Separation of Sunlight and Temperature Effects on the Composition of *Vitis vinifera* cv. Merlot Berries

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Anthocyanin and phenolic profiles of berry skins from *Vitis vinifera* cv. Merlot in the Yakima Valley of Washington were influenced by sun exposure and temperature in 1999 and 2000. Growing degree days (base 10°C) accumulated between veraison and harvest were lower in 2000 than in 1999. Total skin monomeric anthocyanin (TSMA) concentrations were higher in 2000 than in 1999 in any given treatment. Berry temperature was increased as much as 13°C above ambient and shaded cluster temperatures when clusters were exposed to sunlight, regardless of aspect for north-south oriented rows. However, maximum fruit temperatures were higher for clusters on the west side of the canopy because ambient temperatures were higher after 1200 hr. Temperatures of west-exposed clusters at times exceeded 40°C. East-exposed clusters had higher TSMA concentrations than west-exposed or shaded clusters. To separate light and temperature effects, west-exposed clusters were cooled to the temperature of shaded clusters and shaded clusters were heated to the temperature of west-exposed clusters. Exposure to sunlight increased TSMA concentrations regardless of temperature in both years. In 1999 and 2000, cooling sun-exposed clusters increased TSMA concentrations. Heating shaded clusters decreased TSMA concentrations in 1999, but had no effect during the cooler ripening period of 2000. Ultraviolet (UV) light barriers did not influence either cluster temperature or TSMA concentrations. Decreased TSMA concentrations in berry skins from west-exposed clusters were due to temperature and not to UV radiation. Exposure to solar radiation increased concentrations of the 3-glycosides of quercetin, kaempferol, and myricetin. In 2000, sun-exposed clusters, regardless of aspect, had almost 10 times greater concentrations of total flavonols than shaded clusters. UV-light barriers significantly reduced individual and total flavonol concentrations, while temperature had little to no effect on their concentrations.

Key words: Canopy management, light, microclimate, UV, color, heat stress, photoregulation, soluble solids, acidity, pH

Canopy management has received considerable research attention during the past several decades [5,18,26,27,28]. The intent of this research was to develop viticultural practices that provided adequate exposure of fruit to sunlight while providing adequate, but not excessive, leaf area to ripen the fruit and improve fruit composition at harvest. These practices included trellis systems and leaf, shoot, or partial shoot removal (hedging). Cool climates, where fruit maturation may be limited, were to a great extent the impetus for much of this research. However, viticultural practices developed for climates with limited solar radiation are at times adopted in other regions with little consideration for the effects of excessive exposure of fruit to solar radiation. To achieve maximum color development in warm regions, prolonged exposure of clusters to sunlight should be avoided [1]. With the advent of viticultural practices that resulted in much more open canopies (for example, deficit irrigation, leaf removal, and reduced use of nitrogen fertilizer) in eastern Washington, increased sunburning of fruit was observed, particularly on the west side of north-south oriented rows and on the south side of east-west oriented rows.

The relationship between sunlight exposure and temperature of grape clusters is important to berry composition and metabolism. Millar [22] showed that berry temperatures paralleled the diurnal solar radiation curve. Differences in temperature between ambient air and exposed fruit increased as solar radiation increased and wind speed decreased, as one might expect from heat transfer principles. Smart and Sinclair [29], who pursued an energy balance approach, indicated that solar radiation and wind velocity were the two most important determinants of fruit temperature: during the day shortwave radiation was the primary source of fruit warming and convection was the primary source of heat transfer away from the cluster. Fruit size, albedo, wind direction, and net long-wave radiation were less important. They reported that shaded grape berries typically were 2.4°C above ambient, while “hot-spot” temperatures on tight and loose clusters were up to 12.4 and 11.1°C above ambient, respectively. Elsewhere, sunlit leaves and clusters were 5 to 10°C higher than shaded leaves and fruit [16]. In another study, shaded clusters were cooler than sun-exposed clusters during the day, but were warmer than sun-exposed clusters at

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night [6], suggesting greater net radiation loss by the exposed berries at night.

For red-fruited cultivars, shading of clusters decreased color and anthocyanin concentrations [9,30], decreased soluble solids [9,24], and increased titratable acidity [9,24]. Exposure to sunlight increased total phenol concentration in Pinot noir skin disks, but did not affect anthocyanin concentrations [23]. Concentrations of quercetin glycosides were 10-fold greater in skin disks from sun-exposed berries than from shaded berries. The response of berry growth and physiology to light varies during fruit development [7].

The effects of temperature on grape berry composition have been studied extensively, primarily in growth chambers, glasshouses, and phytotrons to compare constant day and/or night temperatures [3,7,15,16,17,21]. In one phytotron study, cool day temperature (15°C) during ripening improved color development in Cardinal, Pinot noir, and Tokay berries, while hot day temperature (35°C) significantly reduced or completely inhibited formation of anthocyanins [17]. A cool night temperature (10 or 15°C) did not reverse the effect of hot day temperature on berry color. Grapes from vines held at warm day (25°C) and cool night (15°C) temperatures developed less color than those from vines held at cool day and night temperatures (both 15°C). The higher anthocyanin content at the cooler temperatures was not related to juice soluble solids concentrations, which tended to be greater at the higher day temperatures. In a glasshouse, berry color development for Cabernet Sauvignon was greater when day temperature was a constant 20°C than a constant 30°C, both with a constant night temperature of 15°C [3]. Soluble solids did not differ. At the higher day temperature, berries had higher proline and malate concentrations.

Separating the effects of temperature and sunlight on grape berry composition is difficult because many of the biochemical pathways are both light and temperature sensitive. In a phytotron study using container-grown vines, coloration of Pinot noir grapes was always less under low [5.38×10^3 to 2.15×10^4 Lx(500 to 2000 ft-c)] than high [2.69×10^4 to 5.38×10^4 Lx (2500 to 5000 ft-c)] light conditions at either a low (20°C) or a high (30°C) temperature [15]. With a constant daytime temperature of 30°C, fruit temperatures were between 33 and 35°C. Spectrophotometric measurements indicated a slight reduction in anthocyanin concentration in Pinot noir berries grown at the higher temperature. Cardinal berry color was more sensitive to high temperature (less color) but less sensitive to light intensity than Pinot noir. Daytime fruit temperatures of 32 to 36°C nearly inhibited anthocyanin synthesis in Cardinal.

The objectives of this study were to examine how the composition of Merlot grapes was affected by sunlight exposure and to separate, in situ, the effects of temperature from solar radiation on the anthocyanin and phenol composition of Merlot grapes.

Materials and Methods

The study was conducted during 1999 and 2000 at the Irrigated Agriculture Research and Extension Center (IAREC) in Prosser, Washington (lat. 46.30°N, long. 119.75°W) within a 1.2-ha vineyard planted in 1983. The experimental plot was planted to *Vitis vinifera* L. cv. Merlot, comprising three rows of 13 vines each, oriented N-S. Vines were double-trunked, trained to a bilateral cordon at 1.2 m, and spur-pruned. Each row was treated as a replicate (three replicates). Each cordon was treated independently of its mate in terms of sunlight exposure on the clusters. Clusters of a given cordon were either shaded or sun-exposed by positioning shoots to the side of the canopy that was to be shaded. Shoots were brought over a "wind" wire (1.5 m) and tied to a catch wire (1.2 m) parallel to the cordon and wind wires. Because one layer of *V. vinifera* leaves will absorb 80 to 90% of incident solar radiation [27], this natural shading technique was expected to allow predominantly only diffuse light to strike the shaded clusters.

In 1999, berry samples were collected randomly twice after veraison from clusters that were (1) exposed to direct sunlight on the east side of the canopy, (2) exposed to direct sunlight on the west side of the canopy, (3) shaded from sunlight on the east side of the canopy, and (4) shaded from sunlight on the west side of the canopy. Because no differences were found in the 1999 preharvest samples from shaded clusters, fruit from the two shaded treatments was pooled for analysis at harvest in 1999 and both before and at harvest in 2000. Only one preharvest sample was obtained in 2000 because a frost (-2.2°C) on 23 September curtailed the experiment.

