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Influence of Vine Vigor on Grape (*Vitis vinifera* L. Cv. Pinot Noir) Anthocyanins. 1. Anthocyanin Concentration and Composition in Fruit

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The relationships between grapevine (*Vitis vinifera*) vigor variation and resulting fruit anthocyanin accumulation and composition were investigated. The study was conducted in a commercial vineyard consisting of the same clone, rootstock, age, and vineyard management practices. The experimental design involved assigning vigor zones in two vineyard sites based upon differences in vine growth. Fruits and wines were analyzed by HPLC from designated vigor zones in 2003 and 2004. Average berry weight (grams), average dry skin weight (milligrams), degrees Brix, and pH were higher and titratable acidity (grams per liter) was lower in 2003 compared to 2004. In 2003, only the highest and lowest vigor zones had differences in berry weight, whereas there were no differences in 2004. In both years, high vigor zones had lower degrees Brix and higher titratable acidity (milligrams per liter). Accumulation of anthocyanins (milligrams per berry) was greater in 2003 compared to 2004. There was a trend for lower anthocyanin concentration (milligrams per berry) in high vigor zones in both years. In 2004 compared to 2003, there was a higher proportion of malvidin-3-*O*-glucoside and lower proportions of the other four anthocyanins (delphinidin-, cyanidin-, petunidin-, and peonidin-3-*O*-glucosides) found in Pinot Noir. In both years, site A had proportionally higher peonidin-3-*O*-glucoside and lower malvidin-3-*O*-glucoside than site B. Some of these differences may be related to the higher exposure and temperatures found in site B compared to site A and also in the low vigor zones.

KEYWORDS: Temperature; light exposure; yield; soluble solids; berry weight; skin weight; grape; anthocyanin; variation

INTRODUCTION

Anthocyanins are a class of pigmented phenolic compounds responsible for the red color of grapes and wine. In fruit, they generally are considered to play a role in attracting seed dispersal organisms and UV-light protection (*1*). Anthocyanins have also been reported to have human health benefits (*2*). The hydroxylation pattern on the B-ring produces five anthocyanins, which include delphinidin-3-*O*-glucoside (Dp), cyanidin-3-*O*-glucoside (Cy), petunidin-3-*O*-glucoside (Pt), peonidin-3-*O*-glucoside (Pn), and malvidin-3-*O*-glucoside (Mv). Malvidin-based anthocyanins are generally the major forms present in *Vitus vinifera* varieties. In addition to free monoglucosides, most varieties produce acetyl and *p*-coumaryl glucoside derivatives. Significant varietal variation exists in anthocyanin acylation, and some varieties such as Pinot Noir lack acylation altogether (*3*).

Grape berry growth follows a double-sigmoid curve separated by a lag phase (*4*). Ripening begins after the lag phase at a time termed véraison by viticulturalists. Anthocyanin accumulation begins at véraison and continues through ripening. Although the biosynthesis of all anthocyanins commences at véraison, they have been reported to accumulate at different rates during fruit ripening (*5*, *6*).

Many environmental factors and viticultural practices influence the accumulation and composition of anthocyanins in the fruit. High vine vigor resulting from excessive soil moisture and high levels of available nitrogen can modify the vine microclimate (*7*) and influence the accumulation of anthocyanins (*7*, *8*). Low vine vigor vineyards are characterized by greater light exposure in the fruiting zone (*7*−*9*). Spatial variation in vineyard topography, climatic conditions, physical and chemical characteristics of the soil, and pests and diseases have been associated with spatial variation in vine vigor, yield, and fruit soluble solids (*10*, *11*). A relationship between grapevine canopy size and vine vigor measured as the normalized difference vegetation index (NDVI) and grape color has been reported (*12*).

Various viticultural practices have been found to influence anthocyanin accumulation and composition including nitrogen

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Table 1. Phenology Dates in 2003 and 2004

site	budbreak (50%)	bloom (50%)	veraison (50%)	harvest
2003	April 14	June 13	Aug 14	Sept 21
2004	April 1	June 4	Aug 6	Sept 9, 10

supply (*6*, *13*), vine canopy management (*14*−*16*), water deficit (*17*), soil amendments (*18*), and many other examples. A difficulty in interpreting many of these results is that the practices modify vine growth and canopy structure, causing changes in light exposure and temperature in the fruiting zone, and therefore it is unclear if these affects are causal.

Sunlight exposure is thought to be one of the main factors influencing anthocyanin accumulation and composition in grapes (*8*, *9*). Light was found to have its greatest impact on anthocyanin accumulation during the initial stages of growth rather than during the fruit ripening period (post-véraison) (*19*). It has also been reported that anthocyanin accumulation increases linearly with increasing sunlight exposure, whereas high berry temperatures reduced anthocyanin concentration in highly exposed fruit (*20*, *21*). In several studies, higher accumulation of anthocyanins was found in grapes with cool day and night temperatures compared to high day or night temperatures (*22*, *23*). A number of exposure studies show a range of results in terms of anthocyanin accumulation (*6*, *9*, *19*−*21*, *24*−*27*). The variable results may be due to temperature differences or other factors. In addition, a plant's response will depend on the degree of exposure to stress and can be additive in response to both water deficit and UV irradiance (*28*).

Environmental influences can also modify anthocyanin composition in grape skins. Several studies have shown a higher proportion of B-ring hydroxylation in anthocyanin composition in response to UV light (*21*, *27*, *29*, *30*). In Pinot Noir, high night temperatures compared to low night temperatures reduced the proportion of Dp, Cy, and Pt (*23*).

In our assessment of vineyard spatial variations and the impact of vine vigor on phenolic accumulation, we found limited differences in seed proanthocyanidins, whereas substantial differences were found in skin proanthocyanidin accumulation and composition (*31*). There was interest in determining whether differences in composition could be detected in grapes from two specific vineyard sites known to produce wines with distinctly different price points (U.S. $38.00/bottle versus U.S. $75.00/bottle). In this paper, we studied the influence of vine vigor on the concentration and composition of anthocyanins in fruit. The objective was to investigate the importance of vineyard spatial variation on anthocyanin accumulation and composition in the variety Pinot Noir. In part 2 of this series, we address relationships between fruit and wine anthocyanin composition and wine color.

