the exception of Cabernet Sauvignon on 3309C, which had only four replications. Each plot consisted of four consecutive vines spaced 1.8 m apart in-row and 3.0 m between rows. Row orientation was N to S to maximize solar radiation interception (Smart et al.

1990). One guard vine was included at the ends of each row along with a guard row on the east and west sides of the research plot. The entire plot was covered with an overhead bird netting from before véraison to after harvest. Vines were trained to a double-trunked bilateral cordon with a vertical shoot positioned trellis system. Vines were pruned to two node spurs with six spurs per meter. Hedging was performed as needed during the season to 1.2 m above the cordon. When berries were pea-sized, leaves were removed from the fruiting zone on the east side of the canopy. Vineyard disease and insect control was according to recommendations for their control in North Carolina vineyards (Poling and Spayd 2015).

Samples of 100 berries from each cultivar and replicate were taken for chemical analysis to determine harvest date on an approximately bi-weekly schedule from véraison to harvest. Berries were equally sampled from east and west sides and all four vines within a replicate. Samples were transported to the laboratory in Raleigh and held at 0°C overnight for analysis the following day. Average berry weight was calculated using the weight of the 100 berry sample. The samples were homogenized in a blender for 10 seconds with ten drops of pectic enzyme (PEC5L, Scottzyme, Petaluma, California) and then filtered through fluted filter paper (Qualitative P8, Fisher Scientific, Waltham, Massachusetts). Soluble solids concentration (SSC) was determined by a refractometer (Refracto 30P, Mettler Toledo, Schwerzenbach, Switzerland). pH was measured by temperature-compensating pH meter (PHi 510, Beckman Coulter, Fullerton, California) using an Orion 8172BNWP electrode (Thermo Scientific, Beverly, Massachusetts) standardized to pH of 4.0, 7.0, and 10.0. Titratable acidity (TA) was determined by titrating 5 mL of juice diluted with 100 mL boiling deionized water to a pH of 8.2 with 0.1 N NaOH using an auto-titrator (DL 15,

Mettler Toledo, Schwerzenbach Switzerland). Color was measured by diluting a 5 mL juice sample to 25 ml with acidified ethanol (pH 1.0). Absorbance was measured at 520 nm using a spectrophotometer (DU 730, Beckman Coulter, Brea, California).

Date of harvest was determined by a combination of onset of fruit diseases, weather forecast and biweekly fruit chemistry. At harvest, fruit weight and number of clusters per vine were determined. Cluster weight was calculated by dividing vine yield by the number of clusters per vine. Harvest berry samples (100 berries) were also analyzed from each treatment and replicate

Prior to dormant pruning, number of shoots per vine was counted. Vines were double pruned each year with the initial pruning to about 6 nodes in early-mid winter and final pruning in late February to mid-March. At each pruning, 1-year old wood removed was weighed and number of spurs and nodes retained on individual vines were counted. Due to sub-normal cold winter temperatures in winter 2013-2014, two 4-8 bud shoots were collected from each vine after final pruning. The nodes were dissected for an assessment of primary and secondary bud mortality. Injury to phloem and xylem of the 1-year old wood was also rated as no injury, presence of injury, or dead.

Data were analyzed using the SAS GLM procedure (ver. 9.4; SAS Institute, Cary, North Carolina). Least squares means was used for mean separation. A probability value of ≤ 0.05 was required for significance.

Results and Discussion

For the period of this experiment the season with the greatest number of GDD was 2010 and the season with the lowest GDD was 2013 based on GDD (Table 1.1). These

seasons placed the site between Climatic Regions IV and V (Winkler et al. 1974). This site accumulated more GDD than cultivar trials in Parma, Idaho (Shellie et al. 2007), Grand Junction, Colorado (Hamman et al. 1993), and Winchester, Virginia (Wolf and Miller, 2001). Of the five years, precipitation was highest in 2013.

The high precipitation years of 2011 and 2013 had the highest average yields with 4.7 and 3.8 kg/vine respectively (data not shown). The cultivars Aglianico, Merlot, Grignolino, and Lemberger produced the greatest yield per vine averaged across all five years (Table 1.2). The lowest yielding cultivars were Carmenere and Nebbiolo. The low yield was principally due to these cultivars having very few clusters per vine (Table 1.3). Nebbiolo is known to have basal bud infertility (Mannini 1995). In winter 2011-2012 Nebbiolo and Carmenere were cane pruned in an attempt to bolster yield, but without success. Wolf and Miller (2001) found that cane pruning Nebbiolo more than doubled yield when compared to spur pruning. They also found that Nebbiolo fruit ripened to 23% SSC. In Parma, Idaho spur-pruned Nebbiolo produced adequate yields and ripened to 25% SSC (Shellie et al. 2007). The heaviest clusters were seen in Grignolino and the lightest were from Carmenere (Table 1.4). The 5-year average berry mass across all cultivars was 1.8 g with higher berry mass in high precipitation years (data not shown). The berry mass were similar to those reported in Virginia (Wolf and Miller 2001) but higher than those reported in Idaho (Shellie et al. 2007). The lowest berry mass was seen in the sentinel cultivar Cabernet Sauvignon on 101-14 Mgt and the heaviest berries were from Aglianico vines (Table 1.5). The smaller berry size of Cabernet Sauvignon on 101-14 Mgt would be more desirable for making red wines as there is a greater ratio of skin to juice. (Singleton 1972).

Aglianico vines had the lowest pruning mass, while Carmenere and Touriga Nacional vines had the highest (Table 1.6). Pruning weights were highest in years 2011 and 2013 (data not shown) which had the highest precipitation. The yield to pruning weight ratio is a measure of vine balance (Table 1.7). Only Aglianico and Merlot vines were within the ideal range of 6 to 10 (Smart et al. 1990). The other cultivars ranged from 0.8 to 5.7 indicating that they had excessive vegetative growth compared to reproductive growth.

Harvest decisions were based primarily on decreasing fruit condition due to disease and secondarily on SSC. Despite having the lowest yield Carmenere consistently had the latest harvest date (Figure 1.1). Grignolino, Lemberger, and Touriga Nacional had average harvest dates similar to the sentinel Merlot. Other cultivars had harvest dates similar to the sentinel Cabernet Sauvignon on 101-14 Mgt.

The average SSC across all cultivars ranged from 19.3% in 2013 to 21.0% in 2011 (Table 1.8). The low SSC in 2013 was due to higher than normal precipitation which caused larger berries and higher disease pressure that necessitated early harvest. SSC at harvest varied by cultivar with Nebbiolo, Cabernet Sauvignon on 3309C, and Grignolino berries having the highest concentrations. SSC of Aglianico and Lemberger berries averaged less than 20% SSC across the five years and were lower than the SSC of fruit from the sentinel cultivars. The low yields of Carmenere and Nebbiolo vines likely influenced the relatively higher SSC of the fruit. It is also worth noting that sun burning was observed in Nebbiolo.

The average fruit TA ranged from a low of 3.8 g/L for Merlot berries to a high of 7.2 g/L tartaric acid for Aglianico berries (Table 1.9). In 2013 the low TA of Aglianico berries reflects the immaturity of the fruit at harvest. Averaged across the five years, Aglianico,

Grignolino, and Nebbiolo berries had the highest TA. Lemberger, Tinta Cao, and Touriga Nacional berries had moderate TA, similar to the sentinel cultivar Cabernet Sauvignon on 101-14 Mgt. Fruit from the other cultivars had low TA. Aglianico berries had the lowest average pH followed by Grignolino and Nebbiolo berries (Table 1.10). Lemberger berries had a moderate pH that was the same as the sentinel cultivar Cabernet Sauvignon on 101-14 Mgt. Fruit from the other cultivars had high pH similar to the sentinel Merlot. In regions IV and V in California Tinta Cao fruit was reported to have moderate acidity of 4.9 to 7.4 and high pH of 3.55 to 4.16 (Amerine and Winkler 1963). Touriga Nacional fruit grown in regions IV and V in California was reported to have good yields and good sugar-acid balance. Lemberger fruit grown in regions IV in California was reported to have high acid (5.4 to 9.6 g/L) and low pH (3.18 to 3.63). Cabernet Sauvignon on 3309C berries averaged lower TA and higher pH than the sentinel Cabernet Sauvignon on 101-14 Mgt. Fruit from Carmenere, Touriga Nacional, and Merlot had the highest total color while Grignolino consistently had the lowest (Table 1.11).

Cold weather event(s) in the winter of 2013/2014 where temperatures went as low as -18°C caused significant vascular injury to 1-year old canes and primary and secondary buds in the count-node position occurred on Tinta Cao and Touriga Nacional (Table 1.12). Aglianico, Grignolino, Tinta Cao, and Touriga Nacional had substantially reduced yields in 2014 compared to 2013 which is likely due to the lower numbers of live buds of those cultivars. Nebbiolo vines were found to be susceptible to cold injury in Washington (Clare et al. 1976). In northern Virginia Cabernet Sauvignon and Nebbiolo vines were found to be susceptible to cold injury (Wolf and Miller 2001). In the present study Lemberger, Merlot

and Cabernet Sauvignon on 3309C canes and nodes were the least affected by the cold temperatures. Similar to the findings of the present study, Cabernet Sauvignon, Lemberger, and Merlot vines were reported to be cold hardy in Washington (Clare et al. 1976, Ahmedullah 1985). Lemberger vines were also found to be cold hardy in Grand Junction, Colorado (Hamman 1993) and Winchester, Virginia (Wolf and Miller 2001).

Conclusion

The best performing cultivars in this study based upon yield, SSC, pH, and TA were Grignolino, Tinta Cao, and Touriga Nacional, which performed as good as or better than the sentinel cultivars, Cabernet Sauvignon on 101-14 Mgt and Merlot. Despite good performance in these criteria Grignolino had poor color intensity and Tinta Cao and Touriga Nacional were the most susceptible to winter injury. Lemberger and Aglianico also performed well but had slightly lower soluble solids concentration. Aglianico berries split at low SSC in the rainiest year of the study, thereby reducing its potential utility in North Carolina's high precipitation environment. Carmenere and Nebbiolo produced unacceptably low yields and are not recommended for cultivation in North Carolina.

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Table 1.1 Growing degree days, July average daily high temperature, and precipitation at Surry Community College in Dobson, NC (2010-2014).

Year	Growing degree days (base 10°C)	July Average Daily High Temperature (°C)	Precipitation (mm)	
			Annual	April-October
2010	2342	31.5	1068	638
2011	2232	32.0	1095	662
2012	2148	31.7	1044	712
2013	2051	28.9	1408	813
2014	2062	30.5	1011	632

Table 1.2 Yield (kg) per vine of eight *Vitis vinifera* L. cultivars in comparison to the *V. vinifera* sentinel cultivars Cabernet Sauvignon and Merlot, Dobson, NC (2010-2014).

Cultivar	Mean	Year				
		2010	2011	2012	2013	2014
		Yield (kg/vine)				
Aglianico ^a	4.52a ^c	2.35bc	6.63ab	3.81c	5.21ab	3.89ab
Carmenere ^a	0.87e	1.00d	0.30f	0.53g	1.30d	1.38de
Cabernet Sauvignon ^b	3.67bc	1.69cd	5.18cd	4.02bc	3.99c	3.49bc
Grignolino ^a	4.34ab	4.05a	4.48cd	5.44a	5.42a	1.90d
Lemberger ^a	3.94abc	3.20ab	5.81bc	1.85ef	4.51ab	4.34a
Nebbiolo ^a	1.60d	1.65cd	2.55e	1.23fg	1.53d	1.07e
Tinta Cao ^a	3.32c	3.49a	5.08cd	2.63de	4.25bc	1.17de
Touriga Nacional ^a	3.61bc	3.08ab	4.12d	3.43cd	4.47abc	2.95c
<u>Sentinel cultivars</u>						
Cabernet Sauvignon ^a	3.70bc	2.12bc	5.43bcd	3.52cd	3.90c	3.51bc
Merlot ^a	4.59a	3.05ab	7.19a	4.93ab	3.31c	4.49a

^a101-14 Mgt rootstock

^bGrafted to 3309C rootstock.

