JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Influence of Vine Vigor on Grape (*Vitis vinifera* L. Cv. Pinot Noir) Anthocyanins. 2. Anthocyanins and Pigmented Polymers in Wine

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The relationships between grapevine (*Vitis vinifera*) vigor variation and resulting wine anthocyanin concentration and composition and pigmented polymer formation were investigated. The study was conducted in a commercial vineyard consisting of the same clone, rootstock, age, and vineyard management practices. Vine vigor parameters were used to designate vigor zones within two vineyard sites (A and B) to produce research wines (2003 and 2004) and conduct a model extraction experiment (2004 only) to investigate the vine−fruit−wine continuum. Wines and model extracts were analyzed by HPLC and UV−vis spectrophotometry. For the model extractions, there were no differences between sites for pomace weight, whereas juice volume was higher for site A. This was not related to a larger berry size. Site A had a higher anthocyanin concentration (milligrams per liter) in the model extracts than site B specifically for the medium- and low-vigor zones. For anthocyanin composition in the model extraction, site B had a greater proportion of malvidin-3-*O*-glucoside and less of the remaining anthocyanin glucosides (delphinidin, cyanidin, petunidin, and peonidin) compared to site A. In the wines, there was a vintage effect, with the 2003 wines having a higher anthocyanin concentration (milligrams per liter) than the 2004 wines. This appears to have been primarily due to a greater accumulation of anthocyanins in the fruit. In general, the medium-vigor zone wines had higher anthocyanin concentrations than either the high- or low-vigor zone wines. There was also vintage variation related to anthocyanin composition, with the 2003 wines having a higher proportion of delphinidin and petunidin glucosides and lower malvidin-3-*O*-glucoside compared to 2004. In both years, there were higher proportions of delphinidin and petunidin glucosides in wines made from low-vigor-zone fruit. Wines made from low-vigor zones showed a greater propensity to form vitisin A as well as pigmented polymers. Low-vigor-zone wines had a ∼2-fold increase in pigmented polymer concentration (milligrams per liter) over high-vigor-zones wines. There was a strong positive relationship between pigmented polymer concentration, bisulfite bleaching resistant pigments, proanthocyanidin concentration, and color density in wines. Overall, differences found in the wines magnified variation in the fruit.

KEYWORDS: Grapes; pigmented polymers; proanthocyanidins; anthocyanins; flavonols; color density; hue; berry size; vitsin A

INTRODUCTION

The color of red wine is an important sensory attribute that originates from anthocyanins in the fruit. Anthocyanins may also have human health benefits (*1*, *2*). Grape varieties can have a complex profile of up to 20 different anthocyanins (*3*) or a relatively simple profile as in cv. Pinot Noir, a cool climate variety that produces only the five nonacylated forms (*4*).

In addition to the anthocyanin amount in the berry, the extractability of anthocyanins from skins influences the wine profile. Berry size was reported to play a role in extraction (*5*), although recent studies found that berry size alone did not have a major impact on extraction (*6*, *7*). Fruit ripeness was also reported to improve the extraction of anthocyanins and phenolic compounds (*8*−*10*).

Winemaking practices such as skin contact time, fermentation temperature, and the use of macerating enzymes influence the extraction of anthocyanins (*11*). Anthocyanin concentration reaches a maximum early in fermentation followed by a decrease

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10.1021/jf070196n CCC: $37.00
Published on Web 07/18/2007

(*12*−*14*). Once anthocyanins have been extracted into the wine matrix, they rapidly form copigmentation complexes (*15*, *16*) and begin undergoing numerous reactions. Several families of new pigmented compounds have been identified and described (*17*).

One reaction mechanism is the cycloaddition between anthocyanins and vinyl derivatives such as pyruvic acid or acetaldehyde to form pigments known as pyranoanthocyanins (*18*, *19*). Vitisin A, the pyruvic acid adduct of malvidin-3-*O*-glucoside, was one of the first compounds identified in this family (*18*, *19*). At wine pH, the vitisins are orange-red pigments, having an absorbance maximum near 500 nm and resistance to oxidation and bisulfite bleaching (*20*). Additional secondary pigments with various spectral properties have been identified (*21*−*23*). The predominant pigments in aged red wine are generally thought to involve anthocyanins and proanthocyanidins (tannins) (*24*), and these pigmented polymers are generally accepted to account for the majority of observed color in older red wines (*12*, *25*−*27*).

Although the existence of these compounds in wines has been confirmed (*27*, *28*), the structural diversity of pigmented polymers is expected to be large. Vineyard-related fruit differences are thought to play a role in pigmented polymer formation on the basis of the initial concentration and composition of anthocyanins and proanthocyanidins (*22*). The concentration of a specific anthocyanin-derived pigment was found to be related to the initial concentration of native anthocyanin precursors (*29*).

In part 1 of this series (*30*), we addressed the influence of vine vigor on the accumulation and composition of anthocyanins in fruit. In this paper, we address the influence of vine vigor on the concentration and composition of anthocyanins in a model extraction system and in wine. The objective was to investigate the relationship between fruit and wine composition and the propensity to form stable color pigments in wine.