MATERIALS AND METHODS

Vineyard. This study was conducted in the Willamette Valley in Oregon in a 7-year-old commercial *V. vinifera* L. cv. Pinot Noir vineyard (clone Dijon 777 grafted onto *Riparia gloire* rootstock). The vines were trained in a vertical shoot positioned system with each vine having 10−12 buds. Vine spacing was 1 m (within row) × 2.8 m (between rows) with ∼5113 vines per hectare. The delineation of vigor zones was based on a vigor index calculated using measurements of average shoot length, trunk cross-sectional area, and leaf chlorophyll as previously described (*31*). Leaf chlorophyll was measured with a Spad 502 m Minolta meter. Phenology data for ∼50% budbreak, bloom, and véraison are shown in **Table 1** as well as harvest dates in 2003 and 2004. The vigor zones appeared to be closely related to variations in soil depth, although soil sampling was done only at site A. Site A had several soil types, varying from shallow (61 cm) to deep soils (152 cm). At site B, the low vigor zone had very limited topsoil.

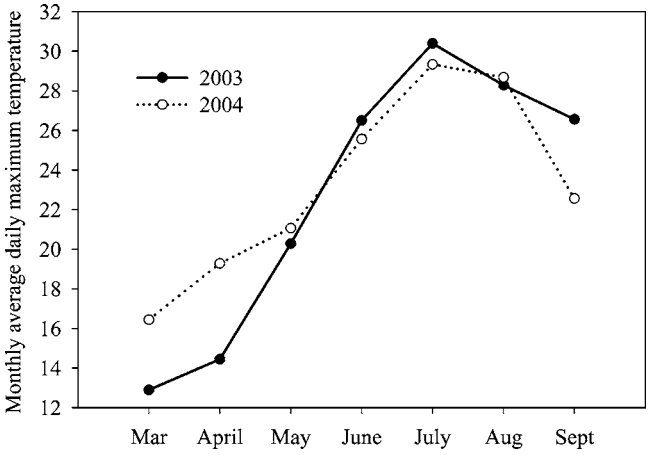

Figure 1. Monthly average of daily maximum temperatures (°C) for 2003 and 2004 from March through September.

Temperature Monitoring. Temperature was monitored in both vineyard sites and all vigor zones. Each vigor zone contained one microstation with four temperature data loggers (Onset Corp., Bourne, MA). One data logger was placed at the top of a vineyard post within a PVC tube open on either end to measure ambient air temperature. The other three data loggers were placed along the fruiting wire in the fruit zone. These data loggers were placed inside small Styrofoam cups open on both ends. The cups were used to prevent failure of the data loggers from direct sunlight. The data loggers collected data at 15 min time intervals throughout the growing season. Some data were lost in the high vigor zone of site A (A-high, with other zones identified in the same manner) due to problems with the microstation.

Surface Maps. In 2003, data for the surface map were collected on a grid across vineyard sites. Fruit was collected from the GPS-located data vine to determine yield, and additional clusters were collected off four to six adjacent vines for analysis. In 2004, five data vines per zone were randomly selected to determine yield, and additional clusters were collected from adjacent vines for analysis. The surface map was made as previously described using ESRI software (Redlands, CA) with the ordinary kriging utility (*31*).

Fruit Sampling and Extraction. Fruit samples were collected as described above for the surface maps and for the model wine extractions in 2004. Fruit samples were also collected across each vigor zone (three replicates per zone) for wine production. This fruit sample consisted of ∼25 clusters pulled randomly out of each replicate used for winemaking. Harvest dates were determined by the cooperating winery. Fruit samples were frozen and stored at −35 °C until processed. Frozen berries were removed from the rachis, and 150 berry samples were randomly collected. Samples were weighed, and skins and seeds were separated and extracted as previously described (*32*).

Chemicals. All solvents were of HPLC grade. Acetonitrile, methanol, ethanol, and acetone were purchased from J. T. Baker (Phillipsburg, NJ). Ammonium phosphate monobasic and orthophosphoric acid were purchased from Fisher Scientific (Santa Clara, CA). Hydrochloric acid was purchased from E. M. Science (Gibbstown, NJ). Malvidin-3-*O*-glucoside was purchased from Extrasynthése (Genay, France).

Instrumentation. An Agilent model 1100 HPLC (Palo Alto, CA) consisting of a vacuum degasser, autosampler, quaternary pump, diode array detector, and column heater was used. A computer workstation with Chemstation software was used for chromatographic analysis.

Reversed-Phase HPLC of Anthocyanins. Anthocyanin content and composition in grape skins was measured by reversed-phase HPLC (*33*). Aqueous extracts were filtered using Teflon filters (0.45 μm, Acrodisc CR13, Pall Corp., East Hills, NY) before injection. Eluting anthocyanins were identified and quantified with a malvidin-3-*O*-glucoside standard.

Statistical Analyses. Statistical data analysis was performed using analysis of variance (ANOVA) and the least significant difference

Table 2. Mean (±SEM) Vigor Index, Yield, Number of Clusters, and Average Cluster Weight for Vigor Zones for Sites A and B in 2003 and 2004

site	year	vigor zone	vigor index	yield (kg/vine)	no. of clusters	av cluster wt (g)
A	2003	high	0.82 ± 0.08	1.07 ± 0.10	16.55 ± 0.98	99.04 ± 6.61
		medium	0.63 ± 0.03	1.22 ± 0.03	15.95 ± 0.27	119.36 ± 1.83
		low	0.44 ± 0.05	1.36 ± 0.07	15.82 ± 0.69	127.92 ± 4.69
vigor *p* value[a]			<0.0001	0.0770	0.8712	0.0065
B	2003	high	0.49 ± 0.03	1.08 ± 0.07	10.55 ± 0.72	102.14 ± 4.90
		medium	0.35 ± 0.02	1.27 ± 0.05	11.00 ± 0.46	115.89 ± 3.13
		low	0.80 ± 0.03	0.80 ± 0.05	8.97 ± 0.55	87.04 ± 3.71
vigor *p* value[a]			<0.0001	<0.0001	<0.0001	<0.0001
A	2004	high	0.87 ± 0.07	0.59 ± 0.14	15.00 ± 1.45	38.73 ± 9.80
		medium	0.63 ± 0.02	0.78 ± 0.14	16.40 ± 1.45	47.59 ± 9.80
		low	0.42 ± 0.05	0.91 ± 0.14	14.60 ± 1.45	64.94 ± 9.80
vigor *p* value[a]			<0.0001	0.0136	0.3708	0.0130
B	2004	high	0.52 ± 0.04	0.71 ± 0.14	12.60 ± 1.45	56.73 ± 9.80
		medium	0.36 ± 0.03	0.90 ± 0.14	14.20 ± 1.45	63.39 ± 9.80
		low	0.11 ± 0.03	0.30 ± 0.14	7.40 ± 1.45	42.02 ± 9.80
vigor *p* value[a]			<0.0001	<0.0001	0.0001	0.0049
site			<0.0001	0.0406	<0.0001	0.1833
year			0.9452	<0.0001	0.6776	<0.0001
site × year			0.7649	0.8370	0.1582	0.0364