^c Means within a column having the same letters do not differ, $p \leq 0.05$.

Table 1.3 Number of clusters per vine of eight *Vitis vinifera* L. cultivars in comparison to the *V. vinifera* sentinel cultivars Cabernet Sauvignon and Merlot, Dobson, NC (2010-2014).

Cultivar	Mean	Year				
		2010	2011	2012	2013	2014
	Clusters per vine					
Aglianico ^a	32cd ^c	12b	30d	31b	31bc	48b
Carmenere ^a	12f	12b	7f	10d	15d	22d
Cabernet Sauvignon ^b	37abc	22a	39abc	38a	32bc	52ab
Grignolino ^a	19e	22a	18e	19c	20d	18d
Lemberger ^a	29d	23a	32d	17c	32bc	40c
Nebbiolo ^a	11f	13b	13e	10d	10e	10e
Tinta Cao ^a	24e	23a	33cd	17cd	30c	18d
Touriga Nacional ^a	34bc	26a	36bcd	29b	35ab	46bc
<u>Sentinel cultivars</u>						
Cabernet Sauvignon ^a	41a	24a	44a	39a	38a	60a
Merlot ^a	39ab	26a	42ab	39a	32bc	56a

^a101-14 Mgt rootstock

^bGrafted to 3309C rootstock.

^cMeans within a column having the same letters do not differ, $p \leq 0.05$.

Table 1.4 Cluster weight at harvest of berries from eight *Vitis vinifera* L. cultivars in comparison to the *V. vinifera* sentinel cultivars Cabernet Sauvignon and Merlot, Dobson, NC (2010-2014).

Cultivar	Mean	Year				
		2010	2011	2012	2013	2014
Cluster weight (g)						
Aglianico ^a	156b ^c	200a	220b	124c	166b	84b
Carmenere ^a	64f	77e	42g	53e	84e	63c
Cabernet Sauvignon ^b	102de	77e	135ef	107cd	122de	68bc
Grignolino ^a	222a	178ab	249a	287a	272a	107a
Lemberger ^a	137bc	143bc	184cd	108cd	142bc	110a
Nebbiolo ^a	138bc	122cd	189c	118c	153bc	108a
Tinta Cao ^a	136bc	149bc	156de	160b	152bc	64c
Touriga Nacional ^a	108de	116cd	115f	119c	126cd	63c
<u>Sentinel cultivars</u>						
Cabernet Sauvignon ^a	92e	85de	124f	89d	105de	59c
Merlot ^a	120cd	115cd	172cd	129c	103de	81b

^a101-14 Mgt rootstock

^bGrafted to 3309C rootstock.

^cMeans within a column having the same letters do not differ, $p \leq 0.05$.

Table 1.5 Berry mass of eight *Vitis vinifera* L. cultivars in comparison to the *V. vinifera* sentinel cultivars Cabernet Sauvignon and Merlot, Dobson, NC (2010-2014).

Cultivar	Mean	Year				
		2010	2011	2012	2013	2014
Berry mass (g/berry)						
Aglianico ^a	2.1a ^c	1.9a	2.3a	2.1a	2.3a	1.9a
Carmenere ^a	1.8bc	1.6b	1.8cd	1.8b	2.0b	2.0a
Cabernet Sauvignon ^b	1.6d	1.4c	1.7de	1.7b	1.8d	1.6d
Grignolino ^a	1.9b	1.6b	2.2ab	2.0a	2.0bc	1.7b
Lemberger ^a	1.8bc	1.6b	1.9cd	1.8b	1.9cd	1.9a
Nebbiolo ^a	1.6d	1.5c	1.7de	1.7b	1.7e	1.7bc
Tinta Cao ^a	1.7c	1.6b	1.9cd	1.8b	1.9cd	1.6bc
Touriga Nacional ^a	1.8bc	1.6b	2.0bc	1.8b	2.1b	1.7bc
<u>Sentinel cultivars</u>						
Cabernet Sauvignon ^a	1.5e	1.5bc	1.6e	1.4c	1.6e	1.4d
Merlot ^a	1.8c	1.6b	2.0bc	1.8b	1.9cd	1.6bcd

^a101-14 Mgt rootstock

^bGrafted to 3309C rootstock.

^cMeans within a column having the same letters do not differ, $p \leq 0.05$.

Table 1.6 Pruning weight at harvest of berries from eight *Vitis vinifera* L. cultivars in comparison to the *V. vinifera* sentinel cultivars Cabernet Sauvignon and Merlot, Dobson, NC (2010-2014).

Cultivar	Mean	Year				
		2010	2011	2012	2013	2014
Pruning weight (kg/vine)						
Aglianico ^a	0.48d ^c	0.54d	0.60g	0.44d	0.46e	0.37f
Carmenere ^a	1.18a	0.75bc	1.33a	1.29a	1.61a	0.93a
Cabernet Sauvignon ^b	0.91b	0.80bc	1.13bcd	0.77c	1.18b	0.70bc
Grignolino ^a	0.77bc	0.67cd	1.10cde	0.67c	0.81d	0.61cde
Lemberger ^a	0.89b	0.76bc	1.12bcd	0.80c	1.11b	0.67cd
Nebbiolo ^a	0.86bc	0.67cd	0.83f	0.77c	1.20b	0.82ab
Tinta Cao ^a	0.88bc	0.98a	1.00def	0.82c	1.10bc	0.54de
Touriga Nacional ^a	1.08a	0.89ab	1.30ab	0.99b	1.48a	0.74bc
<u>Sentinel cultivars</u>						
Cabernet Sauvignon ^a	0.91b	0.83b	1.24abc	0.76c	1.02bcd	0.70bc
Merlot ^a	0.75c	0.79bc	0.92ef	0.71c	0.84cd	0.48ef

^a101-14 Mgt rootstock

^bGrafted to 3309C rootstock.

^c Means within a column having the same letters do not differ, $p \leq 0.05$.

Table 1.7 Yield/pruning weight of eight *Vitis vinifera* L. cultivars in comparison to the *V. vinifera* sentinel cultivars Cabernet Sauvignon and Merlot, Dobson, NC (2010-2014).

Cultivar	Mean	Year				
		2010	2011	2012	2013	2014
Yield/pruning weight						
Aglianico ^a	9.5a ^c	4.5b	11.1a	8.9a	11.3a	10.5a
Carmenere ^a	0.8e	1.3d	0.3f	0.4g	0.8d	1.5f
Cabernet Sauvignon ^b	4.1c	2.3cd	4.6cd	5.4c	3.4c	5.0c
Grignolino ^a	5.7b	6.3a	4.0cde	8.1ab	6.7b	3.1de
Lemberger ^a	4.5c	4.2bc	5.2c	2.3ef	4.1c	6.5b
Nebbiolo ^a	1.9d	2.4bcd	3.0e	1.6fg	1.3d	1.3f
Tinta Cao ^a	3.7c	3.6bc	5.2c	3.2de	4.2c	2.2ef
Touriga Nacional ^a	3.6c	3.6bc	3.2de	3.6de	3.3c	4.2cd
<u>Sentinel cultivars</u>						
Cabernet Sauvignon ^a	4.1c	2.5bcd	4.4cd	4.6cd	4.0c	5.2c
Merlot ^a	6.5b	4.1bc	7.9b	6.7b	4.0c	9.4a

^a101-14 Mgt rootstock

^bGrafted to 3309C rootstock.

^cMeans within a column having the same letters do not differ, $p \leq 0.05$.

Table 1.8 Soluble solids concentration at harvest of berries from eight *Vitis vinifera* L. cultivars in comparison to the *V. vinifera* sentinel cultivars Cabernet Sauvignon and Merlot, Dobson, NC (2010-2014).

Cultivar	Mean	Year				
		2010	2011	2012	2013	2014
Soluble solids concentration (%)						
Aglianico ^a	19.4d ^c	19.7bc	19.4f	20.4d	16.7d	21.0cd
Carmenere ^a	21.0bc	20.6bc	21.4cd	20.9bcd	20.0bc	22.0b
Cabernet Sauvignon ^b	21.4ab	20.7bc	21.3cd	20.8cd	21.0a	23.5a
Grignolino ^a	21.3abc	23.0a	22.3b	20.3d	19.4c	21.5bc
Lemberger ^a	19.6d	19.0c	21.1cde	21.8ab	17.1d	19.1e
Nebbiolo ^a	21.9a	20.2bc	23.5a	22.6a	21.3a	21.8b
Tinta Cao ^a	20.7bc	20.5bc	21.6bcd	21.1bcd	19.6c	20.6d
Touriga Nacional ^a	20.8bc	21.2b	22.0bc	21.4bc	20.1bc	19.3e
<u>Sentinel cultivars</u>						
Cabernet Sauvignon ^a	20.5c	20.5bc	20.7de	20.3d	19.7bc	21.4bc
Merlot ^a	20.7bc	20.7bc	20.2ef	21.1bcd	20.6ab	20.7cd

^a101-14 Mgt rootstock

^bGrafted to 3309C rootstock.

^cMeans within a column having the same letters do not differ, $p \leq 0.05$.

Table 1.9 Titratable acidity at harvest of berries from eight *Vitis vinifera* L. cultivars in comparison to the *V. vinifera* sentinel cultivars Cabernet Sauvignon and Merlot, Dobson, NC (2010-2014).

Cultivar	Mean	Year				
		2010	2011	2012	2013	2014
Titratable acidity (g tartaric acid/L)						
Aglianico ^a	7.2a ^c	5.1c	6.2a	6.2a	11.6a	6.9b
Carmenere ^a	4.3d	3.4g	4.0d	4.1de	5.4f	4.5e
Cabernet Sauvignon ^b	4.1d	4.1ef	3.9ed	4.2cde	4.5g	4.1e
Grignolino ^a	6.6b	5.9b	5.6b	6.3a	7.5c	7.6a
Lemberger ^a	5.5c	4.8cd	4.8c	5.3b	6.5d	6.2c
Nebbiolo ^a	6.9ab	6.8a	5.5b	5.3b	8.7b	8.0a
Tinta Cao ^a	5.4c	4.0f	4.7c	4.7bcd	7.1c	6.6b
Touriga Nacional ^a	5.2c	4.5de	4.7c	4.9bc	6.0e	5.8cd
<u>Sentinel cultivars</u>						
Cabernet Sauvignon ^a	5.3c	4.6d	4.8c	5.3b	6.4de	5.6d
Merlot ^a	3.8d	3.4g	3.5e	3.9e	4.3g	4.1e

^a101-14 Mgt rootstock

^bGrafted to 3309C rootstock.

^cMeans within a column having the same letters do not differ, $p \leq 0.05$.

Table 1.10 pH at harvest of berries from eight *Vitis vinifera* L. cultivars in comparison to the *V. vinifera* sentinel cultivars Cabernet Sauvignon and Merlot, Dobson, NC (2010-2014).