In 2000, an additional fruit exposure treatment was added to determine the influence of ultraviolet radiation (UV). Acrylic sheets (0.9 m x 1.2 m x 3.75 mm thick) that either absorbed (98% below 400 nm; Acrylite OP-2, Cyro Industries, Mt. Arlington, NJ) or transmitted UV (Acrylite OP-1, Cyro Industries) were installed at a 45° angle, 0.5 to 0.8 m in front of west-facing, sun-exposed fruit. The sheets extended from just above the canopy to just below the fruiting zone. Cluster temperatures were recorded for four clusters per cordon (n=8 per screening material). A temperature-stabilized UV-B sensor (280 to 320 nm; model CUV-B1, Kipp and Zonen, Delft, The Netherlands) was used to determine the actual transmittance of the screening materials.

In situ temperature control study. All clusters used were on the west side of the canopy of three adjacent vines. Four clusters were monitored in 1999 and 2000 in each of six treatments: (1) sun-exposed (sun control); (2) shaded by shoots (shade control); (3) sun-exposed and chilled to the temperature of shaded clusters (sun-cooled); (4) shaded and heated to the temperature of sun-exposed clusters (shade-heated); (5) sun-exposed with ambient air blown on the cluster at same rate as chilled air (sun-blower); and (6) shaded with ambient air blown on the cluster at same rate as heated air (shade-blower). The sun-blower and shade-blower treatments served as controls for the effects of forced convection. The system used for heating and cooling the clusters is described elsewhere [31]. Briefly, control clusters were used as "thermostats" to determine the temperature to which the treated clusters were to be heated or cooled. Shaded clusters set the temperature for sun-exposed, cooled clusters, while sun-exposed clusters set the temperature for shaded, heated clusters. The temperature of the treated clusters provided feedback to the system to control delivery of

heated or chilled air. Temperatures were controlled from around bunch closure to harvest. This period was from 13 Aug to 12 Oct 1999 and from 28 July to 24 Sept 2000.

Because of the limited size of the experimental plot and the small number of clusters involved, fruit was sampled only when clusters were harvested. In 1999, clusters were divided along the vertical axis into exterior and interior faces. Berries from all clusters within a treatment were pooled and then divided into three repetitions for laboratory analysis. In 2000, each cluster was analyzed separately. Interior and exterior face berries were not segregated.

Micrometeorological measurements. Several berries on individual clusters were used to estimate average cluster temperature and the difference between exterior and interior faces of the cluster. A four-junction, fine-wire (36 American Wire Gauge [AWG]) thermocouple (type T [copper-constantan]) wired in parallel was used as a unit for each measurement. Individual junctions (2 mm long) were manually inserted just beneath the berry skin at approximately the equator of the sphere. Berries were selected from near the cluster shoulder and midway along its vertical axis. Before veraison, some berries developed necrosis around the thermocouple insertion hole. These junctions were moved to nearby berries on the same face of the cluster. After veraison, no necrosis was observed at the thermocouple entry points.

Ambient air temperatures inside the canopy and at 2 m above the canopy were measured by shielded, aspirated, fine-wire thermocouples (36 AWG; type T). Global irradiance was measured by a pyranometer (model 8-48, Eppley Laboratories, Newport, RI). Irradiance at the fruiting zone was measured parallel to shaded and sun-exposed cordons by 1-m long tube solarimeters (model TSL, Delta-T Devices, Cambridge, UK). All signals were scanned at 10-sec intervals and averaged every 12 min by a datalogger (CR-10X, Campbell Scientific, Logan, UT) that also controlled multiplexers designed specifically for thermocouples (AM25T, Campbell Scientific, Logan, UT). Data were collected continuously for 60 days in 1999 and 58 days in 2000, approximately from bunch closure to harvest. Wind speed at 2 m was measured by a 3-cup anemometer (Wind Sentry, R.M. Young, Traverse City, MI) at the Public Agriculture Weather System station at the IAREC facility. Signals were recorded every 10 sec and averaged every 15 min.

Laboratory analyses. Fifty to 100 berries from each treatment were counted, weighed, and used for fresh berry analyses. Twenty berries were subsampled from each treatment and frozen at -35°C for later anthocyanin and phenol analyses. Rohapect D5-L (Scott Laboratories, San Rafael, CA) was added to the equivalent of 1 mL of enzyme/kg of fresh berries. Berries were pureed in a laboratory blender at high speed for 1 min. The homogenate was filtered through fluted filter paper (no. 588, Schleicher and Schuell, Keene, NH). The filtrate was used for determination of percent soluble solids, pH, titratable acidity (TA), and total color at 520 nm (TC520). Percent soluble solids was measured using a temperature-compensating Abbé refractometer (Model 10450, American Optical Corp., Buffalo, NY). The pH was measured with a pH meter (model 455, Corning Inc., Kennebunk, ME) standardized to pH 7.0 and 4.0. Titratable acidity was determined by titrating 5 mL of juice diluted with 100 mL of boiling distilled water to pH 8.2 with 0.1 *N* NaOH and was expressed as g tartaric acid/L. For TC520, a 5 mL-aliquot of juice was diluted to a volume of 25 mL with acidified ethanol (pH 1.0). Absorbance was measured at 520 nm using a spectrophotometer (DU 600, Beckman, Irvine, CA). TC520 was calculated by multiplying absorbance at 520 nm by the dilution factor of five and expressed as absorbance units per mL of juice.

Ten frozen berries per sample were removed from the freezer the day of extraction and allowed to thaw slightly to facilitate removal of the skins. Whole skins were peeled from berries. Loose pulp was removed from the back of the skins by blotting with a Kim-Wipe® (Kimberly-Clark Corporation, Roswell, GA). Using an 8-mm diameter cork borer, two skin disks were removed from each berry for a total of 20 disks per sample, representing about 10 cm^2 of skin surface. Disks were placed in a 10-mL test tube to which were added 2 mL of 100% ethanol containing 0.2% HCl and homogenized (model PRO250, PRO Scientific Inc., Monroe, CT). The homogenizer was rinsed with an additional 2 mL of acidified ethanol that was added to the 10-mL tube containing the homogenized grape skin. Samples were placed in the refrigerator overnight (ca. 16 hr). The following morning samples were centrifuged at 1200 x *g* for 15 min. The supernatant was transferred to a 10-mL volumetric flask. Sample pellets were extracted two more times by mixing the pellet with 2 mL of acidified ethanol, holding for 2 hr under refrigeration, centrifuging, and transferring the supernatant to the 10-mL volumetric flask. Three milliliters of ultrapure water (18 MΩ) were added to each volumetric flask. The extract was taken to 10.0 mL with acidified ethanol. A more exhaustive extraction performed (7 x 1 mL) on four of the samples indicated that 97% of the anthocyanins and flavonols were extracted using the triple extraction described above (data not presented). Samples were stored under nitrogen headspace at -5°C. At the time of analysis, extracts were thawed at room temperature (ca. 20°C) and diluted 1:1 with ultrapure water.

All HPLC hardware was from Dynamax (Rainin, Oakland, CA) and was controlled by proprietary software (Dynamax 1.9). The system consisted of three pumps (model SD-200), an autosampler (model AI-1A), a photodiode array detector (PDA; model PDA-1), and a fluorescence detector (model FL-2). Column temperature was maintained at 35°C for all separations.

The method of Lamuela-Raventos and Waterhouse [20] was used for the 1999 grape skin extracts. The column was a Waters (Milford, MA) Novapak C18 (3.9 x 150 mm) with 4 µm particle size and a guard column of the same packing. In 2000 eluant A was omitted because flavonoids and anthocyanins were the only phenols identified from the 1999 skin extracts (Table 1). Additionally, the eluant ramp for B and C was adjusted and column length was increased to 250 mm to provide improved peak separation. Concentrations of monomeric anthocyanins and total monomeric anthocyanins were expressed as µg malvidin 3-glucoside/cm^2 skin.

Experimental design and statistical analyses. For the cluster location study, the design was randomized complete block with rows as replicates for all berry samples (3 replicates x 3

Table 1 Gradients for separation of anthocyanins and flavonols from grape berry skin extracts.