MATERIALS AND METHODS

Vineyard. The study was conducted in 2003 and 2004 in a 7-year-old commercial *Vitis vinifera* L. cv. Pinot Noir vineyard (clone Dijon 777 grafted onto *Riparia gloire* rootstock) located in the Willamette Valley in Oregon. Vine spacing was 1 m (within row) × 2.8 m (between rows) with approximately 5113 vines per hectare. The training was a vertical shoot positioned system with each vine pruned to 10−12 buds. The delineation of vigor zones was previously described (*30*, *31*).

Fruit Sampling. A fruit sample was collected across each vigor zone (three replicates per zone) to reflect the fruit used for wine production. Harvest date, yield, and fruit composition were previously reported (*30*). Fruit samples were harvested and processed as described in part 1 of this series (*30*).

Model Extraction. Each replicate of the model extractions consisted of ∼15 clusters collected from 5 random data vine locations within each vigor zone. Fruit was collected off of the data vine and three to four adjacent vines. The berries were carefully removed from the rachis to avoid losing juice. The berries were mixed, and then a 300 g sample was taken for each replicate. The number of berries in the 300 g sample was counted prior to extraction. Berries were passed through a small crusher (providing ∼50% berry crush) and then placed into a 950 mL wide-mouth canning jar. A 40% v/v ethanol solution containing 100 mg/L SO_2 was prepared. Three hundred milliliters of the ethanol solution was added to the 300 g berry sample, resulting in an ∼20% v/v ethanol solution. Samples were sparged with nitrogen and then placed on a shaker table for 48 h at 38 °C. After 48 h, musts were pressed using a Büchner funnel (69 cm^2 surface area) with an applied vacuum of 1.6 bar. The pressed pomace was weighed and frozen. The must volume was determined before and after pressing. After pressing, musts were frozen at −10 °C until analyzed.

Winemaking. Triplicate wines were produced from each vigor zone in 2003 and 2004. The winemaking protocol for the 2003 wines was previously described (*31*). In 2004, wines were made with the same protocol as in 2003 except that replicates consisted of 22 kg of fruit. In 2004, samples were taken at the end of cold soak and every day during fermentation until pressing. Samples were taken before and after pressing in the finished wines. This differed from 2003, when wines were not analyzed until after malolactic fermentation at 3−4 months of age. The samples were frozen at −10 °C until analyzed. Wines were not filtered as part of the processing; however, they were centrifuged and filtered before HPLC analysis.

Chemicals. All solvents were of HPLC grade. Some chemicals were described previously (*30*). Additional chemicals used included potassium metabisulfite and potassium hydroxide purchased from J. T. Baker (Phillipsburg, NJ). *N*,*N*-Dimethylformamide (DMF) was purchased from Burdick and Jackson (Muskegon, MI). Hydrochloric acid was purchased from E. M. Science (Gibbstown, NJ), and lithium chloride was purchased from Mallinckrodt (Phillipsburg, NJ).

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. Anthocyanin content and composition in grape skins and wine were measured by reversed-phase HPLC (*32*). Aqueous extracts and wines were filtered using Teflon filters (0.45 μm, Acrodisc CR13) before injection. Eluting anthocyanins were identified and quantified with a malvidin-3-*O*-glucoside standard.

Gel Permeation Chromatography (GPC). GPC was used to analyze monomeric 520 nm absorbing material and pigmented polymers. By using GPC, information on the size distribution as well as pigment content could be obtained. The GPC method used has been described (*33*). Samples were prepared as previously described (*31*); however, after freeze-drying, the samples were dissolved in mobile phase. Malvidin-3-*O*-glucoside was used as a standard for GPC analysis at 520 nm, and material eluting prior to malvidin-3-*O*-glucoside was considered to be pigmented polymer.

Bisulfite Bleaching of Wines. Wine color density was calculated as absorbance at 420 nm + 520 nm. Hue was determined as 420 nm/520 nm absorbance. Percent red pigment in wine was determined as 520 nm/520 nm + HCl × 100 absorbance. Wines were subjected to bisulfite bleaching using a previously described method (*34*).

Statistical Analyses. Statistical data analysis was performed using analysis of variance (ANOVA) and the least significant difference (LSD) test to determine statistically different values at a significance level of $\alpha \leq 0.05$. For vineyard site and year comparisons, weighted averages were calculated and analyzed to take into account the vigor zone differences in area within each vineyard site. All statistical analyses were performed using SAS version 8.2.

RESULTS AND DISCUSSION

Anthocyanins in Model Extracts. The influence of vine vigor on fruit composition in 2003 and 2004 has been described (*30*). In 2004, in addition to making wines, model fruit extracts from the different vigor zones were prepared. The goal of the model extractions was to have the ability to study the physical−chemical extraction of phenolics in more detail. Consistent with berry size differences (*30*), there was a trend toward a greater number of berries with increasing vine vigor, particularly for A-high, although no differences were observed in pomace weight (**Table 1**). Juice volume was higher for site A than for site B, specifically for A-medium and A-low. The juice volume and pomace weight are not in agreement with what would generally be expected, where a smaller berry size would produce a lower juice volume and higher pomace weight per model extract. Although site B had a higher average berry weight in this model extraction, it had a lower juice volume.