Cultivar	Mean	Year				
		2010	2011	2012	2013	2014
		pH				
Aglianico ^a	3.44e ^c	3.80c	3.45g	3.51d	3.14d	3.33g
Carmenere ^a	3.91a	4.09a	3.97a	3.91a	3.76a	3.81a
Cabernet Sauvignon ^b	3.82b	3.98b	3.82bc	3.80ab	3.76a	3.77ab
Grignolino ^a	3.56d	3.70d	3.62f	3.56cd	3.50c	3.43ef
Lemberger ^a	3.66c	3.76cd	3.74cde	3.81ab	3.50c	3.50de
Nebbiolo ^a	3.58cd	3.56e	3.70def	3.79ab	3.50c	3.37fg
Tinta Cao ^a	3.83b	4.08a	3.89ab	3.89a	3.66b	3.63c
Touriga Nacional ^a	3.78b	3.95b	3.84bc	3.75b	3.73ab	3.63c
<u>Sentinel cultivars</u>						
Cabernet Sauvignon ^a	3.66c	3.92b	3.67ef	3.63c	3.56c	3.54d
Merlot ^a	3.78b	3.91b	3.80bcd	3.78b	3.72ab	3.70bc

^a101-14 Mgt rootstock

^bGrafted to 3309C rootstock.

^cMeans within a column having the same letters do not differ, $p \leq 0.05$.

Table 1.11 Total color at harvest of berries from eight *Vitis vinifera* L. cultivars in comparison to the *V. vinifera* sentinel cultivars Cabernet Sauvignon and Merlot, Dobson, NC (2010-2014).

Cultivar	Mean	Year			
		2011	2012	2013	2014
Total color (AU520/ml)					
Aglianico ^a	5.9 ^c	5.3 ^b	6.5 ^{cd}	4.4 ^e	7.3 ^d
Carmenere ^a	11.3 ^a	6.7 ^a	12.1 ^a	11.1 ^a	17.3 ^a
Cabernet Sauvignon ^b	6.0 ^c	3.7 ^c	5.6 ^d	4.3 ^e	10.4 ^{bc}
Grignolino ^a	2.8 ^d	--- ^d	2.3 ^e	1.5 ^f	4.9 ^e
Lemberger ^a	9.1 ^b	--- ^d	10.9 ^a	5.6 ^{de}	10.2 ^c
Nebbiolo ^a	9.2 ^b	7.0 ^a	7.3 ^{bcd}	10.6 ^{ab}	12.1 ^{bc}
Tinta Cao ^a	7.0 ^c	5.7 ^b	6.3 ^d	5.4 ^{de}	10.4 ^{bc}
Touriga Nacional ^a	11.2 ^a	--- ^d	8.9 ^b	6.4 ^{cd}	18.2 ^a
<u>Sentinel cultivars</u>					
Cabernet Sauvignon ^a	8.9 ^b	5.7 ^b	6.0 ^d	7.4 ^c	16.6 ^a
Merlot ^a	10.1 ^{ab}	--- ^d	8.1 ^{bc}	9.4 ^b	12.6 ^b

^a101-14 Mgt rootstock

^bGrafted to 3309C rootstock.

^cMeans within a column having the same letters do not differ, $p \leq 0.05$.

^dMissing data

Table 1.12 Phloem and xylem injury and percent live primary and secondary buds in the two basal count nodes March 2014.

							Live buds (%)			
	Phloem			Xylem			First Node		Second Node	
	Live (%)	Injured (%)	Dead (%)	Live (%)	Injured (%)	Dead (%)	1°	2°	1°	2°
Aglianico ^a	83abc ^c	14abc	3a	86b	11ab	3a	67bc	67abc	50d	46bc
Carmenere ^a	83abc	15abc	2a	86b	12ab	2a	82ab	73ab	58cd	67abc
Cabernet Sauvignon ^b	97ab	3c	0a	94ab	6ab	0a	90ab	57bcd	81abc	52bc
Grignolino ^a	77c	23ab	0a	88ab	9ab	0a	70ab	79ab	60bcd	80ab
Lemberger ^a	96ab	4c	0a	100a	0b	0a	100a	94a	83abc	67abc
Nebbiolo ^a	87abc	11bc	2a	96ab	4ab	0a	82ab	65abc	100a	88a
Tinta Cao ^a	72c	26ab	2a	94ab	6ab	0a	50c	31d	44d	69abc
Touriga Nacional ^a	72c	29a	0a	97ab	3ab	0a	64bc	36cd	44d	43c
<u>Sentinel cultivars</u>										
Cabernet Sauvignon ^a	77bc	23ab	0a	85b	15a	0a	73abc	52bcd	90ab	50bc
Merlot ^a	100a	0c	0a	100a	0b	0a	83ab	63abcd	92ab	62abc

^aGrafted to 101-Mgt rootstock

^bGrafted to 3309C rootstock

^cMeans within a column having the same letters do not differ, $p \leq 0.05$.

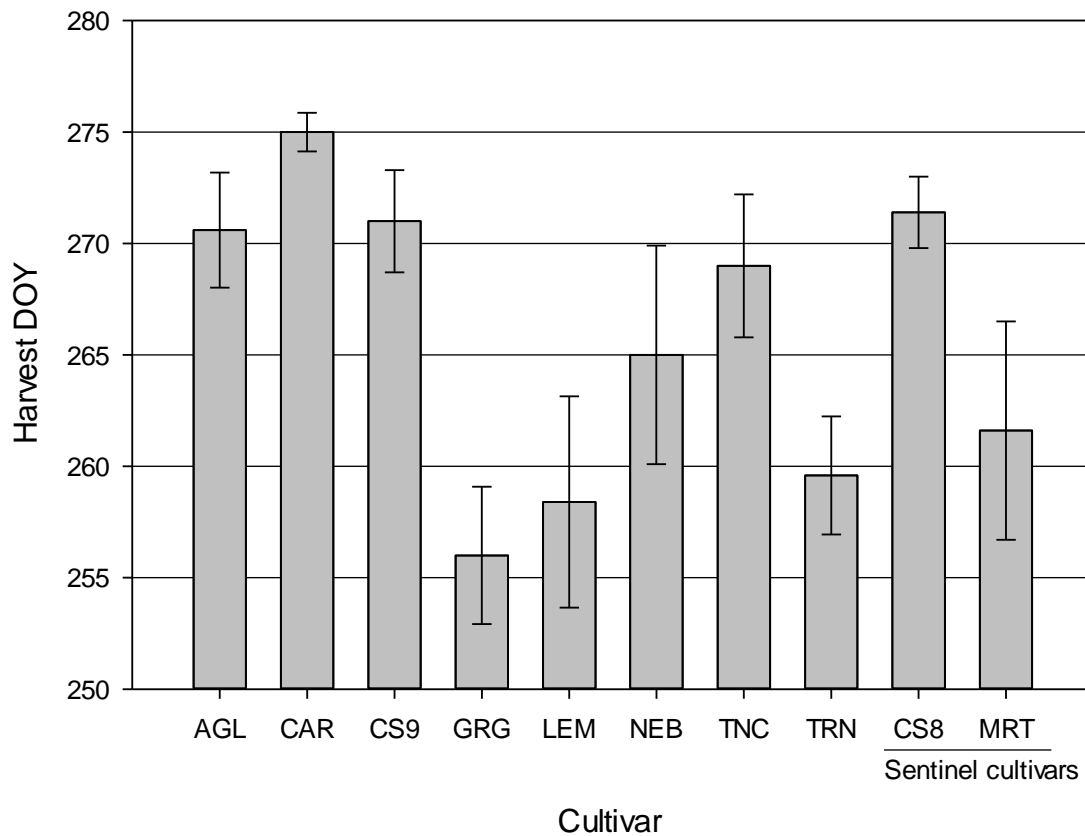


Figure 1.1 Average harvest day of year of eight *Vitis vinifera* L. cultivars in comparison to the *V. vinifera* sentinel cultivars Cabernet Sauvignon and Merlot, Dobson, NC (2010-2014). Error bars represent 95% confidence intervals. AGL: Aglianico, CAR: Carmenere, CS9: Cabernet Sauvignon on 3309C, GRG: Grignolino, LEM: Lemberger, NEB: Nebbiolo, TNC: Tinta Cao, TRN: Touriga Nacional, CS8: Cabernet Sauvignon, MRT: Merlot.

CHAPTER 2

Impact of Leaf Removal on Grape Phenolic Content

Abstract

The practice of leaf removal from the fruiting zone of grapevines is common within vineyards and provides multiple important functions including increased light exposure, berry temperature, spray penetration, and airflow to reduce disease pressure. However, it can result in sunburning of berries. The purpose of this study was to determine the influence of three different leaf removal treatments on grape composition with a focus on phenolic compounds. The study was established at a commercial vineyard in Lexington, North Carolina using *Vitis vinifera* cv. Cabernet Franc. In 2013 and 2014, three different leaf removal treatments (control, medium, high) were applied to the grapevines at beginning of bunch closure. Control vines had no leaf removal (2013) or basal leaf removal up to the first cluster on the east side only (2014). Medium leaf removal vines had basal leaves removed up to the first cluster on both sides of the canopy (2013) or up to the second cluster on both sides of the canopy (2014). High leaf removal vines had basal leaves removed up to the second cluster on both sides (2013) or to the second node above the second cluster on both sides (2014). Canopy density, light penetration, fruiting zone temperature, and internal berry temperature were measured throughout the growing season. Berry samples were assayed for: soluble solids concentration, pH, titratable acidity, total berry color, and phenolic composition and concentration. Basal leaf removal increased sunlight exposure resulting in berry temperatures that were 1.4°C warmer after noon than berries from control vines. The concentration of total skin phenolics was higher in fruit from medium and high leaf removal

vines in one out of two years. Total berry color was highest in fruit from high leaf removal vines in one out of two years. Concentration of tannin and individual anthocyanins and flavonols were unaffected by either leaf removal treatment. Exposure to elevated temperatures may be responsible for the lack of consistent differences in the phenolic concentration of more exposed fruit. The influence of leaf removal on grape composition in North Carolina may be less profound than that observed in cooler climates.

Introduction

Bunch exposure is regarded as a major factor influencing the flavonoid composition of *Vitis vinifera* L. grapes (Jackson and Lombard 1993). Many studies have found that various forms of shading of red grapes lowers the concentration of anthocyanins in the fruit (Wicks and Kliewer 1983, Rojas-Lara and Morrison 1989, Dokoozlian and Kliewer 1996, Bergqvist et al. 2001, Spayd et al. 2002, Chorti et al. 2010). Increased light exposure can increase the concentration of anthocyanins (Bergqvist et al. 2001, Tardaguila et al. 2010, Kotseridis et al. 2012, Jogaiah et al. 2013). However, excessive sunlight can have a negative impact on anthocyanin concentrations (Bergqvist et al. 2001, Jogaiah et al. 2013). Other studies that have shaded grapes found no difference in anthocyanin concentration (Hunter 1995, Downey et al. 2004, Ristic et al 2007). The effects of light on anthocyanin concentrations are heavily dependent on the increase in berry temperature caused by increased sunlight exposure (Spayd et al. 2002). In hot climates, leaf removal may result in sunburning of the fruit and reduce wine quality (Greer et al. 2006). Grapes fully exposed to the sun can reach temperatures more than 12°C higher than ambient (Smart and Sinclair 1976). High berry temperatures have a negative effect on anthocyanin concentrations

(Tomana et al. 1979, Haselgrove et al. 2000, Spayd et al. 2002, Tarara et al. 2008). Artificial cooling of sun exposed clusters to the temperature of shaded clusters increased skin anthocyanin concentrations over non-cooled, sun-exposed clusters (Spayd et al. 2002). Additionally, when shaded clusters were heated to sun-exposed cluster temperatures skin anthocyanin concentrations were lower than those of shaded clusters that remained at ambient temperature. The length of time clusters were above 35°C was negatively correlated with anthocyanin concentration. Temperatures of 30°C can reduce the concentration of abscisic acid, which may reduce the expression of genes that are involved in anthocyanin biosynthesis (Yamane et al. 2006). Grapes grown at temperatures from 15 to 26°C accumulate more anthocyanin than grapes grown at higher temperatures (Kliewer 1970, Kliewer and Torres 1972, Tomana et al. 1979, Jackson and Lombard 1993, Mori et al. 2007).