1999		2000	
Time interval (min)	Proportion of eluant A in eluant B[a] (%)	Time interval (min)	Proportion of eluant A in eluant B[b] (%)
0 to 10	18 to 20	0 to 2	100
10 to 30	20 to 40	2 to 10	100 to 94
30 to 32	40 to 80	10 to 40	94 to 88
32 to 33	held at 80	40 to 60	88 to 75
33 to 36	80 to 18	60 to 64	75 to 10
		64 to 69	held at 10
		69 to 70	10 to 100

[a]Eluent A was 20% ultrapure water and 80% acetonitrile (v/v) containing 0.25 mL 85% phosphoric acid/L. Eluent B was 85% phosphoric acid diluted to 1.0 L with ultrapure water and adjusted to pH 1.5 as required.
[b]Eluent A was ultrapure water containing 0.25 mL 85% phosphoric acid/L, adjusted to pH 2.6 as required. Eluent B was 20% ultrapure water and 80% acetonitrile (v/v) containing 0.25 mL 85% phosphoric acid/L.

cluster locations). Preharvest berry samples taken on more than one date were treated as a split-plot in time where appropriate. Data were subjected to analysis of variance using the general linear model, F-tests, orthogonal polynomial regression, stepwise multiple regression, and Duncan's new multiple range test ($p = 0.05$) using SAS (Cary, NC).

Results

At the IAREC, growing degree day (GDD) accumulations from 1 April to 31 Oct (base temperature 10°C) were 1247 in 1999 and 1384 in 2000. The average GDD for the period 1955 to 2000 is 1389. The pattern of GDD accumulation differed between years. In 1999, GDD accumulation was slow from April through July, the third lowest during the period 1955 to 1999. During the same period in 2000, GDD accumulation was the twelfth highest between 1955 and 2000. Conversely, between 1 Aug and harvest, the trend was reversed between the two years, with 2000 cooler than 1999 (Figure 1). Temperatures for this period were warmer than average in 1999 and lower than average in 2000.

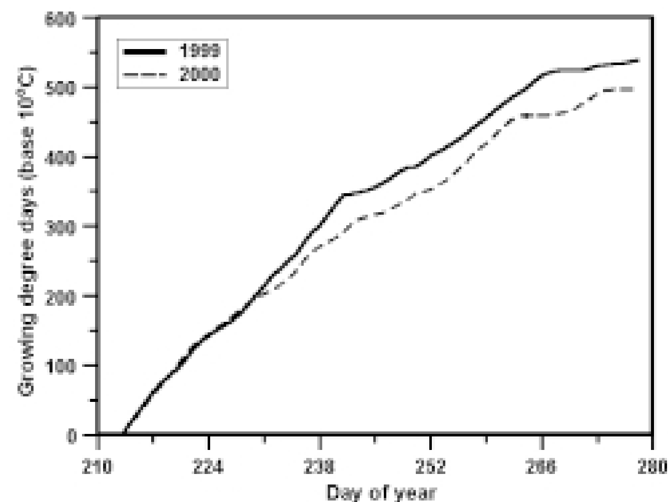

Figure 1 Growing degree day accumulation (base 10°C) at WSU, Prosser from DOY 213 to 278, 1999 and 2000.

In situ temperature control study. Fruit temperature accumulation was greater from bunch closure to harvest in 2000 than in 1999 (Table 2). Between initiation of veraison for east-exposed clusters, which we will refer to as veraison in the remainder of the text, and harvest there were 54 days in 1999 and 33 days in 2000. Absolute temperature accumulation by the fruit was greater in 1999 than 2000 because of the longer period. However, on average, more growing degrees days were accumulated per day in 2000. Generally, temperature accumulations by heated and cooled clusters reflected the temperature regimens we were trying to achieve. Temperature accumulations of cooled, sunlit clusters were within 3 GDD of shaded clusters in both growing seasons. Shade-heated clusters were within 14 GDD of sunlit control clusters for both years and measurement periods. In both years, temperature accumulations for the shade-blower and sun-blower fruit were intermediate to their control and temperature-manipulated counterparts. During the 1999 ripening period, minimum berry temperatures ranged from -1.2 to +0.4°C and occurred before dawn on either 29 Sept or 3 Oct. In 2000, a frost occurred on the night of 23 to 24 Sept. Air temperature was below 0°C for 3.8 hr with the minimum air temperature reaching -2.4°C. Minimum berry temperatures ranged from -2.1 to -3.6°C and were below 0°C for 3.4 to 4.0 hr.

Ambient global irradiance accumulated from veraison to harvest was 1,007 MJ/m^2 in 1999 and 643 MJ/m^2 in 2000. The difference in accumulated irradiance was due to the difference in the length of time between veraison and harvest between the two years. Solar radiation exposure at the fruiting zone of exposed clusters was about 60% of ambient, whereas about 10% of ambient levels of solar radiation were incident on shaded clusters (Table 2). Between 19 Aug and 21 Sept, corresponding to the veraison to harvest period in 2000, cumulative solar radiation exposure was similar in both years (data not shown).

Neither temperature nor exposure to sunlight affected berry mass within either year, but berry mass was greater in 2000 (Table 3). Although there were some differences in soluble solids among the six temperature-sunlight treatments, there were no consistent patterns. Whether based on juice concentration or per berry (data not shown), TA of sun-control berries was higher in 1999 than in 2000, while it was similar in concentration between the two years for shade-control berries. In both years, sun-cooled fruit had the highest mean concentration of soluble solids, but this treatment did not differ from the other two sunlit treatments. Also in both years, shade-heated and sun-control berries had the lowest and shade-control berries had the highest TA. Titratable acidity did not differ among any of the sunlit treatments in either year. Titratable acidity of shade-blower fruit was lower than shade-control fruit and higher than shade-heated fruit; it was similar to that of sun-blower and sun-cooled fruit. Fruit pH was inversely related to TA.

In 1999, sun-cooled clusters had the highest and shade-heated clusters had the lowest TC520 of the six in situ tempera-

Table 2 Temperature accumulation by clusters, expressed as growing degree days, as calculated from actual fruit temperatures using a base temperature of 10°C. Cumulative exposure to solar radiation determined from tube solarimeters placed in the fruiting zone.

	1999			2000		
	Growing degree days (°C)			Growing degree days (°C)		
Treatments	Bunch closure to harvest[a]	Veraison to harvest[b]	Solar radiation (MJ/m²)	Bunch closure to harvest[a]	Veraison to harvest[b]	Solar radiation (MJ/m²)
Sun						
Control	495	434	549	586	289	360
Blower	467	406	—	565	272	—
Cooled	442	385	—	519	252	—
Shade						
Control	439	382	129	521	250	69
Blower	443	385	—	551	266	—
Heated	505	443	—	602	297	—

[a]1999 = 13 Aug to 12 Oct; 2000 = 9 Aug to 25 Sept.
[b]1999 = 20 Aug to 12 Oct; 2000 = 23 Aug to 25 Sept.

ture control treatments (Table 3). TC520 was lower and differences in TC520 among treatments were not as great in 2000 as in 1999. Lower TC520 in 2000 may have resulted from higher berry mass. Trends indicated that shade-control, shade-heated, and sun-control clusters had the least TC520, while sunlit-cooled clusters had the most TC520. In 1999, cluster "face" had no effect on berry composition, whether or not the cluster was shaded or sunlit. Because TC520 did not differ in 1999, the following procedures were followed: (1) in 1999, berries from the exterior and interior cluster "faces" were pooled for individual anthocyanin determinations and (2) individual anthocyanins were not determined for sun- and shade-blower treatments; and (3) in 2000 no differentiation in cluster "face" was made.

Although TC520 of juice from a whole berry macerate was greater in 1999, total anthocyanin concentrations in skin extracts were about 50% higher in 2000 (Table 4), suggesting the dilution of TC520 in the whole berry extracts due to larger berry mass (Table 3). Total monomeric skin anthocyanins (TSMA) were affected by sunlight and temperature in both years, but not to the same degree (Table 4). Of the four treatments in 1999 and six treatments in 2000, sun-cooled clusters tended to have the highest TSMA concentrations. In 1999 shade-heated clusters had the lowest TSMA concentrations of the four treatments. In 2000, the three shade-cluster treatments tended to have the lowest TSMA concentrations. However, shade-blower clusters did not differ from sun-control clusters.