In addition to the concentration of anthocyanins in skins, much emphasis has been placed on the influence of berry size on wine composition as it can modify the ratio of skin and seed material to pulp and influence the final amount extracted into

Table 1. Mean and SEM of Fruit Composition in the Model Extraction in 2004 Including Number of Berries in 300 g, Pomace Weight, and Juice Volume

site	vigor zone	no. of berries in 300 g	pomace wt (g)	juice volume (mL)
A	high	480a	113.1a	447.6b
	medium	451ab	108.9a	458.6a
	low	399b	115.1a	453.6ab
SEM		25.5	3.5	3.4
p value[a]		0.0886	0.4803	0.1360
B	high	414a	106.8a	413.8a
	medium	442a	107.0a	416.6a
	low	393a	115.5a	413.4a
SEM		19	2.9	2.0
p value[a]		0.2525	0.0705	0.3696

[a] ANOVA to compare data (*p* indicated), $n = 5$: values sharing the same letter within each site are not significantly different at $p \geq 0.05$.

Table 2. Mean and SEM of Total and Individual Anthocyanin Concentrations in 2004 Model Extracts (Weighted Average of Sites)

site	total (mg/L)	delphinidin[a] (mg/L)	cyanidin (mg/L)	petunidin (mg/L)	peonidin (mg/L)	malvidin (mg/L)
A	183.36	5.31	2.81	8.84	39.75	126.64
B	162.66	3.27	2.12	5.95	31.49	119.84
SEM	6.89	0.30	0.11	0.46	1.39	5.08
p value[b]	0.0426	<0.0001	0.0002	0.0001	0.0002	0.3515

[a] 3-*O*-Glucosides of individual anthocyanidins. [b] ANOVA to compare data (*p* indicated), $n = 15$.

wine (*5*). This idea of reduced berry size has been explored in deficit irrigation experiments (*6*, *35*). Two studies have confirmed two types of response to water deficit: one is an indirect but positive effect on the concentration of anthocyanins due to berry size reduction, and the second is a direct influence on biosynthesis. In another study on berry size, in general, small berries had a similar skin to fruit ratio and similar juice yield compared to large berries (*7*). This may help to explain why we saw no differences in pomace weight and a higher volume of juice produced from the same weight of berries from vigor zones with smaller berries.

Model extracts from site A had a higher total anthocyanin concentration than those from site B. For individual anthocyanins, site A had significantly higher concentrations of delphinidin-3-*O*-glucoside (Dp), cyanidin-3-*O*-glucoside (Cy), petunidin-3-*O*-glucoside (Pt), and peonidin-3-*O*-glucoside (Pn) and a similar concentration of malvidin-3-*O*-glucoside (Mv) compared to site B (**Table 2**). Anthocyanin concentration in the model extract (milligrams per liter) increased with a reduction in vine vigor in site A, but no differences were found for site B (**Table 3**). The lack of significant differences in vigor zone model extract anthocyanin concentrations for site B is similar to the minimal differences found in the fruit. B-low may have had reduced anthocyanins from inconsistent ripening as there were pink and green berries in the clusters at harvest. This failure for fruit to mature has been previously described (*36*). For site A, Pt, Pn, and Mv concentrations increased with a reduction in vine vigor, whereas there were no differences among individual anthocyanins at site B (**Table 3**).

The A-low model extracts had the highest concentration of anthocyanins. Although A-low had one of the highest average berry weights, it also had the highest anthocyanin concentration per berry and on a fruit weight basis (*30*). However, A-medium, which had the second highest anthocyanin concentration in the model extraction, had the smallest berry size and one of the lowest anthocyanin concentrations per berry. The A-high extraction had a small average berry size and low anthocyanin content per berry and on a fruit weight basis, which resulted in the lowest anthocyanin concentration of the vigor zone model extracts. From these results, it appears that anthocyanin concentration per berry played a more important role than berry size in the anthocyanin concentration in the model extracts except for A-medium, for which small berry size may have been an important positive factor. These results agree with previous research that showed that berry size alone does not explain observed differences in wine anthocyanin concentration (*6*, *7*).

There was a trend toward differences in the percent extraction of anthocyanins. Previous research has shown a higher percent extraction of anthocyanins in less ripe fruit as was seen in the A-high vigor zone, which also had lower soluble solids (*37*). A-high had approximately 75% extraction and B-low had only ~45% extraction (**Table 4**). The percent extractions for the vigor zones at site B were similar. Within site A, a potential explanation for the observed variation in percent extraction may be differences in the total skin surface area. However, whereas approximate calculations for total surface area correlated well with percent extraction for site A ($R^2 = 0.91$), this relationship was not found for site B. On the basis of the variation in percent anthocyanin extraction, the model extracts suggest that measuring fruit anthocyanin concentration alone might not be a sufficient predictor of wine anthocyanin concentration.