Light exposure increases flavonol concentration in grapes (Spayd et al. 2002, Ristic et al. 2007, Gregan et al. 2012, Jogaiah et al. 2013). Downey et al. (2004) showed that shading decreases flavonols in grapes along with the gene encoding the enzyme flavonol synthase. Blocking UV light reduces flavonol accumulation (Spayd et al. 2002, Gregan et al. 2012, Koyama et al. 2012). Similar to anthocyanin metabolism, high daytime temperatures (30-35°C) can inhibit flavonol synthesis (Kliewer 1977).

The practice of removing leaves from around grape clusters is commonly used to increase exposure of the fruit to light and air movement and to improve disease management. Basal leaf removal can increase the concentration of anthocyanin and flavonol (Tardaguila et al. 2010, Gregan et al. 2012, Kotseridis et al. 2012, Jogaiah et al. 2013). In the present study

we examine the impact of basal leaf removal on the temperature and phenolic composition of Cabernet Franc grapes grown in North Carolina.

Materials and Methods

The study was conducted in 2013 and 2014 at a commercial vineyard in Lexington, North Carolina (lat. 35°84'20"N; long. 80°28'45"W; elevation 229m) in a block of *V. vinifera* cv Cabernet Franc (Clone 332/ Rootstock 101-14 MGT) planted in clay loam soil in 2004. Rows were oriented north to south. Vines were trained to a bilateral cordon and spur pruned to 2-nodes/spur on a vertically shoot positioned trellis. Vines were top and side hedged by the grower throughout the growing season. Vines were pruned by the grower to about 35 nodes/vine. Vines were harvested when determined by the grower for commercial use. Yield and number of clusters were recorded for each vine at harvest.

The experimental design was a randomized complete block replicated four times with four vines per plot. In 2013, three leaf removal treatments (control, medium, high) were applied to the grapevines on day 172, Eichhorn-Lorenz (E-L) stage 32 (Coombe 2004). The control consisted of no leaf removal. The medium treatment was applied by removing the leaves from around the basal most cluster on each shoot down to the cordon. The high treatment was applied by removing leaves from around the basal two clusters. The treatments were applied to east and west aspects of the vines. In 2014 similar three levels of leaf removal were applied on day 161 (E-L stage 29). On day 184 (E-L stage 33) the medium leaf removal treatment was inadvertently imposed on the east aspect of all vines in the study by vineyard employees. About two leaves per shoot were removed from the medium and control treatments and about one leaf per shoot was removed from high treatment vines on the east

side only. Therefore, on day 190 (E-L stage 33), new leaf removal treatments were applied to medium and high treatment vines on east and west aspects. Control vines were left with approximately two leaves removed from only the east aspect. Leaves were removed from the second cluster down to the cordon on medium treatment vines. Leaves were removed from the second leaf node above the second cluster down to the cordon on high treatment vines.

Light and temperature measurements. Sensors were calibrated to 40°C in a water bath to confirm an accuracy of $\pm 0.5^\circ\text{C}$. In 2013 HOBO Pendant light and temperature sensors (Onset, Cape Cod, Massachusetts) were affixed to one vine from each treatment and replicate. The sensors were placed in the fruiting zone (1 m above ground): one exposed to sunlight on the west side, one exposed to sunlight on the east side, and one facing upwards, shaded by the canopy. The sensor “face” was oriented outward and parallel to the cordon on east and west aspects. Temperature and illuminance (150-1200 nm wavelength absorption with peak at 900 nm) were recorded every 15 minutes for 34 days, from day 242 (E-L stage 36) to day 276 (harvest). In 2014 sensors were placed on day 175 (E-L stage 32) and logged until day 211 (E-L stage 34) as described for 2013. Due to a malfunction, no data were collected for a 21-day period that included véraison. Data collection resumed on day 234 (E-L stage 36) until day 258 (harvest). A total of 61 days were recorded in 2014. In 2014, temperature sensors inside solar radiation shields (Onset, Cape Cod, Massachusetts) were placed above vines (2.2 m above ground) in each replication to measure ambient conditions outside of the canopy. Growing degree hours (base 10°C) were calculated by using hourly average temperature and summed over the entire data collection period.

Internal berry temperatures were measured for two clusters from the east and the west side of the canopy from each plot using a handheld Omega HH-25TC thermometer (Omega, Stamford, Connecticut). Temperatures were measured after véraison (E-L stage 36-38) at three different times of the day (before, during, and after solar noon) on two separate days in 2013 and two days in 2014. The thermocouple was inserted into the center of three berries (top, middle, and bottom of cluster) on each sampled cluster. Canopy density was measured using enhanced point quadrat analysis (EPQA) (Meyers and Vanden Heuvel, 2008). In 2013 EPQA was performed three times; at 1 week (E-L 32), 5 weeks (E-L 35), and 9 weeks (E-L 37) after the treatments were applied and three times in 2014; at 1 week (E-L 32), 3 weeks (E-L 33), and 7 weeks (E-L 35) after treatments were applied. Irradiance in the fruiting zone of each vine was measured on a clear, cloudless day in 2014 by AccuPAR LP-80 ceptometer (Decagon Devices, Pullman, Washington) 3 weeks after the treatments were applied (E-L 33). Cloud cover throughout the 2013 growing season prohibited measurement of irradiance. Total incident photosynthetically active radiation (PAR; $\mu\text{mol m}^{-2}\text{s}^{-1}$; 400-700 nm wavelength) was measured in the middle of the row with the ceptometer sensor oriented upright at the height of the fruiting zone. Percentage of ambient light intensity was measured with the ceptometer held parallel to the row within the canopy in the fruiting zone. An average was determined by taking three readings; 45° to the east side, straight up through the canopy, and 45° to the west side. Ceptometer readings were used to estimate the percentage of total incident PAR in the fruiting zone (Meyers and Vanden Heuvel, 2008).

Grape composition. In 2013 two-one hundred berry samples were collected from each plot on day 228 (E-L 35), 253 (E-L 36), 266 (E-L 37), and 276 (E-L 38). Equal numbers of

grapes were sampled from each vine and east/west aspect. In 2014 two-one hundred berry samples were collected from east and west aspects separately. Sampling occurred on day 211 (E-L 34), 231 (E-L 35), 252 (E-L 38), and 258 (E-L 38). Half of the fruit from each sample set were weighed and analyzed for fresh basic chemistry and the other half were frozen at 0°C for later phenolic analysis.

For fresh analyses, five drops of PEC5L (Scott Laboratories, Petaluma, California) pectic enzyme were added to whole berries and pureed in a blender at high speed for one minute. The puree was filtered through fluted filter paper (Qualitative P8, Fisher Scientific, Pittsburgh, Pennsylvania). Soluble solids concentration (SSC) was measured by temperature compensating refractometer (Refracto 30P, Mettler Toledo, Switzerland). pH was measured by pH meter (model 510, Beckman Coulter, Fullerton, California) using a Orion 8172BNWP electrode (Thermo Scientific, Beverly, Massachusetts) standardized to pH 10.0, 7.0, and 4.0. Titratable acidity (TA) was determined using an automated titrator (model DL15, Mettler Toledo, Switzerland) titrating 5 mL of filtrate diluted with 100 mL of boiling distilled water with 0.1 N NaOH. For color analysis 20 mL acidified ethanol (pH 1.0) to 5 mL of filtrate and filtered through a GHP Acrodisc 0.45 µm filter (Pall, Port Washington, New York). Absorbance at 520nm was measured using a spectrophotometer (model DU 730, Beckman Coulter, Irvine, California).

Phenolic Analyses

Sample Preparation. Samples were shipped overnight via air on ice (2013) and/or dry ice (2014) from Raleigh, North Carolina to Prosser, Washington for analyses at the Washington State University, Irrigated Agriculture Research and Extension Center. Total

anthocyanins were determined by Iland method (Iland et al. 2000) from a 20 berry sample. A sub-sample of 30 berries was used to determine the tannin and total phenolic content and concentration of seeds and skins by protein precipitation and reaction with FeCl_3 , as described by Harbertson et al. (2002) using a Agilent 8453 UV-Visible spectroscopy system (Santa Clara, California).

All samples subjected to HPLC analysis were extracted from berries using the Iland method (Iland et al. 2000). From the 10.0 mL supernatant, a 1.0 mL aliquot was concentrated to a gel using a Thermo Savant ISS110 SpeedVac-concentrator (Waltham, Massachusetts). The supernatant was then dissolved in 1.0 mL of a 1:1 ratio (v/v) of the two HPLC mobile phases.

HPLC analysis. All chemicals used were obtained from VWR (Visalia, California). Water and methanol were HPLC grade. Formic acid (98% purity) was reagent grade. For HPLC analysis, mobile phase A (MPA) consisted of 90% water and 10% formic acid (v/v). Mobile phase B (MPB) consisted of 90% methanol and 10% formic acid (v/v). For HPLC analysis, an Agilent 1100 series analytical HPLC system (Santa Clara, California) equipped with autosampler, thermostat, and diode array detector was used. The column was an Agilent Zorbax Stable Bond 300 C-18 column (4.6 X 250 mm, 5 μm particle size). The method applied was adapted from Downey and Rochfort (2008). Briefly, anthocyanins and flavonols were separated from injected samples by using the following gradient of MPA:MPB: 0 min, 6% B; 10 min, 22% B; 15 min, 28% B; 20 min, 34% B; 30 min, 40% B; 33 min, 100% B; 40 min, 100% B; 40.1-45 min, 6% B.

Identification and quantification of individual flavonoids. For peak area determination, Agilent Chemstation version B.04.01 SP1 was used. Individual flavonoids were identified by recording the maximum absorbance values in the UV/vis spectra. The recorded maximum absorbance of each compound was compared to previously published work (Downey and Rochfort 2008, He et al. 2010). All samples were calibrated with external standards. Malvidin-3-*O*-glucoside was used as the standard for anthocyanin and was obtained by preparative HPLC (Downey and Rochfort 2008). Quercetin (Sigma-Aldrich, St. Louis, Missouri) was used as the external standard for flavonols. Anthocyanins were expressed as mg malvidin-3-*O*-glucoside equivalents/g berry fresh weight, and flavonols were expressed as mg quercetin equivalents/g fresh weight.

Statistical data analysis. Data were analyzed by SAS PROC MIXED (ver. 9.4; SAS Institute, Cary, North Carolina). Sample date was treated as a split plot in time. Least squares means was used for mean separation. A probability value of ≤ 0.05 was required for significance.

Results

Accumulated growing degree days (GDD, base 10°C) were 2167 and 2414 in 2013 and 2014, respectively (Figure 2.1). Historically, GDD in Lexington, North Carolina averaged 2248 for the period from 1939 to 2013. GDD accumulation in 2014 was higher than 2013 for every month of the growing season except for Apr. Though seasonal and yearly total precipitation were similar, precipitation patterns differed between the two years (Table 2.1). Apr, May, Sep, and Oct precipitation was lower in 2013 than in 2014, while the reverse was true for the months of Jun, Jul, and Aug.

Average yield per vine was 8.06 kg in 2013 and 1.82 kg/vine in 2014. The lower yield in 2014 was due to winter injury and a spring frost. Many of the data vines from 2013 were severely injured and different vines were used for 2014. The number of clusters per vine did not differ between years (data not shown). However, average cluster mass was 125 g in 2013 and 40 g in 2014. Within years, there were no differences in yield, cluster weight, or number of clusters between the treatments.

In both 2013 and 2014 canopy characteristics within the fruiting zone were dramatically changed by leaf removal treatments (Table 2.2). Leaf layer number, percentage interior leaves, and percentage interior clusters were decreased and percentage of leaf gaps decreased with increasing severity of leaf removal in both growing seasons. In 2014 cluster exposure flux availability and leaf exposure flux availability also increased with leaf removal. Cluster and leaf exposure flux availability measures were not measured in 2013 due to rainy, overcast weather conditions in Jun, Jul, and Aug when those measures would have been taken.