[a] ANOVA to compare data. Unequal sample sizes for vigor zones and 2003 yield data; $n = 5$ for yield data in 2004.

(LSD) test to determine statistically different values at a significance level of $\alpha \leq 0.05$. Because vigor zones (high, medium, and low) were relative levels within each site and because both year and site were significantly different for most variables, a separate ANOVA was run to compare vigor zones for each site and year combination. For vineyard site and year comparisons, weighted averages were calculated and analyzed to take into account the contribution of the vigor zones to the total area within each vineyard site. Pearson's correlation analysis was used to investigate relationships between anthocyanin concentration per berry and yield, soluble solids, average berry weight, and average dry skin weight. All statistical analyses were performed using SAS version 8.2.

RESULTS AND DISCUSSION

Vine Vigor. Vine vigor differences across the vineyard sites for both years are included (**Table 2**), although relative vigor differences for 2003 were reported in a previous paper (*31*). Differences in vine vigor appeared to be related to variations in soil depth and water holding capacity (*31*). The variation in vine vigor was consistent between 2003 and 2004. Overall, site A had higher vigor than site B. Vines in high-vigor zones were characterized by greater average shoot length, larger trunk cross-sectional areas, and higher leaf chlorophyll as previously described (*31*). Leaf chlorophyll measurements by Spad have been found to reflect relative leaf nitrogen status in assessing spatial variation in vineyards (*34*).

Yield. Although the number of clusters between vigor zones was reasonably consistent between years, yield was greatly reduced in 2004 due to poor fruitset resulting in low cluster weights (**Table 2**). In both years, there was a higher number of clusters per vine at site A than at site B. Yield was lower in the highest vigor zone (A-high) and the lowest vigor zone (B-low) due to smaller cluster weights in A-high and both lower cluster numbers and weights in B-low.

Temperature Differences. Figure 1 shows seasonal monthly daily maximum temperatures for 2003 and 2004. Although temperatures were similar during bloom for both years, it was very warm from March through May in 2004 compared to 2003. The remainder of the ripening period was similar but cooled off more rapidly in 2004 with early September rains.

Sites A and B are oriented differently in the vineyard, and this appeared to influence temperatures in the fruiting zone. Site B, where the rows are oriented north−south, was 2−3 °C warmer in June−August from 4 to 7 p.m. and 1−2 °C warmer from 10 a.m.−1 p.m. in August compared to site A (oriented east−west) (**Table 3**). Although canopy light measurements were not taken, solar radiation and wind velocity are the two most important determinants of fruit temperature (*35*). Therefore, the higher fruiting zone temperatures strongly suggest greater sunlight interception. This is in agreement with a previous study in which east−west rows intercepted considerably less light than north−south rows (*36*).

Site B was also lower in vine vigor than site A (**Table 3**), and this likely was an additional contributing factor to the temperature differences in the canopy (*31*). The impact of vine vigor on modification of the vine microclimate has been previously described (*7*, *8*). There were differences in temperature between the vigor zones at both sites with the greatest temperature differences occurring in the 4−7 p.m. time period, during which vigor zones with reduced vine vigor were generally warmer. The higher temperatures also suggest there was greater light interception in the fruiting zone. These differences in vine microclimate could affect anthocyanin accumulation positively or negatively.

Fruit Composition. The accumulation of soluble solids (degrees Brix) was higher in 2003 compared to 2004 as rains forced an earlier harvest in the latter year (**Table 4**). Soluble solids were higher at site A in both years compared to site B. Soluble solids were about 1 °Brix lower in the high-vigor zone than in the medium- and low-vigor zones at site A in 2003 (**Table 5**). At site B, soluble solids increased with decreasing vine vigor in 2003. There was a similar pattern for site A in 2004 with the high-vigor zone having lower soluble solids than the medium- or low-vigor zones, whereas at site B the medium-vigor zone was higher in soluble solids than the high- or low-vigor zone. These results agree with reported reductions in sugar accumulation due to fruit shading (*15*, *25*, *26*, *37*) and in high-vigor canopies (*8*). However, other studies specifically on fruit

Table 3. Temperature Data (°C) from 2004 Showing Variation by Site, Vigor Zone, Month, and Time Period