We examined the effects of temperature on TSMA concentration by comparing the difference in concentration between treatments within each of the light conditions. In 1999, for sun-exposed clusters, anthocyanin concentration was 87 µg/cm² skin higher in cooled clusters than in the control clusters (Table 4). For shaded clusters, TSMA concentration was 92 µg/cm² skin higher in nonheated, control clusters than in heated clusters. Therefore, under both light conditions, differences in TSMA concentrations between the control and the temperature-modified clusters were about 90 µg/cm² skin. This value represents the influence of temperature on TSMA concentration. Similarly, we compared the effects of light on TSMA concentration by comparing the difference in concentration between treatments within each of the temperature regimes. For the high temperature clusters, TSMA concentration was 120 µg/cm² higher in light-exposed clusters than in shaded-heated clusters. For the cool temperature clusters, TSMA concentration was 115 µg/cm² higher in sun-cooled clusters than in shade-control clusters. Therefore, under both temperature conditions, differences in TSMA concentration between sunlit and shaded clusters of the same temperature averaged 117 µg/cm² of skin, representing the influence of light on TSMA concentration.

In 2000 sun-exposed clusters, TSMA concentration was 102 µg/cm² skin higher for cooled clusters than the control clusters. For shaded clusters, heating clusters had no effect on TSMA concentrations. Again, we compared the effects of light on TSMA concentration by comparing the difference in concentration between treatments within each of the temperature regimes. For the high temperature clusters,

Table 3 Effect of sun exposure and temperature on Merlot berry composition at harvest, 1999 to 2000.

	Berry mass (g/berry)	Soluble solids (%)	TA (%)[a]	pH	Total color (AU/mL)[b]
1999					
Treatment[c]					
Sun					
Control	1.00a[d]	23.2c	0.60c	3.59bc	16.2b
Blower	1.08a	24.1ab	0.64bc	3.61b	17.3b
Cooled	1.02a	24.4a	0.62bc	3.63b	21.7a
Shade					
Control	1.13a	23.8b	0.73a	3.56c	16.9b
Blower	1.00a	23.8b	0.66b	3.60bc	17.0b
Heated	1.06a	23.2c	0.59c	3.68a	12.5c
Cluster face[e]					
Exterior	1.03a	23.7a	0.63a	3.62a	16.8a
Interior	1.06a	23.8a	0.65a	3.60a	16.9a
2000					
Treatment[f]					
Sun					
Control	1.32a	22.3ab	0.51cd	3.78bc	11.6b
Blower	1.23a	22.2ab	0.59bc	3.70c	14.3ab
Cooled	1.15a	23.0a	0.59bc	3.70c	15.2a
Shade					
Control	1.33a	21.2c	0.76a	3.73bc	10.7b
Blower	1.28a	22.3ab	0.65b	3.78b	13.1ab
Heated	1.27a	21.6bc	0.46d	3.89a	10.9b

[a]Expressed as tartaric acid.
[b]Absorbance at 520 nm of an acidified ethanol extract, pH 1.0.
[c]Means pooled across three repetitions and two cluster faces.
[d]Mean separation within columns within treatment, cluster face, and years by Duncan's new multiple range test ($p = 0.05$). Means followed by the same letter do not differ.
[e]Means pooled across three repetitions and six heating/cooling treatments.
[f]Means pooled across four repetitions.

Table 4 Influence of cluster temperature and exposure to sunlight on anthocyanin and flavonol concentrations in Merlot berry skins, 1999 to 2000.

	1999				2000					
	Sun		Shade		Sun			Shade		
	Control	Cooled	Control	Heated	Control	Blower	Cooled	Control	Blower	Heated
Total anthocyanins[a]	349.0ab[b]	436.2a	321.3b	228.9c	529.9b	597.7a	631.9a	414.7c	501.0b	415.2c
Delphinidin 3-glucoside[a]	60.1b	83.9a	59.9b	29.8c	110.7c	132.0b	159.3a	75.6d	96.2c	54.6e
Cyanidin 3-glucoside[a]	17.9b	31.7a	16.8b	7.50c	39.0b	47.4b	60.7a	20.9c	22.6c	10.1c
Petunidin 3-glucoside[a]	39.3b	51.8a	37.3b	20.6c	63.2bc	71.8ab	80.8a	45.4d	56.3c	38.5d
Peonidin 3-glucoside[a]	31.1b	49.3a	29.8b	17.2c	45.0bc	53.2ab	60.4a	32.6de	35.6cd	22.0e
Malvidin 3-glucoside[a]	96.7ab	107.2a	84.0b	68.1b	135.8a	148.2a	138.4a	118.3a	142.0a	138.0a
Delphinidin 3-glucoside acetate[a]	11.5a	13.3a	12.0a	6.3b	18.4bc	22.0ab	24.2a	17.2c	20.3abc	12.5d
Cyanidin 3-glucoside acetate[a]	4.40a	5.53a	4.20a	2.10b	6.65ab	7.30ab	8.45a	4.45cd	5.52bc	2.90d
Petunidin 3-glucoside acetate[a]	10.5a	11.9a	10.3a	.10b	14.7a	15.8a	16.3a	13.5ab	16.0a	11.8b
Peonidin 3-glucoside acetate[a]	7.57ab	8.63a	7.37ab	5.60b	8.48a	9.08a	8.65a	7.82a	8.92a	8.30a
Malvidin 3-glucoside acetate[a]	32.1a	33.4a	28.5a	29.8a	44.5bc	45.0bc	37.3c	43.6bc	51.8ab	60.7a
Delphinidin/cyanidin 3-glucoside coumarate[a]	6.10ab	7.27a	5.53b	5.13b	6.60a	7.28a	7.20a	4.00b	4.88b	4.25b
Petunidin 3-glucoside coumarate[a]	7.00a	7.00a	5.33ab	4.67b	7.98a	8.80a	7.38a	5.58b	7.40a	7.38a
Peonidin/malvidin 3-glucoside coumarate[a]	25.0a	25.7a	20.3a	25.0a	28.7b	30.1b	22.8b	25.8b	33.6b	44.1a
Total flavonols[c]	—[d]	—	—	—	82.7a	82.7a	76.0a	10.2b	22.9b	17.8b
Quercetin 3-glucoside[c]	22.0b	31.7a	7.13c	7.27c	59.9a	62.8a	56.6a	7.50b	14.6b	12.5b
Myricetin 3-glucoside[c]	—	—	—	—	9.12a	8.50a	8.15a	0.01b	3.15b	1.80b
Kaempferol 3-glucoside[c]	—	—	—	—	13.7a	11.4a	11.3a	2.68c	6.72b	4.80bc

[a]Expressed as μg malvidin 3-glucoside chloride/cm^2 skin; abbreviated TSMA in text.
[b]Mean separation within years within rows by Duncan's new multiple range test ($p = 0.05$). Means followed by the same letter do not differ.
[c]Expressed as μg quercetin 3-glucoside/cm^2 skin.
[d]Not determined in 1999.

TSMA concentration was 115 μg/cm^2 skin higher in exposed clusters than in shade-heated clusters. For the cool temperature clusters, TSMA concentration was 217 μg/cm^2 skin higher in sun-cooled clusters than in shade-control clusters.

In 1999, the response of individual anthocyanins to light and temperature treatments tended to follow the pattern of TSMA concentration with lower individual pigment concentrations in berry skins that were exposed to higher temperatures or were shaded (Table 4). These trends were not as clear in 2000 as in 1999, although sun-cooled clusters tended to have the highest concentration of pigments. Using stepwise multiple regression, 92% of the variability in total anthocyanin concentration in the skins was accounted for by petunidin 3-glucoside.