In addition to measuring total anthocyanin concentrations within model extracts, the individual anthocyanin proportions were determined. Model extracts from site A were proportionally higher in Dp, Cy, Pt, and Pn and lower in Mv than those from site B (**Table 5**). The model extract site differences were greater than differences found across the vigor zones within each site. In the model extracts for site A, there were no vigor-related differences in anthocyanin proportions (**Table 6**). There were no notable patterns in the anthocyanin proportions for the site B model extracts either. During the model extraction there was a shift toward a higher proportion of Mv and Pn concomitant with a reduction in the other three anthocyanin glucosides in comparison to the fruit proportional composition (*30*). This selectivity toward more stable anthocyanins during winemaking has been previously reported (*38*). Anthocyanins were also incorporated into pigmented polymers as discussed later in this paper.

Anthocyanins in Wine. Wines were made from grapes in both 2003 and 2004. In 2004, anthocyanin extraction was measured during fermentation. As expected from the predominant Mv proportion in fruit, the fermentation curve through pressing for total anthocyanin extraction most closely resembled the extraction curve for Mv (**Figures 1** and **2**). For site A, the Dp and Pt concentrations peaked on day 3 of fermentation (**Figure 1a**,**c**), whereas Cy, Pn, and Mv peaked on day 4 (**Figure 1b**,**d**,**e**). Dp, Pt, and Pn peaked on day 3 (**Figure 2a**,**c**,**d**), Cy (**Figure 2b**) on day 2, and Mv (**Figure 2e**) on day 5 at site B.

The high concentration of individual anthocyanins found on day 3 or 4 specifically in the medium- and low-vigor zones dropped substantially with pressing, so that differences in monomeric anthocyanins between vigor zones in the finished wines were minimized (**Figures 1** and **2**). Previous studies have shown that anthocyanin extraction usually reaches a maximum in the first days of fermentation and then the concentration drops, and this was found in the present study as well (*11*). From these data, it is not possible to assess what percentage of the

Table 3. Mean and SEM of Total and Individual Anthocyanin Concentrations in 2004 Model Extract Vigor Zones

site	vigor zone	total (mg/L)	delphinidin[a] (mg/L)	cyanidin (mg/L)	petunidin (mg/L)	peonidin (mg/L)	malvidin (mg/L)
A	high	157.6b	5.43a	2.70a	7.97b	33.7b	107.9b
	medium	180.5ab	4.92a	2.67a	8.35b	38.9b	125.7ab
	low	210.2a	6.77a	3.45a	11.30a	46.7a	142.0a
SEM		9.9	0.69	0.28	0.85	2.32	7.1
p value[b]		0.0126	0.2056	0.1322	0.0401	0.0089	0.0176
B	high	149.0a	3.38a	2.17a	5.82a	28.3a	109.3a
	medium	171.9a	3.21a	2.07a	6.03a	32.4a	128.2a
	low	159.3a	3.29a	2.14a	5.91a	32.9a	115.0a
SEM		11.1	0.33	0.16	0.54	2.0	8.4
p value[b]		0.3829	0.9305	0.9086	0.9615	0.2479	0.3111

[a] 3-*O*-Glucosides of individual anthocyanidins. [b] ANOVA to compare data (*p* indicated), $n = 5$: values sharing the same letter within each site are not significantly different at $p \geq 0.05$.

Table 4. Mean and SEM of Anthocyanin Amount in 300 g of Fruit, Amount in Extract Volume, and Percent Extraction in Model Extraction Experiment

site	vigor zone	amount in fruit (mg)	amount in extract (mg)	extraction (%)
A	high	95.4b	70.5b	74.6a
	medium	142.1ab	82.7ab	62.8a
	low	191.3a	95.4a	52.8a
SEM		19.9	4.4	9.7
p value[a]		0.0524	0.0093	0.1870
B	high	119.8a	61.7a	52.4a
	medium	149.1a	71.6a	48.6a
	low	148.3a	65.9a	45.2a
SEM		12.7	4.7	3.0
p value[a]		0.1850	0.3758	0.1195

[a] ANOVA to compare data (*p* indicated), $n = 5$: values sharing the same letter within each site are not significantly different at $p \geq 0.05$.

Table 5. Mean and SEM of Percent Composition of Anthocyanins in 2004 Model Extracts (Weighted Average of Sites)

site	delphinidin[a] (%)	cyanidin (%)	petunidin (%)	peonidin (%)	malvidin (%)
A	2.9	1.5	4.8	21.7	69.0
B	2.0	1.3	3.7	19.4	73.6
SEM	0.1	0.06	0.1	0.3	0.5
p value[b]	<0.0001	0.0058	<0.0001	<0.0001	<0.0001

[a] 3-*O*-Glucosides of individual anthocyanidins. [b] ANOVA to compare data (*p* indicated), $n = 10$.

anthocyanins was incorporated into pigmented polymers during fermentation and the amount lost due to degradation or precipitation.

Comparison of the anthocyanin concentrations in the wine fermentation to the model extracts showed that the wine extraction concentrations on day 3 for site A and day 4 for site B were comparable, although still somewhat lower than the model extraction, particularly for A-high and B-high. A rough estimate of percent extraction in the wine showed that the model extract had greater extraction than found in the wines, likely in response to the higher temperatures, mixing, and ethanol concentration.