site	vigor zone	12 a.m.–6 a.m.	10 a.m.–1 p.m.	1 p.m.–4 p.m.	4 p.m.–7 p.m.
			May		
A	high	9.98a	18.10b	19.46b	18.45e
	medium	9.56b	18.81ab	20.39a	19.03c
	low	9.77ab	19.16a	20.42a	18.79d
B	high	9.73bc	18.78ab	20.28a	19.38b
	medium	9.56c	19.33a	20.62a	19.76b
	low	9.77b	19.10a	20.73a	19.92a
SEM		0.05	0.28	0.15	0.11
p value[a]		0.0051	0.1220	0.0026	<0.0001
			June		
A	high	12.69ab	22.44d	24.71d	23.02d
	medium	12.86a	22.65cd	25.39bc	23.94c
	low	12.71ab	22.79bcd	25.03cd	23.39cd
B	high	12.76ab	23.59abc	25.55abc	25.12b
	medium	12.59b	23.77ab	25.82ab	25.56ab
	low	12.89a	24.45a	26.18a	26.05a
SEM		0.08	0.33	0.20	0.21
p value[a]		0.1363	0.0129	0.0060	<0.0001
			July		
A	high	13.41d	24.07c	missing	26.03e
	medium	14.82ab	27.56b	30.75ab	29.30c
	low	14.59bc	27.52b	29.98b	27.78d
B	high	14.64bc	28.41ab	30.82ab	30.47b
	medium	14.51c	28.79a	31.19a	30.87b
	low	14.92a	27.70ab	31.44a	32.18a
SEM		0.08	0.35	0.28	0.27
p value[a]		<0.0001	<0.0001	0.0883	<0.0001
			August		
A	high	16.3a	26.1cd	missing	27.64d
	medium	16.09ab	25.38d	29.61b	28.2cd
	low	15.68c	25.98cd	29.06b	26.72e
B	high	15.71c	27.69ab	29.84ab	28.66c
	medium	15.49c	28.3a	30.51a	29.51b
	low	15.78bc	26.76bc	30.7 a	30.84a
SEM		0.27	0.27	0.27	0.27
p value[a]		0.0003	0.0033	0.0228	<0.0001
			September		
A	high	6.99a	22.88ab	missing	missing
	medium	6.68ab	22.00b	24.98ab	23.43c
	low	6.48b	22.56b	24.55a	21.75d
B	high	6.54b	23.48ab	25.07ab	23.19c
	medium	6.38b	24.48a	25.79a	24.12b
	low	6.42b	23.06ab	25.7a	25.16a
SEM		0.25	0.45	0.30	0.14
p value[a]		<0.0001	0.0250	0.1361	<0.0001

[a] ANOVA to compare data (*p* indicated), $n = 3$: values sharing the same letter within each column are not significantly different at $p \geq 0.05$.

shading did not find differences in soluble solids accumulation (*21*, *24*, *27*, *38*, *39*).

In comparing vintages, 2003 was lower in titratable acidity (TA) and had a higher pH than 2004 (**Table 4**). For TA and pH, there were also a site and a site by year interaction. Site A had a higher average pH than site B in 2003 and was similar in 2004. For TA, site B was slightly higher than site A in 2003, whereas site A was substantially higher than site B in 2004. Site A may have had a higher TA than site B in 2004 as it contains higher vigor vines. In both years and at both sites, a reduction in TA was associated with a reduction in vine vigor except that the medium- and low-vigor zones of site A were similar in 2003 (**Table 5**). The high TA in the fruit from the high-vigor zone agrees with other findings where canopies with excessive soil moisture and <60% cluster exposure had higher TAs (*8*). In 2003, pH was higher at site A than at site B possibly due to higher available water and greater cation uptake (**Table 4**) (*8*). In 2004, the sites were similar for pH.

Table 4. Mean and SEM of Average Berry Weight, Average Dry Skin Weight, Soluble Solids, Titratable Acidity (TA), and pH Based on a Weighted Average for Sites A and B in 2003 and 2004

site	year	berry wt (g)	dry skin wt (mg)	soluble solids (°Brix)	TA (g/L)	pH
A	2003	0.91	32.0	24.2	4.8	3.49
B	2003	0.84	27.9	24.0	5.1	3.27
A	2004	0.63	17.5	23.3	6.5	3.23
B	2004	0.73	17.6	22.9	5.2	3.23
SEM		0.02	1.0	0.1	0.1	0.01
site *p* value[a]		0.5541	0.0614	0.0483	0.0012	<0.0001
year *p* value[a]		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
site × year *p* value[a]		0.0004	0.0481	0.9980	<.00001	<0.0001

[a] ANOVA to compare data (*p* indicated), $n = 9$.

Table 5. Mean and SEM of Average Berry Weight, Average Dry Skin Weight, Soluble Solids, Titratable Acidity (TA), and pH for Vigor Zones for Sites A and B in 2003 and 2004

site	year	vigor zone	berry wt (g)	dry skin wt (mg)	soluble solids (°Brix)	TA (g/L)	pH
A	2003	high	0.99a	28.0b	23.5b	5.7a	3.50a
		medium	0.91b	32.8a	24.3a	4.7b	3.50a
		low	0.87b	31.3ab	24.1a	4.6b	3.47a
SEM			0.02	1.2	0.05	0.1	0.02
B	2003	high	0.82a	29.6a	23.7c	5.7a	3.20b
		medium	0.87a	27.7a	24.0b	4.9b	3.30a
		low	0.78a	26.5a	24.4a	4.7c	3.30a
SEM			0.03	2.0	0.05	0.05	0.02
p value[a]			0.2856	0.4758	0.0010	0.0002	<0.0001
A	2004	high	0.63a	16.0a	21.7b	7.4a	3.19b
		medium	0.60a	17.1a	23.6a	6.5b	3.24a
		low	0.72a	20.1a	23.4a	5.9c	3.21ab
SEM			0.05	1.6	0.1	0.1	0.009
B	2004	high	0.69a	17.2a	22.5b	5.8a	3.19c
		medium	0.72a	18.1a	23.3a	5.2b	3.23b
		low	0.78a	17.2a	22.5b	4.8c	3.26a
SEM			0.03	1.8	0.07	0.03	0.003
p value[a]			0.1635	0.9167	0.0014	<0.0001	0.0002

[a] ANOVA (*p* indicated), $n = 3$: values sharing same letter within each site not significantly different at $p \geq 0.05$.

Average berry weight was lower in 2004 than in 2003 (**Table 4**). The average berry weight was higher at site A in 2003 and higher at site B in 2004. The lower average berry weight in 2004 compared to 2003 may have been due to cool rainy weather during bloom and fruitset that reduced ovule fertility and number of seeds per berry (data not included). This agrees with a previous study in which temperature was reported to reduce ovule fertility and number of seeds per berry (*40*). The site by year interaction was also probably related to fruitset problems in 2004, when site A had more small shot berries and site B had compensation for poor fruitset in fewer but larger berries.