Skins from sun-exposed clusters had 3 to 4.5 times higher concentrations of quercetin 3-glucoside than skins from shaded clusters (Table 4). Although sunlight was the overriding factor influencing quercetin 3-glucoside concentrations, cooling the sun-exposed clusters increased quercetin 3-glucoside concentrations by about 40% in 1999. In 2000, quercetin 3-glucoside concentrations were four to eight times greater in skins from sun-exposed clusters than in those from shaded clusters. Kaempferol 3-glucoside was 1.5- to 5-fold greater in skins from sun-exposed than shaded clusters. Cluster temperature did not affect concentration of the three flavonols in 2000.

UV barriers. Berry temperatures behind the UV blocking and UV passing screens were not measurably different from those of unscreened, west-exposed fruit (Figure 2). Thus, any effect of the screening materials on fruit composition was not due to differences in fruit temperature. The acrylic material deemed UV-absorbing transmitted an average of 4.2% of UV-B over the course of a day, whereas the UV-transmitting material only transmitted 56% of incident UV-B (data not shown). Incident UV-B at the fruiting zone averaged 88% of the ambient (sky) value.

Blocking the UV waveband from sun-exposed clusters on the west side of the canopy did not affect berry mass or soluble solids (Table 5). The effects of the two barriers on TA and pH were not consistent, while TC520 of the acidified ethanol ex-

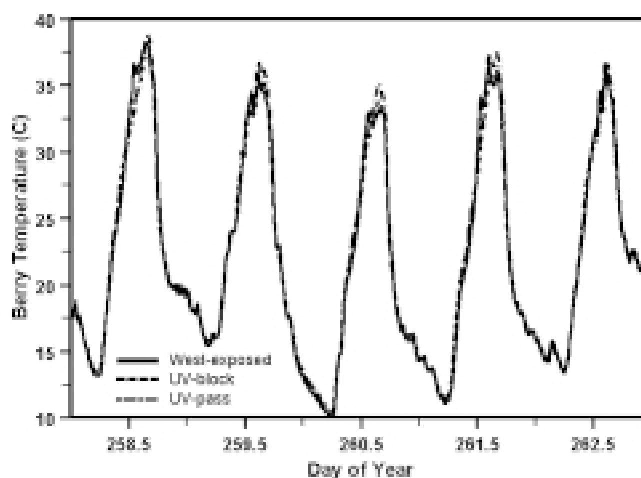

Figure 2 Berry temperatures from clusters that were exposed to the sun on west side of the canopy, west-exposed but behind a UV absorbing material, or west-exposed but behind a UV-transmitting material. Data collected 10 days to one week before harvest, 2000.

Table 5 Effect of UV radiation on anthocyanin and flavonol concentrations in Merlot berry skins, 2000.

	Treatment		
		UV barriers	
	Control	Transmitting	Absorbing
Berry composition			
Berry mass (g)	1.23a[a]	1.25a	1.24a
Soluble solids (%)	22.9a	22.2a	22.0a
Titratable acidity (%)[b]	0.55a	0.48b	0.53ab
pH	3.64b	3.78a	3.75ab
Total color[c]	11.8a	9.6b	8.4b
Skin anthocyanins and flavonols			
Total anthocyanins[d]	592.6a	551.0a	561.8a
Delphinidin 3-glucoside[d]	132.4a	108.3a	108.3a
Cyanidin 3-glucoside[d]	54.0a	33.6ab	31.9b
Petunidin 3-glucoside[d]	69.9a	63.6a	63.6a
Peonidin 3-glucoside[d]	54.6a	43.1b	30.6b
Malvidin 3-glucoside[d]	135.1a	148.7a	151.8a
Delphinidin 3-glucoside acetate[d]	22.2a	19.8a	21.3a
Cyanidin 3-glucoside acetate[d]	8.98a	5.6b	5.6b
Petunidin 3-glucoside acetate[d]	16.0a	14.6a	15.3a
Peonidin 3-glucoside acetate[d]	10.0a	9.0a	9.0a
Malvidin 3-glucoside acetate[d]	43.7a	50.6	49.3a
Delphinidin/cyanidin 3-glucoside coumarate[d]	7.2a	5.8ab	4.7b
Petunidin 3-glucoside coumarate[d]	8.2a	8.9a	8.0a
Peonidin/malvidin 3-glucoside coumarate[d]	30.6b	39.2a	36.6ab
Total flavonols[e]	71.3a	67.0a	40.8b
Quercetin 3-glucoside[e]	52.9a	50.2a	31.2b
Myricetin 3-glucoside[e]	7.4a	6.3	1.9b
Kaempferol 3-glucoside[e]	11.0a	10.5a	7.7b

[a]Mean separation within rows by Duncan's new multiple range test ($p = 0.05$). Means followed by the same letter do not differ. Means were pooled across four repetitions.
[b]Expressed as tartaric acid.
[c]Absorbance at 520 nm of an acidified ethanol extract, pH 1.0.
[d]Expressed as µg malvidin 3-glucoside chloride/cm^2 skin; abbreviated TSMA in text.
[e]Expressed as µg quercetin 3-glucoside/cm^2 skin.

tract was reduced as compared to the control. However, TSMA concentrations did not differ between the two UV panels and the control clusters. Some of the individual anthocyanins were affected by UV screening. Concentrations of the 3-glucosides of quercetin, myricetin, and kaempferol were greatly reduced when UV radiation was blocked from the clusters.

Cluster location: Fruit temperature. Of the three cluster locations (east-exposed, west-exposed, and shaded), absolute fruit temperatures were highest on the east side before solar noon and on the west side after solar noon (Figure 3). East-exposed fruit warmed earlier in the day than west-exposed fruit and remained near ambient shade temperatures during the afternoon. Grape berries exposed to sunlight were warmer than berries shaded from sunlight by the grapevine canopy (Figure 4). The maximum daily differences in temperature (ΔT_{max}) between east-exposed and shaded berries ranged from 3.3 to 11.7°C (between 6.0 and 10.7°C in 2000) on days with clear skies, and generally occurred between 0800 and 1000 hr LST. This sizable range on days with similar irradiance was due to variations in wind speed, with more effective convection away from the exposed clusters than from those in the relatively calm interior of the canopy (data not shown). Daily ΔT_{max} between west-exposed and shaded berries was 2.5 to 9.2°C (between 3.8 and 9.7°C in 2000) on days with clear skies. Again, the variability in ΔT_{max} under similar irradiance was due to differences in convection (wind speed). Generally, ΔT_{max} occurred between 1400 and 1600 hr LST. In both years, on days with clear skies the exposed berries on either side of the canopy were 4 to 13°C warmer than ambient air at reference height at the time of daily ΔT_{max} (data not

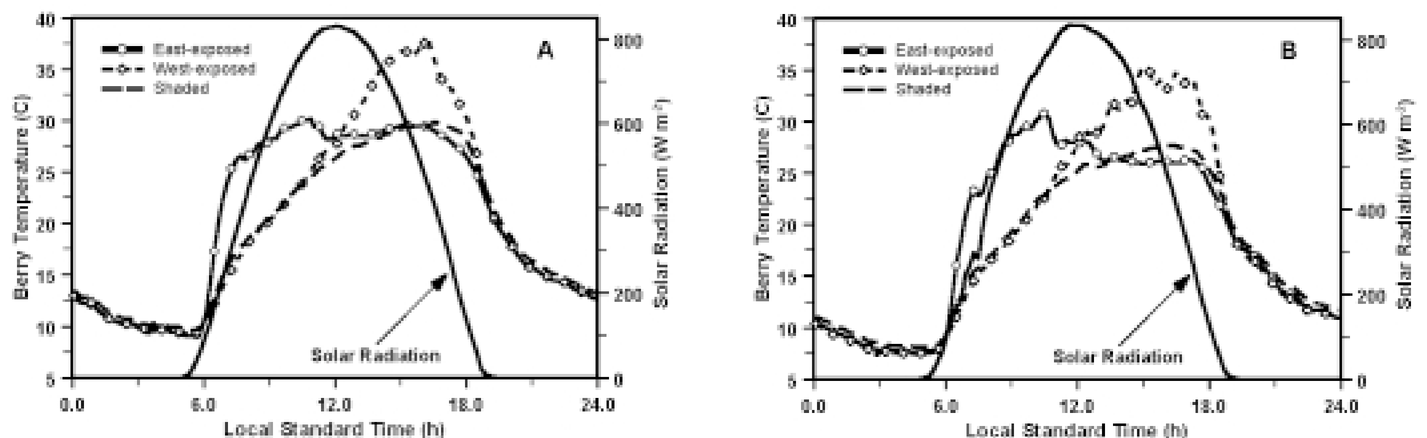

Figure 3 Typical diurnal pattern of berry temperatures for fruit exposed to sunlight on the east or west sides of the canopy, or shaded from sunlight by the canopy, on a day with clear skies. Shaded temperatures are the mean of clusters from both east and west sides of the vine. Solar radiation was measured on-site with a pyranometer. (**A**) DOY 234 in 1999; (**B**) DOY 234 in 2000. In both years, fruit was approaching veraison.