There was a significant difference between vintages for anthocyanin concentration in the finished wines, with 2003 being higher than 2004 (**Table 7**). This agrees with vintage differences found in the fruit (*30*). Site A had a higher concentration of all individual and total plant-derived anthocyanins than site B in both years. Vitisin A, formed during fermentation (*18*, *19*), was higher for site B in 2003. The average wine anthocyanin concentrations found in this study in both years were similar to the concentrations found in other studies on Pinot Noir (*39*−*42*).

Table 6. Mean and SEM of Percent Composition of Anthocyanins in Model Extracts from 2004 Vigor Zones

site	vigor zone	delphinidin[a] (%)	cyanidin (%)	petunidin (%)	peonidin (%)	malvidin (%)
A	high	3.43a	1.70a	5.05a	21.35a	68.46a
	medium	2.74a	1.50a	4.62a	21.67a	69.47a
	low	3.20a	1.63a	5.35a	22.18a	67.64a
SEM		0.34	0.13	0.32	0.64	1.16
p value[b]		0.3780	0.5577	0.3261	0.3385	0.4450
B	high	2.28a	1.48a	3.90a	19.04b	73.31ab
	medium	1.86ab	1.20b	3.50a	18.92b	74.51a
	low	2.04b	1.34ab	3.69a	20.75a	72.18b
SEM		0.12	0.07	0.14	0.44	0.56
p value[b]		0.0976	0.0656	0.1720	0.0344	0.0547

[a] 3-*O*-Glucosides of individual anthocyanidins. [b] ANOVA to compare data (*p* indicated), $n = 5$: values sharing the same letter within each site are not significantly different at $p \geq 0.05$.

When segregated by vigor zone, A-high wines had a lower anthocyanin concentration in both years (**Table 8**). The highest anthocyanin concentration was found in A-medium and B-high in 2003 and in A-medium in 2004. The lower concentration found in A-high and B-high was consistent with a lower amount of anthocyanins in the fruit compared to the other zones. In 2003, concentrations of Dp, Cy, Pt, and Pn were higher for A-medium and A-low compared to A-high (**Table 6**). At site B, B-high had the highest concentration of all individual native anthocyanins in 2003. For both sites, vitisin A increased in wines with a reduction in vine vigor. In 2004, all anthocyanins including vitisin A were higher in A-medium and A-low compared to A-high. In 2004 at site B, all anthocyanins except for Mv were higher in the B-medium and B-low compared to B-high.

It has been suggested that having high concentrations of total phenolics and specifically copigments such as flavonols may help to keep anthocyanins in solution through copigmentation (*16*, *43*, *44*). Copigmentation has also been suggested to be the first step toward the formation of more stable pigments (*15*, *16*, *45*). In addition to having higher overall anthocyanin concentrations, the 2003 wines had double the proanthocyanidin concentration (*32*) found in the 2004 wines (**Table 9**). The low-

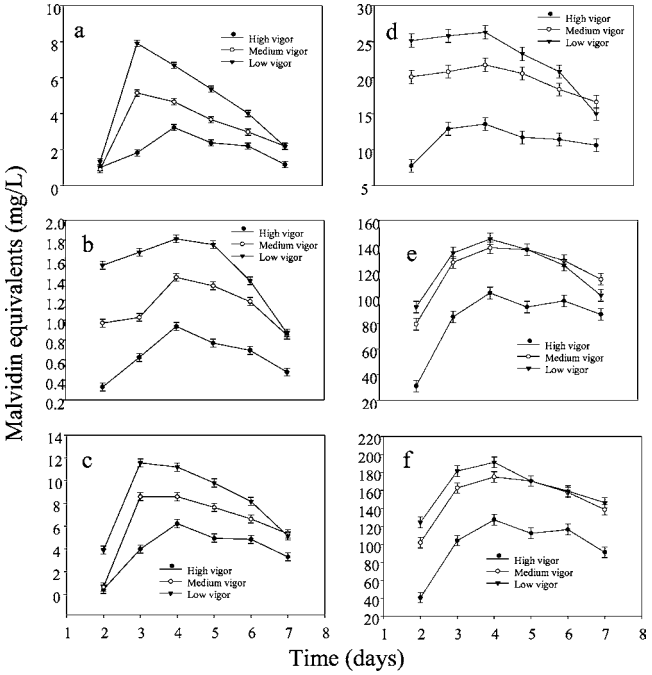

Figure 1. Site A anthocyanin evolution during fermentation for (**a**) delphinidin, (**b**) cyanidin, (**c**) petunidin, (**d**) peonidin, (**e**) malvidin, and (**f**) total anthocyanins from the start of fermentation (day 2) through pressing (day 7).