Comparison of the vigor zones at site A showed that the high-vigor zone had a higher average berry weight than the medium- and low-vigor zones in 2003 (**Table 5**). There were no differences between vigor zones at site B in 2003. In 2004, there were no differences between vigor zones at either site. The larger

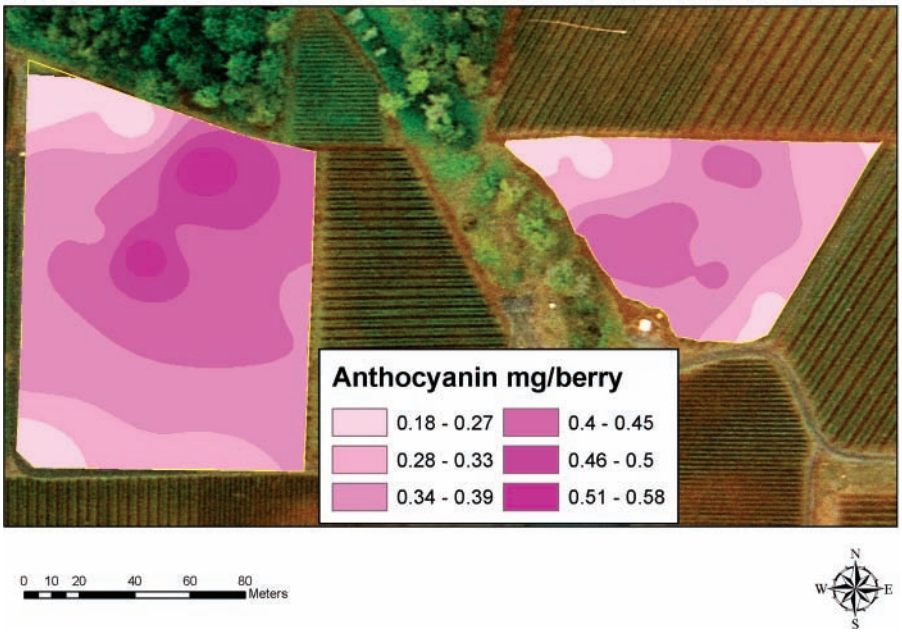

Figure 2. Surface map of spatial variation in total anthocyanin accumulation in milligrams per berry in 2004.

Table 6. Mean and SEM of Total and Individual Anthocyanins for Vine Vigor Zones in 2004[a]

site	vigor zone	total ACY (mg/berry)	delphinidin[b] (mg/berry)	cyanidin (mg/berry)	petunidin (mg/berry)	peonidin (mg/berry)	malvidin (mg/berry)
A	high	0.20b	0.007b	0.003b	0.011b	0.043c	0.13b
	medium	0.31b	0.013b	0.005b	0.020b	0.066b	0.21b
	low	0.48a	0.024a	0.009a	0.036a	0.10a	0.31a
SEM		0.04	0.002	0.0007	0.003	0.006	0.02
p value[c]		0.0067	0.0094	0.0060	0.079	0.0036	0.0096
B	high	0.29a	0.009b	0.003b	0.015b	0.055a	0.21a
	medium	0.34a	0.013ab	0.005ab	0.020ab	0.064a	0.24a
	low	0.38a	0.015a	0.005a	0.023a	0.068a	0.26a
SEM		0.03	0.002	0.0005	0.002	0.006	0.02
p value[c]		0.2027	0.0400	0.0758	0.0704	0.3294	0.2430

[a] Fruit samples were used for spatial surface map and model extractions (part 2). [b] 3-*O*-Glucosides of individual anthocyanidins. [c] ANOVA to compare data (*p* indicated), $n = 5$: values sharing the same letter within each site are not significantly different at $p \geq 0.05$.

berries in A-high in 2003 agree with other studies in which larger berries were found due to fruit shading (*38*, *41*).

For average dry skin weight (milligrams), there was a year effect with the higher berry weight in 2003 also resulting in a higher dry skin weight (**Table 4**). Skin weight appeared to be more strongly related to berry size than to vigor differences. Differences in average dry skin weights were found at site A in 2003, where the medium-vigor zone was higher than the high-vigor zone, whereas no differences were found at site B (**Table 5**). No differences were found in average dry skin weight between vigor zones at either site in 2004. Although research on deficit irrigation has shown an increase in skin weight (*42*, *43*) and a higher skin to pulp ratio (*17*), the lack of differences at site B and in 2004 make it difficult to draw conclusions on the basis of these results.

Anthocyanin Accumulation in Fruit. Spatial variation across vineyard sites for total anthocyanin accumulation in milligrams per berry in 2004 is shown in **Figure 2**. There was a response of greater anthocyanin accumulation in the low-vigor zones of both vineyard sites. In 2004, there was an increase in the total amount per berry and for all individual anthocyanins at site A with a reduction in vine vigor (**Table 6**). For site B, there were no significant vine vigor differences for total, Pn, and Mv amount per berry, whereas Dp, Cy, and Pt increased with a reduction in vigor. This berry sample was taken at five random locations (five replicates) within each vigor zone. The fruit was also used for the model extraction discussed in part 2 of this series.

Fruit samples were also collected to reflect the fruit used to make wines. For these fruit samples, there was higher total anthocyanin accumulation (milligrams per berry) in 2003 than in 2004 (**Table 7**). In other studies, some reported vintage variation (*21*, *44*), whereas others reported minimal influence of the season on anthocyanin accumulation (*45*). Comparison of the sites showed that there were no differences between sites or a site by year interaction. In the present study, it seems to be more probable that the low accumulation of anthocyanins in 2004 was in response to rapid vine growth due to warm spring weather during bloom. High bloom time nitrogen, particularly with low light irradiance, was previously found to interfere with phenolic biosynthesis, leading to a lower total amount at maturity (*6*).

In 2003, the medium- and low-vigor zones at site A were higher in anthocyanin concentration (milligrams per berry) compared to the high-vigor zone (**Table 8**). In 2004, there were no differences between zones at site A. At site B there were no differences in total anthocyanin concentration (milligrams per berry) in either year. In 2003, there was a trend toward higher

Table 7. Mean and SEM of Total and Individual Anthocyanins Based on a Weighted Average for Sites A and B in 2003 and 2004

site	year	total ACY (mg/berry)	total ACY (mg/kg)	delphinidin[a] (mg/berry)	cyanidin (mg/berry)	petunidin (mg/berry)	peonidin (mg/berry)	malvidin (mg/berry)
A	2003	0.81	896	0.045	0.021	0.061	0.21	0.48
B	2003	0.87	1043	0.049	0.020	0.065	0.19	0.55
A	2004	0.25	411	0.011	0.004	0.017	0.05	0.17
B	2004	0.30	413	0.012	0.004	0.018	0.05	0.21
SEM		0.03	51	0.003	0.0009	0.004	0.008	0.02
site *p* value[b]		0.8871	0.1484	0.3908	0.9909	0.5171	0.2752	0.0110
year *p* value[b]		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
site × year *p* value[b]		0.1525	0.1610	0.6364	0.6852	0.8305	0.1205	0.4267

[a] 3-*O*-Glucosides of individual anthocyanidins. [b] ANOVA to compare data (*p* indicated), $n = 9$.