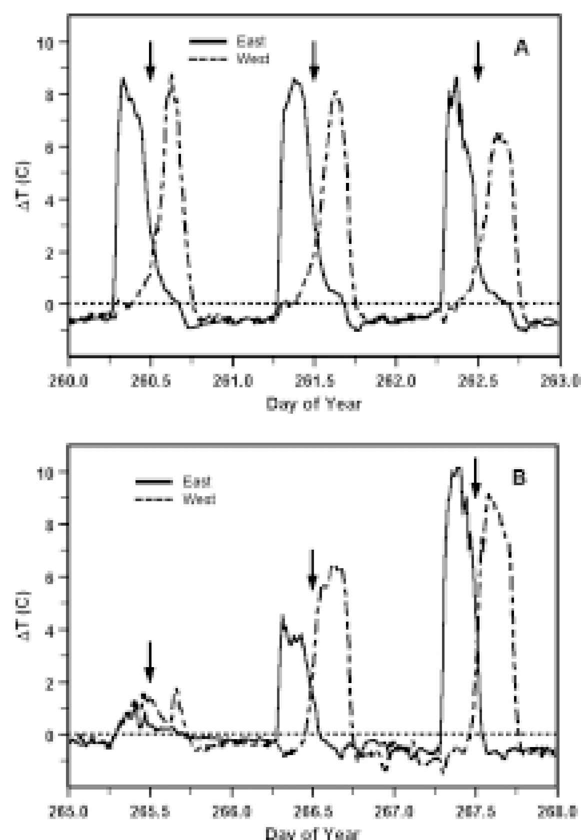

Figure 4 Difference in fruit temperature (ΔT) between fruit exposed to sunlight (east-exposed or west-exposed) and fruit shaded by the canopy on the same side of the vine. Arrows indicate solar noon. (**A**) Postveraison, 1999. DOY 261 to 264 had clear skies and maximum mid-day irradiance of 720 to 725 W/m^2. (**B**) Postveraison, 2000. DOY 265 was cloudy with average mid-day irradiance of 250 W/m^2. DOY 266 and 267 mid-day irradiance was 705 W/m^2 but average wind speed differed between the days.

Table 6 Influence of cluster position on accumulation of growing degree hours and days based on Merlot berry temperatures between initiation of monitoring (ca. bunch closure) and harvest and between veraison and harvest in 1999 and 2000 (10°C used as base temperature).

Cluster position	Start of monitoring to harvest: Degree hours (°h)	Start of monitoring to harvest: Degree days (°d)	Veraison to harvest: Degree hours (°h)	Veraison to harvest: Degree days (°d)
1999	*13 Aug to 12 Oct*		*20 Aug to 12 Oct*	
East exposed	12,422	504	10,617	442
East shaded	10,422	434	9,089	379
West exposed	11,821	493	10,407	434
West shaded	10,554	440	9,227	384
2000	*9 Aug to 25 Sept*		*23 Aug to 25 Sept*	
East exposed	10,015	417	7,399	308
East shaded	8,852	369	6,507	271
West exposed	9,979	416	7,441	310
West shaded	8,935	372	6,603	275
UV pass	9,913	413	7,360	307
UV block	9,739	406	7,219	301

Table 7 Number of hours Merlot berry temperature exceeded selected temperature thresholds from about bunch closure to harvest, 1999 to 2000.

Cluster position	Threshold temperature (°C) >30	>35	>40
1999 (*1437 total hours of monitoring from 13 Aug to 12 Oct*)			
East exposed	148.2	3.2	0.0
East shaded	53.2	0.0	0.0
West exposed	181.4	73.6	2.8
West shaded	83.0	0.0	0.0
2000 (*1094 total hours of monitoring from 9 Aug to 25 Sept*)			
East exposed	114.6	7.6	0.0
East shaded	45.0	2.6	0.0
West exposed	149.6	67.4	2.6
West shaded	61.0	2.6	0.0

shown). Only the time of ΔT_{max} differed; its magnitude was similar for the two sides of the canopy.

An indicator of fruit exposure to heat was constructed by calculating GDD from berry temperatures at each cluster location (Table 6). In both years, between bunch closure and harvest or between veraison and harvest, there was little difference (~2%) in the accumulation of GDD between east- and west-exposed clusters. Shaded clusters consistently accumulated fewer GDD than sun-exposed clusters. Clusters screened by the UV materials accumulated similar numbers of GDD as unscreened, west-exposed clusters.

Accumulated temperature, although an important indicator, may not be as critical to fruit physiology as the length of time the berries are subjected to specific temperatures. Threshold temperatures of 30, 35, and 40°C were selected to determine differences in high temperature duration among the four cluster exposures (Table 7). The number of hours above 30°C was less in 2000 than in 1999 for all cluster locations. During both growing seasons, east-shaded clusters experienced the lowest number of hours at temperatures greater than 30°C, while west-exposed clusters had the highest number of hours. Perhaps the greatest differences in fruit temperatures among the four cluster locations were in the number of hours above 35°C. In both years, west-exposed clusters were above 35°C for more than 65 hr between bunch closure and harvest, while clusters in the other three locations were above 35°C for no more than 7.6 hr. Of the four locations, only west-exposed clusters reached temperatures greater than 40°C in either year.

Cluster location: Preharvest berry composition. In 1999 and 2000, sample date and cluster location influenced the composition of berries in the preharvest samples. There were no interactions between sample date and cluster location (data not shown). Therefore, only the main effects will be presented (Tables 8 and 9). Berry mass decreased between sampling dates in both 1999 and 2000 (Table 8). In 1999, soluble solids, pH, and TC520 increased, while TA did not differ between sampling dates. In 2000, soluble solids, TA, and TC520 were lower on 25 Sept than on 21 Sept. The decrease in soluble solids and

TC520 probably resulted from several hours below 0°C on 23 Sept that damaged the fruit, forcing harvest on 25 Sept. In 1999, berries on the west side of the canopy weighed less than berries on the east side of the canopy. Shaded berries on the west side of the canopy tended to have lower soluble solids than their east-side counterparts, but the maximum difference in soluble solids among all cluster locations was only 0.8%. Titratable acidity was highest in shaded fruit, followed by east-exposed and the lowest concentration in west-exposed fruit. West-exposed fruit had the highest pH of the four cluster locations. TC520 was highest for east-exposed and lowest for west-exposed clusters. In 2000, shaded clusters were sampled from both sides of the canopy for a combined shaded fruit sample. Cluster location did not affect berry mass. As in 1999, the maximum difference in soluble solids was 0.8% among all cluster locations, with east-exposed fruit having the highest and shaded fruit having the lowest concentrations. Titratable acidity reflected berry temperatures. Shaded berries had the highest TA followed by east-exposed berries. Berry pH was inversely related to TA. TC520 was highest in east-exposed fruit with no difference in TC520 between shaded and west-exposed fruit. On 5 Sept 2000 on the same vine, based on berry color, west-exposed clusters apparently were still in the early stages of veraison, while most east-exposed clusters were fully colored. This extreme difference was not noticed in 1999. Preveraison temperatures in 2000 were warmer than during the same period in 1999.

Table 8 Main effects of sample date and cluster position on berry mass and composition of Merlot, 1999 and 2000.