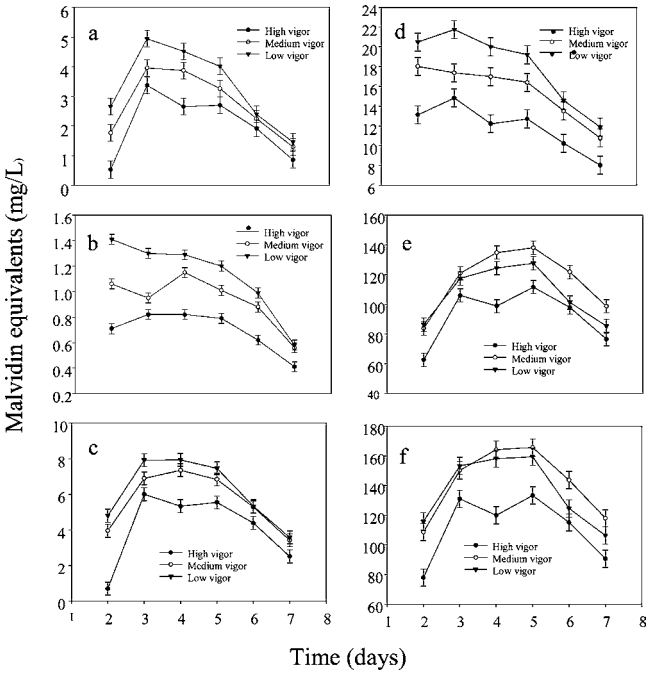

Figure 2. Site B anthocyanin evolution during fermentation for (**a**) delphinidin, (**b**) cyanidin, (**c**) petunidin, (**d**) peonidin, (**e**) malvidin, and (**f**) total anthocyanins from the start of fermentation (day 2) through pressing (day 7).

vigor wines had a substantially higher flavonol concentration as well as proanthocyanidin concentration than the high-vigor-zone wines.

An estimate of total extraction based upon anthocyanin concentration in the fruit and corresponding wines indicates that 9−16% and 10−35% of total fruit anthocyanins were extracted in 2003 and 2004, respectively. In both years, A-medium had the highest percent extraction compared to the other zones (data not included).

In addition to vintage variation in the anthocyanin concentration in wines, there were differences in the proportional composition between years (**Table 10**). In 2003, the proportions of Dp and Pt were higher in wines, whereas Mv was lower in comparison to 2004. This reflects the compositional vintage differences in the fruit except that, in the fruit, all of the anthocyanins were higher except for Mv in 2003 compared to 2004 (*30*). The Mv proportion was about 10% higher in the 2004 wines than in the 2003 wines.

Comparison of wine to model extract anthocyanin proportions showed that there was a greater proportion of Mv, no difference in Pt, minimal reduction in Dp, and large reductions in Cy and Pn in the wines. The reduction in the proportion of Pn in the wines compared to the model extractions may be due to yeast cell wall adsorption in the wines as the model extracts did not undergo yeast fermentation. Pn has been reported to be strongly adsorbed to yeast cell walls compared to other anthocyanins (*46*). Although model extractions can give some ideas about the solubility and extractability of anthocyanins from berries between treatments, caution is needed in making direct comparisons between model extracts and wines due to different extraction parameters and the influence of yeast in a traditional wine system.

There were differences in the anthocyanin proportion in wines between vineyard sites (**Tables 10** and **11**). In 2003, Pn and Mv were higher at site A than at site B. Vitisin A, formed in the wine during fermentation, was higher for site B compared to site A in 2003 but not in 2004. In 2004, site differences were minimal among the grape-derived anthocyanins in the wines.

In 2003, the major change between fruit and wine proportional composition was for site B, having a much greater formation of vitisin A (18.2%) and minimal change in the Mv percent going from ∼63% in the fruit to ∼64% in wine. For site A, there was only about 6% vitisin A production, and Mv went from ∼59% in the fruit to ∼74% of the total proportion in the wines. It is possible that the greater formation of vitisin A for site B in 2003 had to do with the lower pH and also possibly higher production of pyruvic acid (*47*) due to low nitrogen and other nutrients (data not included), resulting in a sluggish fermentation (*48*). In 2004, there were no differences in pH or vitisin A formation between sites. In 2004, the must yeast assimilable nitrogen (YANC) concentrations were higher from all vigor zones than in 2003 (data not included).

Wines made from vine vigor zones also resulted in proportional variations in anthocyanin composition (**Table 11**). In both years and at both sites, there was a higher proportion of Dp and Pt in wines made from fruit from low-vigor zones. For Cy, there was a higher proportion in the wines made from fruit from low-vigor vines in 2004 and a trend in 2003. The Pn proportion was lowest in wines made from the high-vigor zones in both years and both sites except that there were no differences for site B in 2003. This differs from the variation in the fruit as A-high had the highest proportion of Pn in the fruit (*30*) and one of the lowest proportions in the wine. The Mv proportion increased in wines made from fruit with higher vine vigor in both years and both sites. The Mv proportion was also higher in high vigor fruit (*30*).