Berry temperatures. As expected, the time \times aspect interaction was highly significant for berry temperature both years (Table 2.3). When means were pooled across replications and leaf removal treatments east-exposed berries were warmer than west-exposed berries before solar noon and around solar noon. West-exposed grapes had the lowest temperature increase above ambient before noon and the highest temperature and greatest increase above ambient after noon. West-exposed grapes were closer to ambient temperature before-noon than east-exposed grapes were after noon. From 1300 to 1430 hr EST east-exposed berries were heated to a higher degree above ambient than west-exposed berries were.

In 2014 leaf removal \times aspect, leaf removal \times time (Table 2.4) interactions were highly significant. The internal berry temperature of west-exposed grapes increased with increasing leaf removal. Fruit on the eastern-exposure had increased berry temperatures for the medium to high leaf removal treatments. East-exposed grapes were heated to a higher degree above ambient than west-exposed grapes in control and high leaf removal treatments. East-exposed grapes were heated to the highest temperature above ambient in high leaf removal and west-exposed grapes were heated most in medium and high leaf removal treatments. From 1300 to 1430 hr EST and from 1530 to 1700 hr EST, berries from high leaf removal vines were warmer than berries from control vines. From 1000 to 1130 hr EST berries from control and high leaf removal vines were heated to the highest degree above ambient. From 1300 to 1430 hr EST berries from high leaf removal vines were heated to the highest degree above ambient. From 1530 to 1700 hr EST the berries from medium and high treatments were heated above ambient more than control.

Pendant sensor light and temperature. Pendant sensors recorded diurnal changes in light and temperature differences experienced on east and west sides of the canopy (Figure 2.2 and 2.3). Generally the east-exposed sensors recorded higher illuminance and temperatures before noon than west-exposed sensors. Similarly, west-exposed sensors recorded higher illuminance and temperatures after noon than east-exposed sensors. When illuminance was low such as in the afternoon on day 235 there was no difference between the temperature of east and west sensors.

In 2013 leaf removal did not affect either the number of growing degree hours, illuminance or number of hours above the three designated threshold temperatures (Table

2.5). Sensors on the west side of the canopy received the most total illuminance followed by the east side and then shade. East and west-facing sides of the canopy received more growing degree hours (GDH) than the shade. Sensors on the east and west sides logged more hours above 30°C than shade sensors. The west side of the canopy accumulated more hours above 35°C than the east side which accumulated more than shade. The west side accumulated more than seven times the number of hours above 40°C than either east or shade.

In 2014 sensors on control vines received about 23% less total illuminance and logged fewer GDH than those on medium and high treatment vines (Table 2.6). Sensors on medium and high leaf removal vines recorded more hours above 30, 35, and 40°C than control vines. Sensors on the west side of the canopy received the most total illuminance followed by the east side and then shade. GDH did not differ between east, west, and shaded sensors. There were no differences in number of hours above 30°C between aspects. East and west side sensors logged more hours above 35°C than shade, while the west side sensors logged more than twice as many hours above 40°C than either east or shade sensors.

Basic berry composition. In 2013 berry mass was heaviest on day 276 (E-L 38) (Table 2.7). SSC increased on each consecutive sample date. pH was lowest and TA was highest at day 253 (E-L 36). TC520 did not differ between sample dates. Basic berry chemistry did not vary due to leaf removal treatments.

In 2014 berry mass increased with time and SSC peaked at day 258 (E-L 38) (Table 2.8). Berry mass was higher on the east side than west side of the canopy, while SSC, TA, pH and TC520 did not differ due to aspect. Berry pH increased with time and was inversely related to TA. Berries from control vines were heavier than berries from medium and high

leaf removal vines. SSC and TA did not differ due to leaf removal. There was an interaction between leaf removal treatments \times sample date for TC520 (data not shown). On day 231 (E-L 35) and day 252 (E-L 38) TC520 was highest in berries from high leaf removal vines. TC520 peaked around day 252 (E-L 38) and then declined. On day 258 (E-L 38) there was no difference between leaf removal levels in TC520.

Fruit tannin content. In 2013 anthocyanin content per berry and per gram fresh weight were highest on day 276 (E-L 38) (Table 2.9). Seed tannin per berry, seed tannin per gram fresh berry weight, and skin tannin per berry and per gram fresh weight were highest on day 228 (E-L 35). Total skin phenolics per gram were highest on day 231 (E-L 35). Leaf removal treatments had no effect on skin or seed tannins in 2013.

In 2014 anthocyanin content per berry increased at each consecutive sample date (Table 2.10). Anthocyanin concentration was highest on day 231 (E-L 35). Seed tannin content per berry and per gram of berry fresh weight decreased at each sample date. Skin tannin per berry was highest on day 231 (E-L 35) and day 258 (E-L 38). Skin tannin per gram of berry fresh weight was highest on day 211 (E-L 34) and day 231 (E-L 35) and dropped by day 258 (E-L 38). Total skin phenolics per berry were highest on day 231 (E-L 35) and day 258 (E-L 38). Total skin phenolics per gram of berry fresh weight were higher in medium and high leaf removal vines than control vines. In 2014, seed tannin per berry was higher in fruit from the east side than fruit from the west side of the canopy.

HPLC. Of the three sample dates analyzed in 2013, peonidin-3-glucoside, malvidin-3-glucoside, and malvidin-3-acetylglucoside concentrations were highest on day 276 (E-L 38), while petunidin-3-glucoside concentrations were highest on day 253 (E-L 36) (Table

2.11). Quercetin-3-glucuronide concentration was higher on day 253 (E-L 36) than the two later sample dates. Laricitin-3-galactoside concentration was higher on day 266 (E-L 37) than on day 253 (E-L 36). Kamepherol-3-glucoside concentration increased on each sample date from day 253 (E-L 36) to day 266 (E-L 37) to day 276 (E-L 38). There were no differences in the concentration of individual anthocyanins or flavonols between leaf removal treatments.

In 2014 individual anthocyanins were all lowest on day 211 (E-L 34), highest on day 231 (E-L 35), and declined by day 258 (E-L 38) (Table 2.12). Individual flavonol concentrations of laricitin-3-galactoside and kamepherol-3-glucoside followed the same pattern as anthocyanins with regard to change in concentration with date of sampling. The concentration of the flavonols quercetin-3-glucuronide and quercetin-3-glucoside were highest on day 211 (E-L 34). Delphinidin-3-glucoside, cyanidin-3-glucoside, petunidin-3-glucoside, and malvidin-3-glucoside concentrations were higher in west-exposed grapes than east-exposed grapes. Leaf removal treatments did not affect the concentration of individual anthocyanins or flavonols.

Discussion

Leaf removal increased fruit exposure as measured by EPQA. In 2013 the medium treatment decreased the percent interior clusters (PIC) by 27% compared to control and high reduced PIC by 45%. In 2014 the medium treatment decreased the PIC by 46% and the high treatment reduced PIC by 81%. The increased openness of the canopy in the 2014 medium and high treatments was due to the increased magnitude of the leaf removal in 2014 as compared with 2013. In comparison to control and cluster thinned Vignoles vines in New York (Meyers and Vanden Heuvel, 2008) the control and medium leaf removal vines in the

present study are similar in all EPQA metrics except the flux metrics. Control vines in Hector, New York had 40% cluster exposure flux availability and 51% leaf exposure flux availability compared to 18% cluster exposure flux availability and 36% leaf exposure flux availability in the present study. This suggests that although fruiting zone density was similar the above-fruiting zone canopy density is higher in the present study, likely due to higher vigor vines. The denser canopy reduced the amount of sunlight flux that reached the fruiting zone around noon when measurements were taken.

Cloud cover was variable on days that the internal berry temperatures were taken in 2013. Average ambient PAR was lowest from 1000 to 1130 hr ($227 \mu\text{mol m}^{-2}\text{s}^{-1}$), highest from 1300 to 1430 hr ($1023 \mu\text{mol m}^{-2}\text{s}^{-1}$), and intermediate from 1530 to 1700 hr ($576 \mu\text{mol m}^{-2}\text{s}^{-1}$). Level of leaf removal did not affect the degree to which the berries were heated above ambient on the two sample dates in 2013. The reduction in solar radiation due to clouds likely reduced the degree of heating of the exposed berries. In 2014, on days that the internal berry temperatures were recorded there were very few clouds. Average ambient PAR was lowest from 1000 to 1130 hr ($1376 \mu\text{mol m}^{-2}\text{s}^{-1}$), highest from 1300 to 1430 hr ($1771 \mu\text{mol m}^{-2}\text{s}^{-1}$), and intermediate from 1530 to 1700 hr ($1475 \mu\text{mol m}^{-2}\text{s}^{-1}$). With increasing fruit exposure due to leaf removal the degree to which the berries were heated above ambient increased. The highest temperature increase above ambient observed was an east-exposed cluster before noon that was 13.0°C above ambient. West-facing clusters before noon were close to ambient temperature. East-exposed clusters were warmer than west-exposed clusters before noon. Similarly the west exposed clusters were warmer than east exposed clusters after noon. The highest ambient temperatures in both years were around 1800 hr EST.

Pendant sensors within the canopy recorded temperatures that were not different from ambient temperatures before sunrise and after sunset. After sunrise there was increasing variability in the temperature recorded between east-facing, west-facing, and shaded sensors. East-facing sensors generally recorded higher temperatures before noon than shaded or west-exposed sensors. West-exposed sensors recorded higher temperatures after noon than shaded or east-exposed sensors. Average ambient temperature recorded by pendant sensors was correlated to ambient temperature measured by the probe exposed to the air ($r^2 = 0.62$, $p < 0.0001$). Pendant sensors were not correlated ($r^2 = 0.007$, $p = 0.6$) with actual berry temperatures as determined by direct probe (data not shown). Pendant sensors recorded temperatures that were on average 0.7°C higher than the corresponding internal berry temperatures in the morning. Around noon the difference was 1.9°C and after noon the difference was 5.2°C . The increasing temperature difference throughout the day suggests that the pendant sensors retained heat from solar radiation longer than the corresponding berries did. Although the pendant sensors were not correlated to internal berry temperatures they responded to the diurnal changes in daily temperatures in a similar pattern as temperatures found with the direct berry probe suggesting that the data is a worthwhile indicator of berry temperatures throughout the day .

In both years there were no differences between leaf removal treatments in flavonol concentration. Previous studies found that shaded grapes had lower flavonol concentrations than sun-exposed grapes (Price et al. 1995, Haselgrove et al. 2000, Ristic et al. 2006, Spayd et al. 2002). Downey et al. 2004 found that shaded Shiraz grapes had greatly reduced expression of the gene encoding flavonol synthase which significantly reduced flavonol

synthesis compared to unshaded grapes. In the present study control grapes may have received enough light that flavonol synthesis was not inhibited by the degree of shading they experienced.

In 2014 the berries which were exposed to less high temperatures (control and east) had higher berry weight than berries that were exposed to longer periods of high temperatures (medium, high, and west). Crippen and Morrison (1986) related the higher transpiration of sun-exposed fruit to reduced berry weight. In contrast Jogaiah et al. (2013) found higher berry mass from leaf removal vines compared to control. In the present study in 2014, anthocyanins/berry increased at each date as berry mass increased. However, anthocyanins/g FW dropped from day 231 (E-L 35) to day 258 (E-L 38) due to a faster rate of engustation of water than anthocyanin accumulation. Similarly, in 2014 skin tannins/g FW decreased from day 231 (E-L 35) to 258 (E-L 38) while skin tannins/berry did not change.