Table 8. Mean and SEM of Total and Individual Anthocyanins for Vine Vigor Zones for Sites A and B in 2003 and 2004

site	year	vigor zone	total ACY (mg/berry)	total ACY (mg/kg)	delphinidin[a] (mg/berry)	cyanidin (mg/berry)	petunidin (mg/berry)	peonidin (mg/berry)	malvidin (mg/berry)
A	2003	high	0.61b	619.5b	0.03b	0.02a	0.04b	0.15a	0.38a
		medium	0.83a	913.7a	0.05ab	0.02a	0.06ab	0.22a	0.48a
		low	0.87a	994.5a	0.06a	0.03a	0.07a	0.22a	0.50a
SEM			0.08	79.9	0.007	0.002	0.008	0.02	0.04
p value[a]			0.1394	0.0610	0.0929	0.2449	0.1174	0.1527	0.1755
B	2003	high	0.86a	1056.2a	0.04a	0.02a	0.06a	0.19a	0.55a
		medium	0.87a	996.4a	0.05ab	0.02a	0.06a	0.19a	0.55a
		low	0.87a	1116.4a	0.05b	0.02a	0.07a	0.18a	0.54a
SEM			0.04	81.0	0.004	0.001	0.005	0.01	0.03
p value[a]			0.9756	0.3977	0.0889	0.1523	0.3082	0.4894	0.9494
A	2004	high	0.23a	362.1a	0.009a	0.004a	0.01a	0.05a	0.15a
		medium	0.22a	384.6a	0.009a	0.003a	0.01a	0.04a	0.15a
		low	0.39a	544.4a	0.02a	0.007a	0.03a	0.08a	0.25a
SEM			0.05	86.8	0.004	0.001	0.006	0.01	0.03
p value[a]			0.1187	0.3648	0.1148	0.1135	0.1367	0.1097	0.1295
B	2004	high	0.26a	381.2a	0.009a	0.004a	0.015a	0.05a	0.19a
		medium	0.31a	439.5a	0.013a	0.005a	0.020a	0.06a	0.22a
		low	0.31a	396.6a	0.014a	0.005a	0.020a	0.06a	0.21a
SEM			0.05	82.9	0.003	0.001	0.004	0.01	0.03
p value[a]			0.7087	0.8701	0.3025	0.6267	0.4990	0.7534	0.7468

[a] 3-*O*-Glucosides of individual anthocyanidins. [b] ANOVA to compare data (*p* indicated), $n = 3$: values sharing the same letter within each site are not significantly different at $p \geq 0.05$. Anthocyanins were calculated as malvidin equivalents.

Dp and Pt with a reduction in vine vigor and no differences in Cy, Pn, or Mv at site A. In 2003 at site B, there was a trend for higher Cy with reduced vine vigor and no differences in Dp, Pt, Pn, or Mv. In 2004, there were no vine vigor differences in total or individual anthocyanins at site A or B. However, as seen in **Table 6**, a different sampling strategy with greater replication was able to detect differences. This suggests that in 2004, three replicates of 150 berries was not an adequate sample size, possibly due to high fruit variability.

Anthocyanin accumulation in grapes is light and temperature sensitive (*21*). In 2003, some variation in anthocyanin concentrations was observed between high- and low-vigor zones, and this may have been due to a combination of light and temperature effects as a result of variations in canopy shading. Sunlight exposure has a positive linear effect on anthocyanin biosynthesis, although high resultant berry temperature can reduce anthocyanin accumulation (*20*, *21*, *39*, *46*).

In 2004, small differences in anthocyanin concentrations between vigor zones were observed. The fruit in the B-low vigor zone had inconsistent ripening, where clusters had a combination of purple, pink, and green berries. B-medium and B-high vigor zones in contrast appeared to ripen normally. This may have been due to water deficit as this phenomenon has been reported in fruit that failed to mature due to water deficit (*47*).

Overall, the medium-vigor zones tended to have berries with the highest anthocyanin concentrations. This may have been due to the moderate water deficit and observed moderate light exposure in the fruiting zone compared to the more extreme conditions found in the high- and low-vigor zones. In a number of irrigation studies, greater water stress was found to have a direct effect of increasing the concentration due to higher anthocyanin biosynthesis or an indirect effect from a reduction in fruit size (*17*, *39*, *47*, *48*−*50*). This suggests the importance of maintaining good vine balance with moderate vine vigor for consistent anthocyanin accumulation.

In this study the medium-vigor vines tended to have both higher yields and higher anthocyanins in agreement with others who found improved phenolics in vines with better vine balance based on grape yield to pruning weight ratio (*51*). In 2003, there was also better vine balance, with yield to pruning weight ratios between 1.90 and 4.60 compared to 2004, when the range was from 0.80 to 2.81 (data not included). Nevertheless, some studies have reported that higher yielding vineyards had lower anthocyanins in the fruit than fruit from low-yielding vineyards (*52*, *53*). Overall, the variability in anthocyanin accumulation observed in this study is consistent with the variable results observed in previous studies (*9*, *19*−*21*, *25*−*27*). To summarize relationships between variables, Pearson's correlation analysis