Overall, wines from low-vigor fruit had a greater diversity of anthocyanins, whereas high-vigor wines were dominated by Mv. Comparison of the composition in the wine and fruit showed that the patterns were reasonably consistent. In 2003, low-vigor fruit was higher in Dp and lower in Mv (*30*). This same pattern was expressed in the wine. In a study by Sims and Bates (*8*), wine made from more mature grapes had higher

Table 7. Mean and SEM of Total and Individual Anthocyanins Including Vitisin A Concentration in Wines from Sites A and B (Weighted Average of Sites) in 2003 and 2004

site	year	total (mg/L)	delphinidin[a] (mg/L)	cyanidin (mg/L)	petunidin (mg/L)	peonidin (mg/L)	malvidin (mg/L)	vitisin A (mg/L)
A	2003	186.3	5.79	1.24	10.53	20.62	137.39	10.78
B	2003	177.1	5.09	0.96	8.89	16.08	114.96	31.34
A	2004	137.6	2.42	0.92	5.22	16.08	110.10	2.91
B	2004	117.1	1.93	0.77	4.08	11.81	95.66	2.89
SEM		5.9	0.26	0.10	0.41	0.93	5.62	1.99
site *p* value[b]		0.0180	0.0296	0.0390	0.0018	<0.0001	0.0025	<0.0001
year *p* value[b]		<0.0001	<0.0001	0.0131	<0.0001	<0.0001	0.0002	<0.0001
site × year *p* value[b]		0.3388	0.7038	0.5168	0.5448	0.8892	0.4818	<0.0001

[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 Including Vitisin A Concentration in Wines Made from Vine Vigor Zones in 2003 and 2004

site	year	vigor zone	total (mg/L)	delphinidin[a] (mg/L)	cyanidin (mg/L)	petunidin (mg/L)	peonidin (mg/L)	malvidin (mg/L)	vitisin A (mg/L)
A	2003	high	143.90c	3.30b	0.50b	6.66c	13.71c	114.70b	5.04c
		medium	199.66a	6.08a	1.29ab	11.44a	22.21a	149.41a	9.23b
		low	159.74b	6.14a	1.50a	9.29b	18.57b	103.83c	20.41a
SEM			3.65	0.51	0.31	0.37	0.69	1.82	0.60
p value[b]			0.0004	0.0025	0.0849	0.0010	0.0020	0.0001	0.0001
B	2003	high	204.81a	6.16a	1.32a	10.95a	20.96a	150.32a	15.11a
		medium	162.32c	4.36ab	0.65b	7.86b	13.58b	101.59b	34.29b
		low	177.55b	5.38b	1.19a	8.74b	15.84c	104.27b	42.14c
SEM			2.92	0.30	0.06	0.29	0.38	2.25	0.83
p value[b]			0.0013	0.0327	0.0024	0.0039	0.0004	0.0002	<0.0001
A	2004	high	95.70b	1.48b	0.49c	3.26c	8.98c	79.89b	1.61c
		medium	143.12a	2.42a	0.95b	5.29b	16.59b	115.04a	2.83b
		low	141.73a	3.01a	1.06a	6.14a	18.44a	109.06a	4.03a
SEM			2.35	0.08	0.009	0.11	0.36	1.94	0.08
p value[b]			0.0001	0.0005	<0.0001	0.0001	<0.0001	0.0002	0.0206
B	2004	high	104.24b	1.61b	0.63b	3.47b	9.50c	86.79b	2.24b
		medium	125.04a	1.94a	0.79ab	4.16a	12.14b	102.79a	3.04a
		low	115.44ab	2.21a	0.86a	4.54a	13.52a	91.05b	3.25a
SEM			3.65	0.09	0.06	0.17	0.39	2.81	0.16
p value[b]			0.0357	0.0253	0.0601	0.0300	0.0034	<0.0001	0.0206

[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 $p \geq 0.05$.

proportions of Mv and Pn relative to Dp, Cy, and Pt. Although differences have been observed between ripeness and anthocyanin composition, this was not observed in our study, suggesting environmental influences were a factor.

Pigmented Polymers in Wines. Numerous studies have shown a strong relationship between pigmented polymers and color density (*14*, *26*, *49*). Peng et al. (*50*) reported that pigmented polymers accounted for 50% of wine color after 2 years of aging, and Lee et al. (*20*) found that pigmented polymers accounted for close to 70% of color after 1 year. However, significant pigmented polymer formation is thought to occur during fermentation (*51*, *52*).

During fermentation, the simultaneous extraction of monomeric 520 nm absorbing material and the formation of pigmented polymers was determined by GPC (**Figure 3**). This extraction profile showed rapid formation of pigmented polymers in agreement with others (*53*). In the model extracts, pigmented polymer concentrations increased as well (data not included). The experiment was done using 20% v/v ethanol and at a higher than normal fermentation temperature, which might explain the rapid formation of pigmented polymers in this system. In the model extract system, pigmented polymers likely involved acetaldehyde as pyruvate would not be produced in a system not including yeast. In Pinot Noir, temperature was found to be an important factor in pigmented polymer formation in wines, when a fermentation temperature of 30 °C produced higher levels of pigmented polymers than did a temperature of 20 °C (*14*). The research wines for both years in this study were fermented at temperatures between 30 and 32 °C for 2 days, which likely encouraged the formation of pigmented polymers.