Leaf removal had no significant effects on yield, SSC, TA, and pH in agreement with previous studies (Chorti et al. 2010, Kosteridis et al. 2012). Likewise, leaf removal did not affect total skin anthocyanin concentration, this in agreement with Chorti et al. (2010). This finding could be a result of elevated berry temperatures counteracting the positive effects of increased light exposure (Bergqvist et al. 2001, Tarara et al. 2008). Genes related to anthocyanin biosynthesis are down-regulated above 30°C (Yamane et al. 2006). In 2014 on the days berry temperatures were measured the berries were above 30 or 35°C at 1000 hr EST. In climates with high daytime and nighttime temperatures, such as central North Carolina, differences in cluster exposure to light may have little impact on anthocyanin accumulation. There was no significant increase in anthocyanin as measured by HPLC or

Illand method. Both leaf removal treatments did however improve the content and concentration of total skin phenolics. Total color was greater in berries from high leaf removal vines at post-veraison sampling dates (days 231 and 252) in 2014 but there was no difference at harvest. The increase in total color and skin total phenolic concentration may be due in part to the lower mass of berries from high leaf removal vines which increased the skin to juice ratio. In comparison to Merlot in Prosser, Washington (Spayd et al. 2002) the TC520 for the Cabernet Franc in the present study is approximately 4 to 18 times lower and also experienced approximately 1000 more GDD. Locations that have fewer extreme temperatures may see increased anthocyanin concentrations with leaf removal. The reduced effectiveness of leaf removal in North Carolina should be considered by growers of Cabernet Franc or other red grape cultivars when deciding whether or not to perform the expensive and time-consuming task. Leaf removal may also have other benefits that were not measured by this study such as reduced disease incidence. Leaf removal was shown to reduce incidence of fruit rot in the humid climate of Virginia (Zoecklein et al. 1992). Although disease incidence was not measured there were no observed differences between leaf removal treatments despite the control vines having a measurably denser canopy. Light exposure has been found to decrease the accumulation of methoxypyrazines in Cabernet Franc (Ryona et al. 2008) and Sauvignon Blanc, (Gregan et al. 2012) which is another reason why a grower may want to perform leaf removal. The potential benefits of leaf removal (i.e. improved phenolic content, reduced methoxypyrazines, reduced disease pressure, etc.) most likely outweigh the costs (i.e. high expense, risk of sunburn to grapes, etc.) however, North Carolina growers should

be aware that the benefit to anthocyanin concentration may not be as pronounced as those reported in cooler, dryer climates.

Conclusion

Basal leaf removal increased sunlight exposure in the fruiting zone resulting in increased berry temperatures. The fruiting zone of leaf removal vines experienced more hours above 35 and 40°C than that of control vines. Similarly the west-exposed fruiting zone experienced more hours above 35 and 40°C than the east-exposed fruiting zone. Temperatures above 35°C negatively impact anthocyanin accumulation. Despite experiencing longer periods of elevated temperatures the total skin phenolic concentration and content per berry were elevated in medium and high leaf removal vines in 2014 but not in 2013. In climates with high daytime and nighttime temperatures, such as in central North Carolina, increases in cluster light exposure may have little impact on anthocyanin accumulation due to long periods of time above 35°C. The influence of leaf removal on grape phenolic composition in North Carolina may be less profound than that seen in cooler climates.

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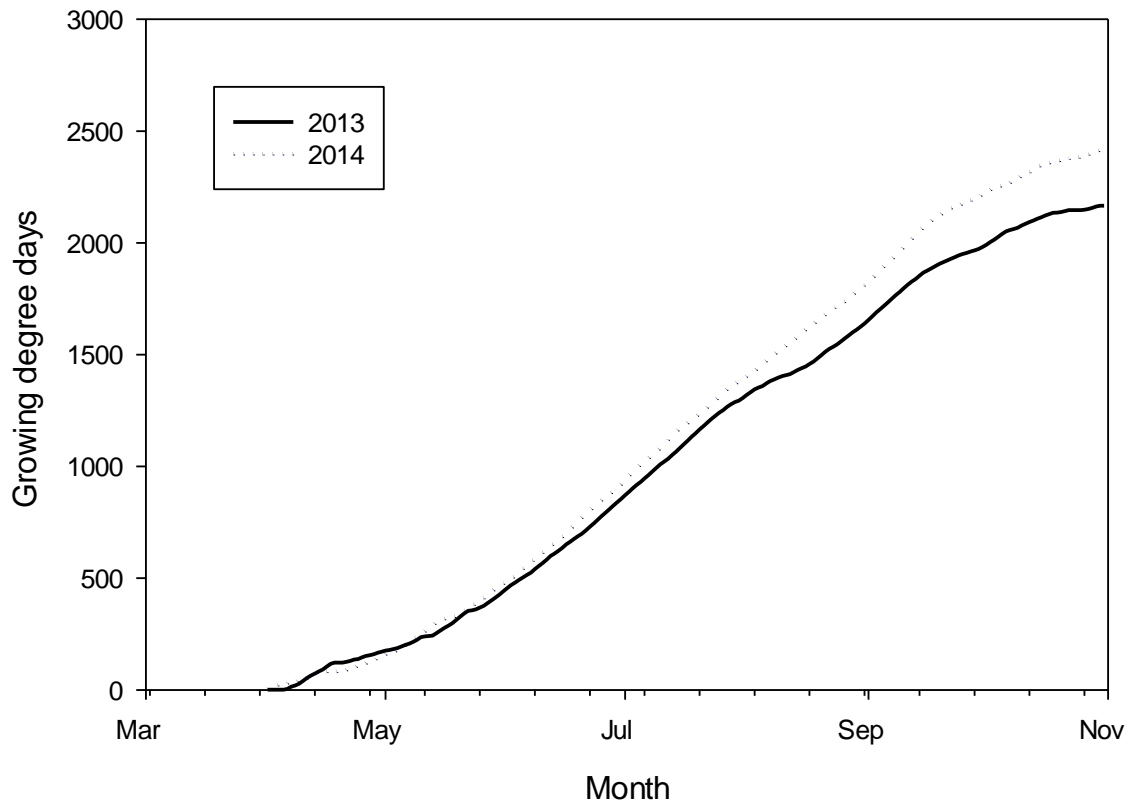


Figure 2.1 Growing degree day accumulation (base 10°C) in Lexington, North Carolina. Missing data filled from CRONOS station 314970, lat. 35°85'N; 80°26'W (<https://www.nc-climate.ncsu.edu/cronos/?station=314970&temporal=D>).

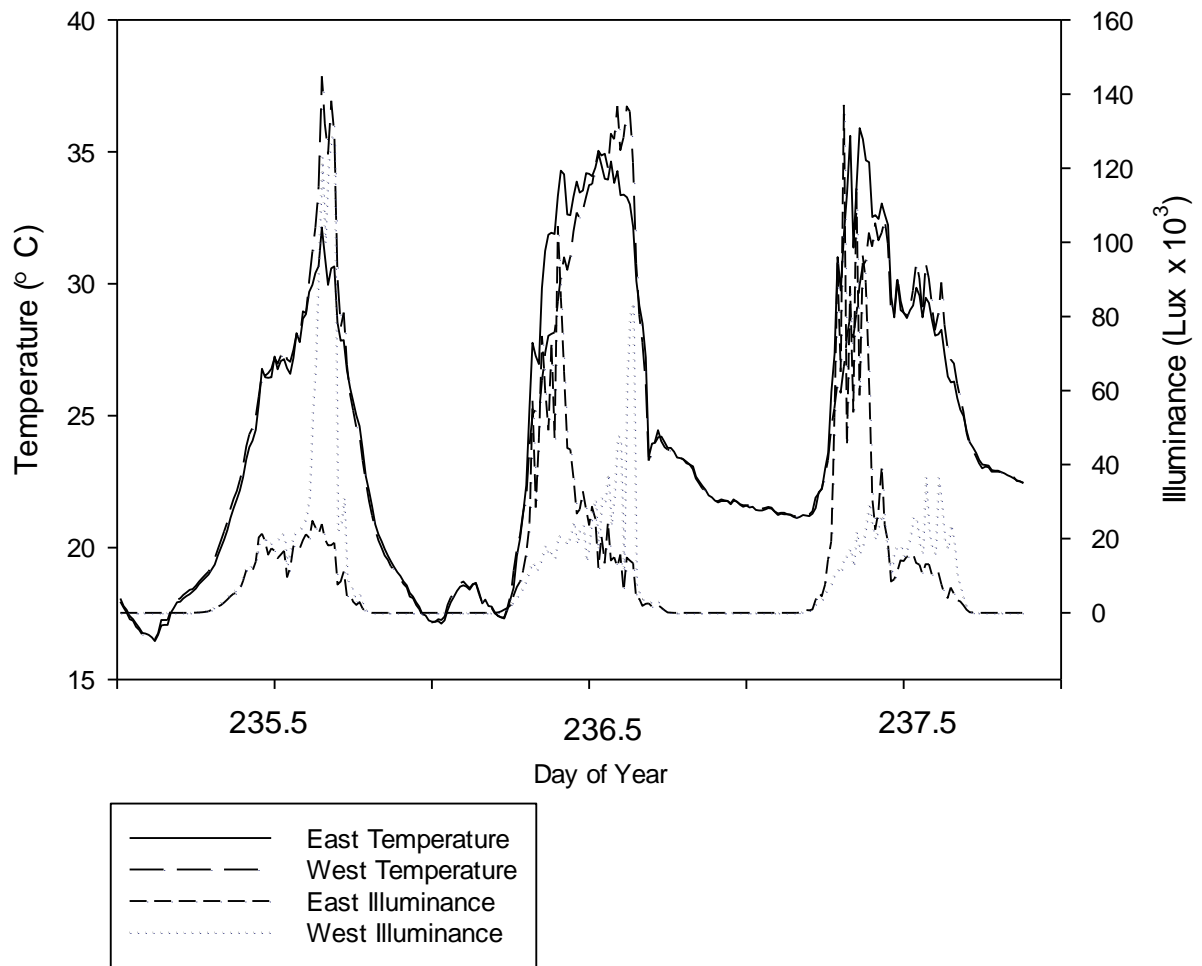


Figure 2.2 Pendant sensor recorded temperature and irradiance (east-exposed or west-exposed) from day 235 to day 237, 2014 in control vines.