Table 9. Mean and SEM of Percent Composition for Individual, 3′,4′-Dihydroxy, and 3′,4′,5′-Trihydroxy Anthocyanins Based on a Weighted Average for Sites A and B in 2003 and 2004

site	year	delphinidin[a] (%)	cyanidin (%)	petunidin (%)	peonidin (%)	malvidin (%)	3′,4′-OH[b] (%)	3′,4′,5′-OH[c] (%)
A	2003	5.5	2.6	7.4	25.9	58.6	28.5	71.5
B	2003	5.7	2.3	7.5	21.7	62.9	24.0	76.0
A	2004	4.2	1.5	6.3	19.3	68.71	20.8	79.2
B	2004	4.10	1.4	6.0	17.6	70.9	19.0	81.0
SEM		0.3	0.1	0.3	0.5	0.6	0.5	0.5
site *p* value[c]		0.6118	0.0662	0.6723	<0.0001	0.0003	<0.0001	<0.0001
year *p* value[c]		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
site × year *p* value[c]		0.8356	0.3599	0.6092	0.0255	0.1841	0.0192	0.0192

[a] 3-*O*-Glucosides of individual anthocyanidins. [b] 3′,4′-Dihydroxy anthocyanins = (cyanidin + peonidin). [c] 3′,4′,5′-Trihydroxy anthocyanins = (delphinidin + petunidin + malvidin). [d] ANOVA to compare data (*p* indicated), $n = 9$.

was used. When data from both years were included, anthocyanin accumulation (milligrams per berry) was positively correlated with yield ($r^2 = 0.72$, $p = <0.0001$), soluble solids ($r^2 = 0.73$, $p = <0.0001$), average berry weight ($r^2 = 0.56$, $p = <0.0001$), and average dry skin weight ($r^2 = 0.77$, $p = <0.0001$).

When the anthocyanin available to be extracted into wine is assessed, both the concentration per berry and the berry size need to be taken into account. Determining the concentration as milligrams per kilogram gives the potential amount that can be extracted into wine. Berry size can affect the skin to pulp ratio as well as the skin surface area. When the same anthocyanin data were expressed on a milligrams per kilogram basis to reflect differences in fruit weight, 2003 was substantially higher than 2004 (**Table 7**). There was no consistent site effect or site by year effect. In 2003, site B was higher than site A in anthocyanin accumulation when expressed as milligrams per kilogram, whereas in 2004 the sites were similar. In 2003 for the vigor zones, there was greater anthocyanin accumulation (milligrams per kilogram) in the medium- and low-vigor zones compared to the high-vigor zone in site A and no differences between vigor zones in site B (**Table 8**).

Anthocyanin Composition in Fruit. There were differences in the anthocyanin proportional composition between years (**Table 9**). In 2003, the proportions of Dp, Cy, Pt, and Pn were higher, and only the proportion of Mv was lower in comparison to 2004. Although a number of authors have concluded that anthocyanin composition is primarily determined by genetic factors (*5*, *44*, *54*, *55*), the proportional composition specific for a variety may be modified by environmental conditions (*18*, *21*, *27*, *56*) and ripening (*56*).

In the present study, the fruit was harvested riper (based on soluble solids) and the weather was warmer near the time of harvest in 2003 compared to 2004 (**Table 3**; **Figure 1**). However, 2004 had a higher proportion of Mv than 2003. This is the opposite of a previous finding of higher Mv in warmer years and with greater fruit maturity (*56*). One possible explanation for this is that the composition was influenced during early berry development by atypical spring weather in 2004 (**Figure 1**). This plausible explanation agrees with a study that found that the percentage of Mv was predominant with high rates of nitrogen at bloom and low light intensity at véraison (*6*). Another possibility is that the variation in proportions is simply related to differences in total accumulation as the total concentration was lower in 2004.

There were differences in the anthocyanin proportion between sites (**Table 9**). The most notable and consistent pattern was in site A having proportionally higher Pn and lower Mv than site B. The higher Pn and lower Mv found in site A might be explained by the lower temperatures (**Table 2**) and associated higher degree of shading at this site as it has an east−west orientation and has overall higher vigor than site B. Although other factors such as nitrogen status and water availability could be contributing factors, a light exclusion experiment done in the low-vigor zone of site A resulted in an increase of around 2 times the proportion of Pn compared to exposed clusters, suggesting that this response may be explained primarily by shading (*57*). Shading in cv. Syrah has also been found to increase the proportion of Pn (*27*).

Although accumulation increased for all anthocyanins in fruit with a reduction in vine vigor (**Table 6**; **Figure 2**), vine vigor resulted in proportional variations in anthocyanin composition (**Table 10**). In 2003, there was a trend toward higher proportions of Dp and Pt at site A with a reduction in vine vigor. At site B, there was a trend toward higher Dp and Cy with a reduction in vine vigor in 2003. In 2004, there was a trend toward higher Dp and Cy with a reduction in vine vigor at site A and no differences between vigor zones at site B. Although shading was found to increase the proportion of Pn (*57*) and site A had a higher proportion of Pn than site B, there were no differences found in the vigor zones in 2003 or 2004. Mv was similar to Pn in that there were no differences between vigor zones within a site for either year.

The higher proportion of Dp and Pt found in the low-vigor fruit at site A for 2003 agrees with previous results showing that sun-exposed fruit was associated with higher Dp and Pt (*27*, *58*). In the associated cv. Pinot Noir shading experiment, exposed fruit had higher proportions of Dp, Cy, Pt, and Mv with only a decrease in the proportion of Pn compared to the shaded treatment (*57*). Hence, it seems likely that higher sunlight exposure in the low-vigor canopy played a role in the higher proportion of Dp and Pt. It does not explain the response of Pn or Mv as clearly.

The percent of 3′,4′-dihydroxy (Cy and Pn) and 3′,4′,5′-trihydroxy (Dp, Mv, and Pt) anthocyanins was calculated. Variations with respect to vintage and site as well as a site by year interaction existed (**Table 9**). In 2003, the proportion of 3′,4′-dihydroxy anthocyanins was approximately 6% higher than in 2004. Site B was consistently lower than site A in the proportion of 3′,4′-dihydroxy anthocyanins in both years. In 2003, there was no apparent pattern in the proportion of 3′,4′-dihydroxy anthocyanins across vigor zones at either site (**Table 10**). In 2004, there was a trend toward a higher proportion of 3′,4′-dihydroxy anthocyanins with a reduction in vine vigor at site A, whereas no differences were found at site B.