	Berry mass (g/berry)	Soluble solids (%)	TA (%)[a]	pH	Total color (AU/mL)[b]
1999					
Date					
28 Sept	1.23a[c]	22.2b	0.74a	3.43b	13.9b
7 Oct	1.19a	23.6a	0.72a	3.61a	16.8a
Cluster position					
East exposed	1.27a	23.0ab	0.71b	3.50b	18.4a
East shaded	1.29a	23.3a	0.81ab	3.49b	16.7ab
West exposed	1.15b	22.5b	0.59c	3.62a	11.6c
West shaded	1.14b	22.9ab	0.82a	3.46b	14.7b
2000					
Date					
21 Sept	1.45a	23.4a	0.67a	3.59b	15.6a
26 Sept	1.29b	22.4b	0.60b	3.65a	14.3b
Cluster position					
East exposed	1.47a	23.2a	0.61b	3.61b	18.0a
Shaded	1.35a	22.4b	0.76a	3.55c	13.2b
West exposed	1.30a	22.7ab	0.52c	3.71a	13.7b

[a]Expressed as tartaric acid.
[b]Absorbance at 520 nm of an acidified ethanol extract, pH 1.0.
[c]Mean separation within columns within year within main effect by Duncan's new multiple range test ($p = 0.05$). Means followed by the same letter do not differ.

Merlot berry skins were analyzed for changes in the distribution of individual anthocyanins on each of the two preharvest sample dates and on the day of harvest in 1999 and on one preharvest sample date in 2000 (Table 9). In 1999 cluster location contributed more to the variability in concentration of individual monomeric anthocyanins and TSMA concentrations

Table 9 Influence of sample date and cluster position on concentration of anthocyanins and flavonols in skins of Merlot berries, 1999 to 2000.

	1999						21 Sept 2000		
	Sample date			Cluster location			Cluster location		
	28 Sept	7 Oct	13 Oct	East	Shade	West	East	Shade	West
Total anthocyanins[a]	353.6a[b]	365.4a	409.4a	433.0a	378.3b	317.2c	784.8a	521.8b	545.3b
Delphinidin 3-glucoside[a]	66.0a	64.4a	70.2a	81.8a	68.6b	50.2c	183.5a	115.9b	112.9b
Cyanidin 3-glucoside[a]	23.4b	25.1a	21.8c	29.4a	24.4b	16.4c	50.3a	38.5a	45.7a
Petunidin 3-glucoside[a]	41.6a	42.8a	45.9a	51.7a	44.4b	34.3c	105.8a	63.1b	63.1b
Peonidin 3-glucoside[a]	36.7a	39.4a	40.2a	45.3a	39.8b	31.3c	64.9a	47.2a	47.2a
Malvidin 3-glucoside[a]	87.0b	93.2ab	108.8a	110.7a	91.4b	86.9b	206.0a	126.3b	134.5b
Delphinidin 3-glucoside acetate[a]	11.7a	12.6a	13.5a	13.5a	14.8a	9.54b	25.7a	21.3a	18.1a
Cyanidin 3-glucoside acetate[a]	4.74a	4.91a	5.08a	5.16a	5.58a	4.00b	6.73a	6.80a	7.07a
Petunidin 3-glucoside acetate[a]	10.4a	23.0a	12.6a	11.7a	24.9a	9.30a	19.1a	14.6a	13.5a
Peonidin 3-glucoside acetate[a]	7.44a	8.32a	9.34a	8.34ab	9.09a	7.67b	9.40a	9.00a	8.70a
Malvidin 3-glucoside acetate[a]	29.6b	29.8b	37.7a	33.9a	32.1a	31.0a	55.5a	42.7b	40.3b
Delphinidin/cyanidin 3-glucoside coumarate[a]	5.77b	6.61ab	7.16a	6.48a	6.39a	6.67a	7.90a	5.60a	6.77a
Petunidin 3-glucoside coumarate[a]	6.50a	5.44a	7.44a	7.67a	6.17b	5.56b	11.9a	6.57b	8.6b
Peonidin/malvidin 3-glucoside coumarate[a]	22.5b	22.2b	29.6a	27.2a	22.9b	24.1ab	38.0a	26.6b	31.7ab
Total flavonols[c]	—	—	—	—	—	—	111.1a	12.7b	111.1
Quercetin 3-glucoside[c]	29.6a	25.6b	18.4c	34.4a	9.47b	32.4a	76.6a	8.48b	82.8a
Myricetin derivative[c]	—	—	—	—	—	—	22.9a	4.23c	15.4b
Kaempferol 3-glucoside[c]	—	—	—	—	—	—	11.6a	0.01b	12.9a

[a]Expressed as µg malvidin 3-glucoside equivalents/cm^2 skin; abbreviated TMSA in text.
[b]Mean separation within rows within years within sample date and cluster position by Duncan's new multiple range test ($p = 0.05$). Means followed by the same letter do not differ.
[c]Expressed as µg quercetin 3-glucoside/cm^2 skin.

in skin tissue than did date of sampling. There were no significant interactions between date of sampling and cluster location. Therefore, only main effect means of date and cluster location are shown. With means pooled across cluster location, malvidin 3-glucoside was the only nonsubstituted anthocyanin to increase in concentration during the sampling period. In 1999, TSMA concentration tended to increase with time.

In 1999, TSMA concentration differed among cluster locations with concentration decreasing from east-exposed to shaded to west-exposed clusters (Table 9). East-exposed clusters had the highest concentrations of each of the nonsubstituted anthocyanins, while west-exposed clusters had the lowest concentrations. Shaded clusters were intermediate to east- and west-exposed clusters, with the exception of malvidin 3-glucoside where shaded and west-exposed clusters had similar skin concentrations. In 2000, concentrations of TSMA and several of the individual anthocyanins were highest in the berry skins of east-exposed clusters, while shaded and west-exposed clusters did not differ.

In both 1999 and 2000, quercetin 3-glucoside concentration was lowest in the skins of shaded berries (Table 9). In 2000, we tentatively identified a derivative of myricetin, based on retention time, and confirmed the presence of kaempferol 3-glucoside. Both of these flavonols responded to sunlight exposure in the manner of quercetin 3-glucoside. Total flavonol concentration was almost 10 times greater in skins of sun-exposed than shaded clusters, regardless of aspect.

Combined light and temperature effects. Forward selection, stepwise multiple regression was used to develop a model for factors contributing to anthocyanin accumulation in berry skins. Mean TSMA concentrations from the in situ and the cluster location studies were regressed against their corresponding veraison-to-harvest GDD accumulation, accumulated solar radiation, and hours fruit temperature exceeded 30, 35, and 40°C. For the cluster location study, the day of harvest sample in 1999 and the preharvest sample in 2000 were used in the regression. To be retained in the model, the contribution of a variable had to be significant at $p \leq 0.15$. Across the two years and two studies, GDD between veraison and harvest was the first independent variable added to the model and accounted for about 45% of the variability in TSMA concentrations. Accumulated solar radiation (partial r^2=0.306) was the second variable added, giving a model that accounted for 76% of the variability in TSMA concentrations (r^2=0.758). Number of hours above 35 and 30°C were included in the final model, in that order. However, these variables accounted for far less variability than GDD and accumulated solar radiation (partial r^2-values of 0.046 and 0.076, respectively). The final model generated was:

$$TSMA=869 + (-1.88*GDD) + (1.67*SR) + (-3.24*H35) + (1.67*H30)$$

where TSMA is total skin monomeric anthocyanin concentration (μg/cm²); GDD is growing degree days (base 10°C) from veraison to harvest; SR is accumulated solar radiation (MJ/m²) from veraison to harvest; H35 is hours above 35°C; and H30 is hours above 30°C. The final model accounted for 88% (p = 0.006) of the variability in TSMA concentration. Recognizing that interrelations exist among the four independent variables, the model was tested for collinearity. The condition index for the model was 15.9. Therefore, although the variables are physically related, their biological effect on anthocyanin concentration had a low degree of collinearity.