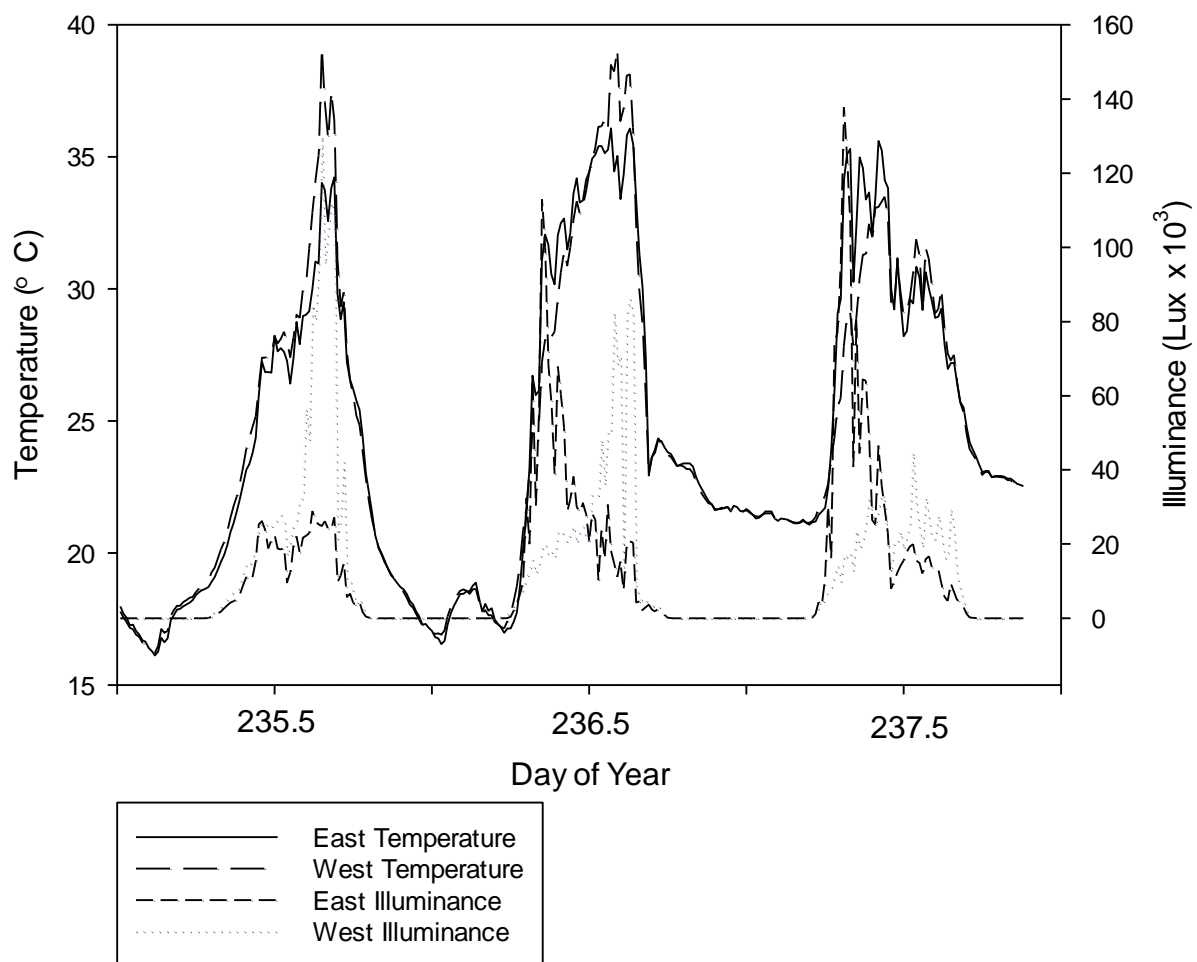


Figure 2.3 Pendant sensor recorded temperature and irradiance (east-exposed or west-exposed) from day 235 to day 237, 2014 in high leaf removal vines.

Table 2.1 Precipitation in Lexington, NC, 2013 and 2014. Historical data from CRONOS station 314970, lat. 35°85'N; 80°26'W
(<https://www.nc-climate.ncsu.edu/cronos/?station=314970&temporal=D>).

	Precipitation (mm)		
	2013	2014	1971-2000
April	76	119	92
May	49	114	100
June	172	97	103
July	128	76	98
August	167	83	92
September	37	52	98
October	24	60	89
Season	653	601	672
Year total	1027	997	1153

Table 2.2 Effect of leaf removal on enhanced point quadrat analysis (EPQA) variables in 2013 and 2014.

EPQA variables	2013			2014		
	Control	Medium	High	Control	Medium	High
Gaps (%)	0.0b ^a	2.8b	7.3a	0.7b	4.9b	22.2a
Leaf layer number	2.6a	2.1b	1.5b	3.0a	2.3a	1.4a
Interior leaves (%)	39.2a	31.0ab	24.1b	38.6a	28.2b	19.4c
Interior clusters (%)	61.4a	45.1ab	33.7b	77.6a	41.8b	14.6c
Occlusion layer number	3.6a	2.7b	2.3c	3.3a	2.5b	1.5c
Cluster exposure layer	0.8a	0.6ab	0.4b	0.9a	0.5a	0.2b
Leaf exposure layer	0.5a	0.3ab	0.3b	0.5a	0.3a	0.2a
Cluster exposure flux availability	--- ^b	---	---	0.18c	0.39b	0.59a
Leaf exposure flux availability	--- ^b	---	---	0.36c	0.42b	0.52a

^aMeans within main effects within years having the same letters are not significantly different, $p \leq 0.05$.

^bCanopy sunlight penetration measures not made in 2013, therefore flux availability values were not obtained that year.

Table 2.3 Influence of aspect and time interaction on internal berry temperature and difference from ambient temperature of Cabernet Franc berries in 2013 and 2014.

Time (hr)	Ambient Temperature (°C)	Berry Temperature (°C)		ΔT^a (°C)	
		East	West	East	West
2013					
1000 - 1130	17.9c ^b	20.1e	18.1f	2.2b	0.2d
1300 - 1430	23.9b	25.1c	24.4d	1.3c	0.5d
1530 - 1700	24.8a	26.2b	29.5a	1.2c	4.9a
2014					
1000 - 1130	29.7c	35.1b	30.1f	5.3a	0.6d
1300 - 1430	31.6b	33.1d	32.3e	1.4c	0.8d
1530 - 1700	32.6a	34.1c	35.9a	1.5c	3.3b

^aDifference in berry temperature from ambient.

^bMeans within year within ambient temperature, berry temperature and ΔT having the same letters are not significantly different at $p \leq 0.05$.

Table 2.4 Influence of leaf removal × aspect and leaf removal × time on internal berry temperature and difference from ambient temperature of Cabernet Franc berries in 2014.

	Berry Temperature (°C)			ΔT (°C) ^a		
	Control	Medium	High	Control	Medium	High
Aspect						
East	34.1ab ^b	33.9b	34.3a	2.7b	2.2c	3.2a
West	32.1e	32.9d	33.3c	1.0d	1.9c	1.8c
<i>p</i> ≤	0.001			0.001		
Time (hr)						
1000 - 1130	32.8de	32.2f	32.8de	3.2a	2.6c	3.1ab
1300 - 1430	32.4ef	32.7de	32.9d	0.9e	0.9e	1.5d
1530 - 1700	34.1c	35.2b	35.7a	1.5d	2.7bc	2.9abc
<i>p</i> ≤	0.001			0.001		

^aDifference in berry temperature (°C) from ambient.

^bMeans within berry temperature and ΔT having the same letters are not significantly different at *p*≤0.05.

Table 2.5 Growing degree hours (base 10°C) and time above threshold temperatures experienced by data loggers from day 242 to 276, 2013.

	Illuminance ^a (lux x 10 ⁷)	Growing degree hours ^b (x 10 ³)	Time above threshold temperature (hr)		
			>30°C	>35°C	>40°C
Leaf removal					
Control	4.53a ^d	9.6a	140.7a	43.5a	7.6a
Medium	4.85a	9.6a	143.5a	44.1a	9.7a
High	4.44a	9.6a	135.6a	40.1a	9.2a
Aspect					
East	4.84b	9.7a	153.4a	38.9b	3.1b
West	5.95a	9.7a	143.6a	66.2a	21.5a
Shaded	3.03c	9.5b	122.7b	22.6c	1.9b
Significance^c					
Leaf removal	ns	ns	ns	ns	ns
Aspect	<0.001	0.02	<0.001	<0.001	<0.001

^aSum of illuminance (lux x 10⁷)

^bGrowing degree hours base 10°C

^c*p-values* of treatment effects and significant interactions (ns = not significant).

^dMeans within main effects having the same letters are not significantly different at $p \leq 0.05$.

Table 2.6 Growing degree hours (base 10°C) and time above threshold temperatures experienced by data loggers from day 175 to 211 and day 233 to 258, 2014.

	Illuminance ^a (Lux x 10 ⁷)	Growing degree hours ^b (x 10 ³)	Time above threshold temperature (hr)		
			>30°C	>35°C	>40°C
Leaf Removal					
Control	8.3b ^d	22.6b	391.9b	118.7b	12.9b
Medium	10.4a	23.2a	422.6a	171.5a	35.4a
High	11.2a	23.2a	432.0a	183.5a	41.5a
Aspect					
East	10.0b	23.0a	431.3a	160.5a	13.9b
West	11.8a	23.2a	407.2a	178.7a	53.4a
Shaded	8.0c	22.9a	408.0a	134.4b	22.6b
Significance^c					
Leaf removal	<0.001	<0.001	0.003	0.005	0.002
Aspect	<0.001	ns	ns	0.02	<0.001

^aSum of illuminance (lux x 10⁷)

^bGrowing degree hours base 10°C

^cMeans within main effects having the same letters are not significantly different, $p \leq 0.05$.

^d*p-values* of treatment effects and significant interactions (ns = not significant).

Table 2.7 Effect of sample date (day of year, DOY) and leaf removal on berry composition in 2013.

	Berry mass (g/berry)	Soluble solids (%)	Titrateable acidity (g/L)^a	pH	Total color (AU at 520 nm/mL)
DOY					
253	1.76b ^c	16.2c	5.6a	3.86b	0.810a
266	1.80a	18.0b	4.8b	3.98a	0.535a
276	1.99a	19.4a	4.7b	4.00a	0.830a
Leaf removal					
Control	1.83a	18.2a	5.0a	3.94a	0.640a
Medium	1.84a	17.6a	4.9a	3.97a	0.734a
High	1.88a	17.8a	5.2a	3.93a	0.800a
Significance^b					
Leaf removal	ns	ns	ns	ns	ns
DOY	<0.001	<0.001	<0.001	<0.001	ns

^aExpressed as g tartaric acid/L

^b*p-values* of treatment effects and significant interactions (ns = not significant).

^cMeans within main effects having the same letters are not significantly different, $p \leq 0.05$.

Table 2.8 Effect of sample date (day of year, DOY), leaf removal, and aspect on berry composition in 2014.

	Berry mass (g/berry)	Soluble solids (%)	Titratable acidity (g/L)^a	pH	Total color (AU at 520 nm/mL)
DOY					
211	0.92d ^c	--- ^d	--- ^d	-- ^d	0.41c
231	1.17c	16.8b	9.05a	3.36c	5.40b
252	1.36b	21.5a	5.03b	3.80b	7.00a
258	1.44a	21.8a	4.71c	3.86a	5.73b
Leaf removal					
Control	1.26a	20.2a	6.22a	3.66b	4.14b
Medium	1.21b	19.9a	6.40a	3.66b	4.52a
High	1.19b	20.1a	6.17a	3.70a	5.24a
Aspect					
E	1.26a	20.0a	6.28a	3.67a	4.70a
W	1.18b	20.1a	6.24a	3.67a	4.57a
Significance^b					
DOY	<0.001	<0.001	<0.001	<0.001	<0.001
Leaf removal	0.03	ns	ns	ns	<0.001
LR x DOY	ns	ns	ns	ns	0.03
Aspect	<0.001	ns	ns	ns	ns

^aExpressed as g tartaric acid/L

^b*p*-values of treatment effects and significant interactions (ns = not significant).

^cMeans within main effects having the same letters are not significantly different at $p \leq 0.05$.

^dInsufficient juice for complete analysis on day 211.

Table 2.9 Influence of sample date (day of year, DOY) and treatment on phenolic content and concentration as determined by Iland method and BSA precipitation, 2013.

	Anthocyanins		Seeds tannins		Skin			
					Tannins		Phenols	
	mg/ berry ^a	mg/g ^a	mg/ berry ^b	mg/g ^b	mg/ berry ^b	mg/g ^b	mg/ berry ^b	mg/g ^b
DOY								
253	0.24b ^d	0.17b	4.80a	3.37a	0.83a	0.59a	1.20a	0.85a
266	0.29b	0.17b	2.69b	1.60b	0.68b	0.41b	1.13a	0.67b
276	0.47a	0.26a	2.32b	1.29b	0.60b	0.34b	1.03a	0.57b
Leaf removal								
Control	0.31a	0.18a	3.30a	2.11a	0.68a	0.44a	1.05a	0.66a
Medium	0.33a	0.20a	3.26a	2.12a	0.69a	0.43a	1.12a	0.71a
High	0.37a	0.21a	3.24a	2.04a	0.74a	0.46a	1.18a	0.73a
Significance^c								
Leaf removal	ns	ns	ns	ns	ns	ns	ns	ns
DOY	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	ns	<0.001

^aAnthocyanins presented in mg malvidin-3-glucoside equivalents per berry and per gram berry fresh weight.