In this study, site B had a lower proportion of 3′,4′-dihydroxy anthocyanins or, in particular, a lower proportion of Pn and higher Mv (**Table 9**). This agrees with other studies in which light exposure was found to cause a shift in biosynthesis toward a higher proportion of 3′,4′,5′-trihydroxy anthocyanins compared

Table 10. Mean and SEM of Percent Composition for Individual, 3′,4′-Dihydroxy, and 3′,4′,5′-Trihydroxy Anthocyanins for Vine Vigor Zones for Sites A and B in 2003 and 2004

site	year	vigor zone	delphinidin[a] (%)	cyanidin (%)	petunidin (%)	peonidin (%)	malvidin (%)	3′,4′-OH[b] (%)	3′,4′,5′-OH[c] (%)
A	2003	high	4.3b	3.1a	6.4b	23.9a	62.2a	27.0a	73.0a
		medium	5.5ab	2.4b	7.4ab	26.5a	58.3a	28.8a	71.2a
		low	6.4a	2.9a	8.0a	25.0a	57.7a	27.9a	72.1a
SEM			0.5	0.1	0.4	1.3	1.3	1.2	1.2
p value[d]			0.0953	0.0009	0.0809	0.2983	0.1305	0.4777	0.4777
B	2003	high	5.1b	2.2b	7.0a	22.2bcd	63.5a	24.4a	75.6a
		medium	5.7ab	2.3b	7.4a	21.8cd	62.8a	24.1a	75.9a
		low	6.3a	2.5a	7.9a	20.9d	62.4a	23.4a	76.6a
SEM			0.3	0.04	0.3	0.5	0.8	0.6	0.6
p value[d]			0.0459	0.0095	0.1499	0.3275	0.6472	0.5235	0.5235
A	2004	high	4.1b	1.6ab	6.0a	20.2a	68.1ab	21.8a	78.2a
		medium	3.8b	1.4b	6.0a	18.9a	69.9a	20.3a	79.7a
		low	5.6a	1.9a	7.6a	20.4a	64.6b	22.3a	77.7a
SEM			0.4	0.1	0.5	0.6	1.5	0.7	0.7
p value[d]			0.0832	0.0608	0.1697	0.1114	0.0813	0.0802	0.0802
B	2004	high	3.6a	1.4a	5.6a	17.6a	71.8a	19.0a	81.0a
		medium	4.1a	1.4a	6.1a	17.4a	71.2a	18.7a	81.3a
		low	4.6a	1.5a	6.5a	18.0a	69.4a	19.5a	80.5a
SEM			0.3	0.1	0.3	0.9	1.4	1.0	1.0
p value[d]			0.0650	0.4069	0.0678	0.9002	0.4657	0.8647	0.8647

[a] 3-*O*-Glucosides of individual anthocyanidins. [b] 3′,4′-Dihydroxy anthocyanins = (cyanidin + peonidin). [c] 3′,4′,5′-Trihydroxy anthocyanins = (delphinidin + petunidin + malvidin). [d] ANOVA to compare data (*p* indicated), $n = 3$: values sharing the same letter within each site are not significantly different at $p \geq 0.05$.

to 3′,4′-dihydroxy through the up-regulation of flavonoid-3′,5′-hydroxylase (*27*, *29*, *30*). As Mv has not been found to be particularly sensitive to light or nitrogen (*6*, *13*), this suggests that Mv is temperature responsive. The proportional increase in Dp and Pt in the low-vigor zones may be more specifically related to canopy light exposure as these have been previously reported to be light responsive (*6*, *27*) and were found to be lower in a shading experiment carried out in the B-low vigor zone (*57*).

With regard to Pn, we have seen increases in the proportion of Pn in response to shading in Pinot Noir (*57*); however, others have found a higher proportion of Pn in response to water stress (*59*) and jasmonic acid (*60*). This shows the complexity of anthocyanin accumulation in response to different environmental factors. In addition, more emphasis needs to be placed on separating out environmental influences from possible indirect responses of ratio-based expressions (proportional composition) that are influenced by denominator size (*61*). This idea of direct and indirect effects on proportional composition will be explored further in a separate paper.

Conclusions. The goals of these papers were to investigate the influence of spatial variation in vine vigor on anthocyanin concentration and composition in fruit (part 1) and to explore the relationship between fruit chemical analyses and wine color (part 2). A number of environmental influences can cause complex relationships in the vineyard related to anthocyanin accumulation in the fruit. In this study, differences were found between vintage, vineyard site, and vigor zone in both the amount and composition of anthocyanins. There were smaller berries, lower soluble solids, and lower yields in 2004, whereas there was a higher concentration of anthocyanins in 2003. Although differences in anthocyanin accumulation in response to vine vigor could involve several factors, differences in the fruiting zone microclimate appeared to be an important factor on the basis of similar results found in a shading experiment conducted simultaneously (*57*). Viticulturalists would like to find a strong relationship between vineyard parameters, fruit anthocyanin concentrations, and wine color; however, although we found some differences in skin anthocyanins, they were not as pronounced or consistent as the variation found in skin proanthocyanidins (*31*).

In relation to wine, accumulation of anthocyanins in the fruit is only part of the story as differences in cell structure and winemaking techniques also play a role in anthocyanin extraction. The ease of anthocyanin diffusion from cell membranes (*62*), berry size, and fruit ripeness (*63*−*65*) may influence extraction. This suggests a need for further investigation of factors that influence the extractability of anthocyanins from fruit.

Once anthocyanins have been released into the wine matrix, they rapidly begin undergoing reactions that can form pigmented polymers. Both anthocyanins and pigmented polymers contribute to wine color; however, as wine begins to age, the pigmented polymers play an increasingly important role in wine color (*66*−*68*). Consequently, both the initial concentration of anthocyanins in the fruit and the rate of pigmented polymer formation need to be considered in the assessment and prediction of stable red wine color. In part 2 of this series, we investigate some aspects of the relationship between fruit anthocyanin composition as modified by vine vigor and the evolution of anthocyanins and formation of pigmented polymers during winemaking.

ABBREVIATIONS USED

TA, titratable acidity (mg/L); Mv, malvidin-3-*O*-glucoside; Dp, delphinidin-3-*O*-glucoside; Cy, cyanidin-3-*O*-glucoside; Pt, petunidin-3-*O*-glucoside; Pn, peonidin-3-*O*-glucoside; CI, 95% confidence interval; ACY, anthocyanins; SEM, standard error of the mean.

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