Discussion

In the present study, sunlight influenced grape berry composition through at least two mechanisms: temperature and solar radiation. Visual evidence of sunburn, browning, or russeting of berry skins was observed on clusters exposed to sunlight on the west side of north-south oriented rows. Berry temperatures exposed to sunlight on either side of the canopy were elevated above ambient to the same degree. However, actual berry temperatures were higher on sun-exposed clusters on the west side of the canopy due to the normally higher ambient temperatures that occurred after solar noon. Shaded berries were at ambient temperature. Maximum berry and ambient temperatures occurred about 1600 hr. Varying magnitudes of temperature elevation due to exposure to sunlight were reported in other studies [1,14,16,22,24,29]. With sun exposure, Smart and Sinclair [29] found that the temperature of tight clusters (12.4°C) increased above ambient more than loose clusters (11.1°C). They reported a maximum increase over ambient of 15.7°C, while we recorded a maximum increase above ambient temperature of 12.3°C in 1999 and 13.0°C in 2000. Merlot clusters in our study were relatively loose. For east-west oriented rows, mid-day berry temperatures of south-exposed clusters were 3 to 4°C higher than north-exposed clusters [1]. Sunburn has been observed in eastern Washington on south-exposed clusters from east-west oriented rows.

To support the effects of temperature and light on phenol components, we focused in part on some of the basic berry measurements (berry mass, soluble solids, TA, and pH). Our data regarding the effect of temperature and light on these measurements did not conflict with previous reports [9,14,15,18,24]. Shaded and exposed berries differed little in mass in our study even though cluster exposure treatments were imposed during stage I (pea size) of berry growth. Heat stress of Napa Gamay vines during Stage I and Stage II of berry growth resulted in greater loss of berry mass than heat stress during Stage III [21]. Crippen and Morrison [6] reported shade berries of Cabernet Sauvignon were heavier and larger than sun-exposed berries. Of the fruit components measured, percent soluble solids was perhaps the least affected by either exposing/shading clusters from sunlight or artificially altering cluster temperature. This was in agreement with Crippen and Morrison [6], who did not differentiate between east- and west-exposed clusters. Reynolds and coworkers [24] reported that soluble solids of east and west exposed clusters were higher than partially and fully shaded clusters. North-exposed clusters of Cabernet Sauvignon were found to have lower soluble solids than south-exposed clusters at a given level of mid-day PAR [1]. In general, TA between the various treatments was inversely related to the overall temperature to which a treatment was subjected: the greater the heat summation the lower the acidity. However, preveraison tem-

peratures as well as postveraison alteration of conditions that influenced fruit temperatures apparently influence TA, as concentrations were higher in 1999 than in 2000. GDD accumulation in 1999 was lower preveraison and higher postveraison than in 2000. Fruit pH was inversely related to TA. For Seyval blanc, TA was higher preveraison and lower postveraison in exposed clusters than shaded clusters with malate concentrations paralleling TA [24]. The pattern of differences in TA and pH between south-exposed and north-exposed Cabernet Sauvignon clusters reported by Bergqvist et al. [1] paralleled differences observed in the present study.

Light and temperature effects on anthocyanins and phenols, primarily flavonols, in berry skins were the focus of our study. Lower anthocyanin concentrations found in skins from west-exposed clusters appear to be due to elevated temperature either through degradation, inhibition of synthesis, or, more likely, both. This is supported by the measured increase in skin anthocyanins when west-exposed clusters were chilled to the temperature of shaded fruit. Temperature had little to no effect on flavonol concentrations. Light increased total concentration of monomeric anthocyanins and flavonols. UV-light stimulated flavonol biosynthesis as evidenced by the decreased concentration in skins of berries shielded by UV-barriers from bunch closure to harvest. The UV-barriers did not affect total monomeric anthocyanin concentrations. Roubelakis-Angelakis and Kliewer [25] reported that total phenol accumulation in Cardinal berry skins responded differently to light and temperature than did anthocyanin accumulation. The authors suggested that in grape berry skins there is independent regulation of these two pools. However, one must consider that anthocyanins and flavonols share the flavonoid biosynthetic pathway to the point of formation of dihydroflavonols [19].

Concentration of the quercetin aglycone was decreased by UV-restriction as were the glycosylated forms of quercetin, kaempferol, and myricetin. There was no apparent proportional inhibition of biosynthesis of the glycosylated forms of the anthocyanins. As revealed in our study, inhibition of biosynthesis in grape berry skins of quercetin and the 3-glycosides of quercetin, kaempferol, and myricetin before formation of dihydrokaempferol and/or dihydroquercetin due to restriction of UV is unlikely, as anthocyanin biosynthesis was not inhibited. Therefore, for at least some period during grape berry ripening, UV is required to stimulate gene transcription for production of flavonol synthase (FLS). FLS catalyzes the conversion of dihydromyricetin to myricetin, dihydrokaempferol to kaempferol, and dihydroquercetin to quercetin [12]. Leucoanthocyanidins are formed from dihydroflavonols in the next step of the anthocyanin biosynthetic pathway [19]. In our study, it was unlikely, but not impossible, that there were sufficient concentrations of leucoanthocyanins to sustain anthocyanin synthesis at the level of fully solar-irradiated clusters during the six-week period of restriction of UV-radiance. Light is essential for anthocyanin synthesis in petunia (*Petunia hybrida* L.) flowers with the active spectrum including most of the visible range, but not UV [13]. Blocking UV-B from apple flowers inhibited anthocyanin accumulation but did not completely inhibit anthocyanin synthesis [8]. Neither UV-B nor UV-C had an effect on anthocyanins of table grapes during postharvest storage [4]. Gallop and coworkers [10] suggested the possible role of UV receptors in induction of gene expression of leucoanthocyanidin dioxygenase (LDOX), the enzyme that catalyzes the conversion of leucoanthocyanidins to anthocyanidins. Their supposition was based on stimulation by white light and calcium of the expression of the LDOX gene in cell suspensions of red-fruited *V. vinifera* cultivars. Of seven genes involved in anthocyanin biosynthesis in grapes that were examined, including LDOX, the gene responsible for encoding UDP-glucose:flavonoid 3-*O*-glucosyltransferase (UFGT) was the only one not detected in flowers and berry skins up to four weeks postflowering [2]. Expression of these genes then declined to veraison and then increased during grape berry ripening [2,11]. UFGT expression was detected only after veraison. Our study was based on end-product concentration rather than gene expression. Concentrations of monomeric anthocyanins were not affected by blocking greater than 95% of UV from bunch closure through harvest.

Roubelakis-Angelakis and Kliewer [25] reported that the rate of anthocyanin accumulation and final anthocyanin concentration in the epidermal layer of excised Cardinal berries was greater at 35°C than at either 14 or 22°C. In the present study, the number of hours above 35°C was associated with a net loss of TSMA. However, number of hours above 30°C was positively related to TSMA concentrations. Apparently, excessively high temperatures were detrimental to anthocyanin accumulation in the skins, but some degree of heat was needed for synthesis. The critical temperature for net anthocyanin accumulation in Merlot berry skins may lie between 30 and 35°C. More information is needed on critical temperature range(s) in vivo for anthocyanin biosynthesis and degradation in grape berry skins, recognizing that it may vary with cultivar. Once fundamental mechanisms are understood, practical methods for balancing cluster temperature with degree of sunlight exposure can be developed.

Conclusions

Excessive absolute fruit temperatures, rather than the difference between fruit temperatures and ambient temperatures, reduced anthocyanin concentrations in sun-exposed grape berries. East- and west-exposed clusters received the same total exposure to solar radiation on cloudless days and were heated to the same degree above ambient temperature at the time of maximum sun exposure. The highest absolute fruit temperatures occurred in west-exposed fruit because ambient temperature typically increases throughout the day. In warm to hot viticultural regions, exposure of grapes to full sunlight on west- and south-facing canopies should be avoided unless some method is used for reducing fruit temperature. Complete fruit shading is not recommended because some sunlight is needed for maximum anthocyanin synthesis and for balancing the composition of other fruit components. Partial shade might be provided in vertical shoot positioned canopies by incomplete positioning of shoots on west- and south-facing canopies. Additionally, leaf stripping should be minimized unless there are multiple layers of leaves.

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