^bTannins and phenols presented in mg catechin equivalents per berry and per gram berry fresh weight.

^c*p-values* of treatment effects and significant interactions (ns = not significant).

^dMeans within main effects having the same letters are not significantly different at $p \leq 0.05$.

Table 2.10 Influence of sample date (day of year, DOY), leaf removal, and aspect on phenolic content and concentrations as determined by Iland method and BSA precipitation, 2014.

	Anthocyanins		Seed tannins		Skin			
					Tannins		Phenols	
	mg/ berry ^a	mg/g ^a	mg/ berry ^b	mg/g ^b	mg/ berry ^b	mg/g ^b	mg/ berry ^b	mg/g ^b
DOY								
211	0.06c ^d	0.08c	5.44a	5.90a	0.67b	0.74ab	1.07b	1.17a
231	0.59b	0.55a	4.50b	3.95b	0.98a	0.87a	1.34a	1.18a
258	0.71a	0.50b	2.23c	1.60c	0.84a	0.61b	1.46a	1.06a
Leaf removal								
Control	0.43a	0.35a	4.03a	3.80a	0.80a	0.71a	1.13b	1.00b
Medium	0.44a	0.38a	3.99a	3.71a	0.87a	0.77a	1.41a	1.24a
High	0.49a	0.40a	4.16a	3.94a	0.82a	0.73a	1.33a	1.18a
Aspect								
E	0.47a	0.37a	4.23a	3.85a	0.84a	0.73a	1.26a	1.10a
W	0.43a	0.38a	3.89b	3.78a	0.83a	0.75a	1.31a	1.18a
Significance^c								
Leaf removal	ns	ns	ns	ns	ns	ns	0.001	0.01
Aspect	ns	ns	0.04	ns	ns	ns	ns	ns
DOY	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	ns

^aAnthocyanins presented in malvidin-3-glucoside equivalents per berry and per gram berry fresh weight.

^bTannins and phenols presented in catechin equivalents per berry and per gram berry fresh weight.

^c*p-values* of treatment effects and significant interactions (ns = not significant).

^dMeans within main effects having the same letters are not significantly different at $p \leq 0.05$.

Table 2.11 Influence of sample date (day of year, DOY), and leaf removal on anthocyanin and flavonol concentrations as determined by HPLC, 2013.

	Anthocyanins (μg malvidin 3-glucoside equivalents/ g fresh weight)					Flavonols (μg quercitin-3-glucoside equivalents/ g fresh weight)			
	P3G^a	M3G	P3AG	M3AG	Pet3CG	Q3GR^b	Q3G	L3G	K3G
DOY									
253	64.4b ^d	123.2b	23.8a	58.1b	63.7a	4.6a	3.6a	0.6b	0.6c
266	45.2b	99.3b	17.7a	53.8b	44.9a	2.8b	3.7a	1.1a	1.4b
276	91.4a	159.5a	20.6a	82.5a	46.9a	2.3b	4.2a	0.9ab	1.8a
Leaf removal									
Control	65.4a	120.4a	19.0a	65.2a	54.1a	3.2a	4.1a	0.9a	1.3a
Medium	65.8a	121.9a	21.5a	56.9a	48.0a	3.0a	3.4a	0.8a	1.2a
High	69.9a	139.7a	21.8a	72.4a	53.3a	3.4a	4.0a	0.9a	1.2a
Significance^c									
Leaf removal	ns	ns	ns	ns	ns	ns	ns	ns	ns
DOY	<0.001	0.001	ns	0.02	ns	<0.001	ns	0.03	<0.001

^aP3G: peonidin-3-O-glucoside; M3G: malvidin-3-O-glucoside; P3AG: peonidin-3-O-acetylglucoside; M3AG: malvidin-3-O-acetylglucoside; Pet3CG: petunidin-3-O-(6-O-coumaryl-glucoside).

^bQ3GR: quercitin-3-O-glucuronide; Q3G: quercitin-3-O-glucoside; L3G: laricitin-3-O-galactoside; K3G: kamepherol-3-O-glucoside.

^c*p-values* of treatment effects (ns = not significant).

^dMeans within main effects having the same letters are not significantly different at $p \leq 0.05$.

Table 2.12 Influence of sample date (day of year, DOY), leaf removal, and aspect on anthocyanin and flavonol concentrations as determined by HPLC, 2014.

	Anthocyanins (µg malvidin 3-glucoside equivalents/ g fresh weight)								Flavonols (µg quercetin-3-glucoside equivalents/ g fresh weight)			
	D3G ^a	C3G	Pet3G	P3G	M3G	P3AG	M3AG	P3CG	Q3GR ^b	Q3G	L3G	K3G
DOY												
211	0.0c ^d	0.0c	0.0c	15.8c	14.9c	1.2c	6.9c	1.5c	11.5a	15.8a	0.0c	0.0c
231	30.3a	36.8a	38.6a	41.0a	42.1a	53.3a	54.1a	61.1a	1.6b	1.6b	1.9a	2.0a
252	22.9b	26.8b	28.5b	30.7b	31.8b	39.4b	40.0b	44.5b	1.1b	1.2b	1.3b	1.3b
Leaf removal												
Control	17.1a	20.0a	21.2a	30.7a	28.5a	30.9a	19.8a	35.7a	4.0a	6.9a	1.1a	1.1a
Medium	18.6a	22.2a	23.4a	28.2a	30.9a	32.3a	19.9a	36.5a	4.5a	5.2a	1.1a	1.1a
High	17.6a	21.5a	22.4a	28.6a	29.3a	30.7a	21.7a	34.9a	5.89a	6.5a	1.0a	1.1a
Aspect												
E	16.7b	19.8b	20.9b	28.8a	28.1b	39.9a	24.8a	34.3a	5.0a	6.7a	1.0a	1.1a
W	18.8a	22.6a	23.8a	29.5a	31.1a	32.7a	25.3a	37.1a	4.5a	5.7a	1.1a	1.1a
Significance^c												
Leaf removal	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Aspect	0.001	0.01	0.001	ns	0.04	ns	ns	ns	ns	ns	ns	ns
DOY	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

^aD3G: Delphinidin-3-glucoside; C3G: Cyanidin-3-O-glucoside; Pet3G: Petunidin-3-O-glucoside; P3G: peonidin-3-O-glucoside; M3G: malvidin-3-O-glucoside; P3AG: peonidin-3-O-acetylglucoside; M3AG: malvidin-3-O-acetylglucoside; Pet3CG: petunidin-3-O-(6-O-coumaryl-glucoside)

^bQ3GR: quercetin-3-O-glucuronide; Q3G: quercetin-3-O-glucoside; L3G: laricitin-3-O-galactoside; K3G: kamepherol-3-O-glucoside

^c*p-values* of treatment effects and significant interactions (ns = not significant).

^dMeans within main effects having the same letters are not significantly different at $p \leq 0.05$.

APPENDICES

Appendix A. Modified Eichhorn and Lorenz (E-L) system.

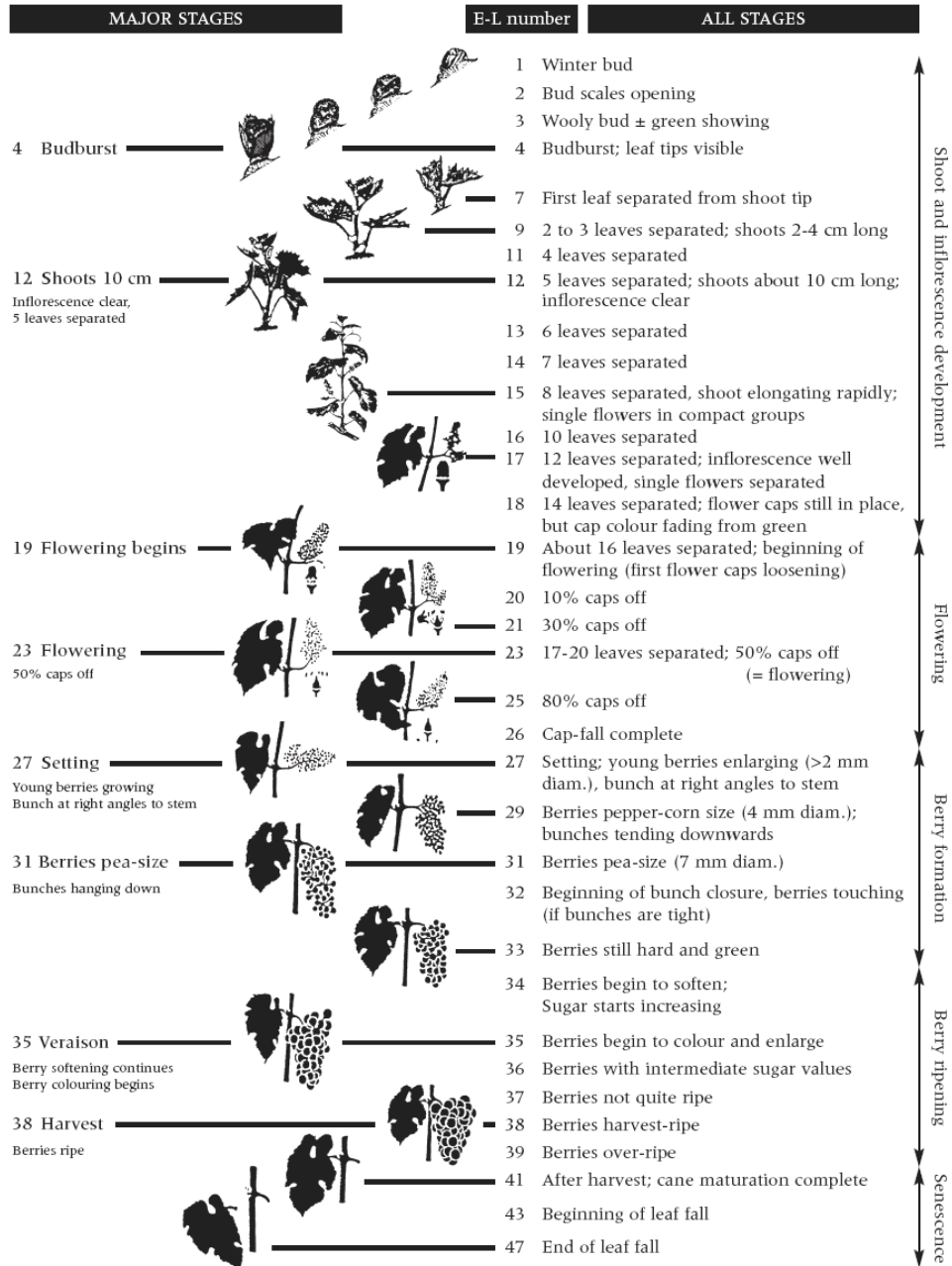


Figure 7.3 Modified E-L system for identifying major and intermediate grapevine growth stages (revised from Coombe 1995). Note that not all varieties show a woolly bud or a green tip stage (May 2000) hence the five budburst stages in the modified original 1995 system have been changed slightly by removing stage 4 and allocating the definition of budburst to what was formerly stage 5. Revised version of "Grapevine growth stages - The modified E-L system" Viticulture 1 - Resources. 2nd edition 2004. Eds. Dry, P. and Coombe, B. (Winetitles)

Revised version of "Grapevine growth stages - The modified E-L system" Dry, P. and B. Coombe. 2004. Viticulture 1 – Resources. Ed. 2 Modified from Coombe (1995). Originally published by Eichhorn, K.W. and H. Lorenz (1977).

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