Analysis of the finished wines showed that 2003 contained higher concentrations of pigmented polymers than those from 2004 (**Table 12**). In both vintages, there was a strong relationship between pigmented polymer concentration in the wine and a reduction in vine vigor (**Table 13**). Pigmented polymer concentration was much higher when the extreme treatments of B-low and A-high in both years were compared. These results are in agreement with a previous study on Pinot Noir in which wines made from highly exposed clusters resulted in a 40% increase in polymeric anthocyanins compared to less exposed treatments (*39*). Low-vigor zones within the vineyard had greater sun exposure in the fruiting zone, and site B was overall lower in vigor compared to site A. Further studies are needed to elucidate the importance of different factors that may influence

Table 9. Mean and SEM of Proanthocyanidins, Skin-Derived Proanthocyanidin, and Flavonols in Wines Made from Vine Vigor Zones in 2003 and 2004

site	year	vigor zone	wine proantho-cyanidin (mg/L)	skin-derived proantho-cyanidin (%)	wine flavonols (mg/L)
A	2003	high	268.6c	52.8b	34.2c
		medium	366.9b	64.3a	56.5b
		low	457.6a	68.0a	81.3a
SEM			6.85	2.92	2.94
p value[a]			0.0001	0.0037	0.0009
B	2003	high	432.8ab	73.9a	83.4c
		medium	423.5b	72.9a	104.3b
		low	504.3a	76.8a	129.1a
SEM			21.21	2.62	2.54
p value[a]			0.0995	0.5920	0.0006
A	2004	high	108.2c	39.9c	8.1c
		medium	140.4b	51.4b	14.2b
		low	177.1a	64.5a	18.7a
SEM			2.47	2.64	0.29
p value[a]			0.0001	0.0071	<0.0001
B	2004	high	154.4c	48.4a	15.8b
		medium	180.9b	56.6a	20.3a
		low	253.1a	52.1a	20.3a
SEM			3.54	3.20	0.69
p value[a]			<0.0001	0.1783	0.0023

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

reaction rates in the formation of pigmented polymers. The pigmented polymer values reported here may be overestimated as previously discussed (*31*).

There was ~3-fold increase in the amount of bisulfite-resistant pigments found in 2003 compared to 2004, in agreement with the higher amount of pigmented polymers and vitisin A found in the wines (**Table 12**). No differences in bisulfite resistance were found between vineyard sites in either year. Bisulfite resistance increased in wines made from vines with reduced vine vigor in both years and sites, similar to what was observed for pigmented polymer concentration in wines (**Table 13**).

Several studies have suggested that pigmented polymer formation can be predicted by anthocyanin to proanthocyanidin ratios (*22*, *52*, *54*, *55*). It was also suggested that increases in pigmented polymer formation appear to be due more to an increase in proanthocyanidin rather than anthocyanin extraction (*51*). In this study, a strong positive correlation between pigmented polymer formation and proanthocyanidin concentration in wine was found ($r^2 = 0.92$) (**Figure 4**). The correlation between wine pigmented polymer formation and other parameters was weaker [wine anthocyanins ($r^2 = 0.56$) and wine proanthocyanidins/anthocyanins ($r^2 = 0.72$)]. Pigmented polymer formation in wine was also compared to proanthocyanidin and anthocyanin concentrations found in the fruit. In 2003, the relationship between pigmented polymer concentration in the wine and proanthocyanidin concentration in fruit (milligrams per kilogram) was stronger ($r^2 = 0.70$) (**Figure 5**) than the relationship between pigmented polymer concentration in the wine and anthocyanin concentration in the fruit (milligrams per kilogram) ($r^2 = 0.47$). However, there did not appear to be similar relationships in the 2004 data. The results of this study suggest that the rate of pigmented polymer formation may be driven by proanthocyanidin amount more than by anthocyanin concentration. However, other factors are anticipated to also play a role.

UV−Vis Spectrophotometry of Wines. Although vintage differences in spectrophotometric color were observed, they were not included as the wines were analyzed at different ages. Whereas pigmented polymers form during primary fermentation (*53*), oxidation and acid-catalyzed reactions during storage are likely to modify the color profile further. In this study, the 2003 wines were much darker in color than the 2004 wines after fermentation and storage. Comparison of the two sites showed that color density was substantially higher at site B than at site A in 2003, whereas there were no differences in 2004 (**Table 12**). Although there were minimal differences in monomeric anthocyanins in the fruit in 2003, color density was substantially higher in wines made from low-vigor fruit in both sites (**Table 13**). In 2004, color density increased with a reduction in vine vigor except for B-low. In both years and particularly in 2003, there was a good correlation between pigmented polymer concentration and wine color density (**Figure 6**). This agrees with previous results in Pinot Noir, for which pigmented polymers were important in wine color intensity (*14*). In another study on several varieties including Pinot Noir, viticultural practices that increased cluster sun exposure resulted in wines with higher phenolics and improved wine color density (*39*).

Hue (yellow/brown color) was higher in wines from site A compared to site B in 2003, and there were no differences in 2004 (**Table 12**). Percent red pigment was higher for site B in 2003 (**Table 12**) and higher at site A in 2004. For both years and sites, hue was lower in wines made from vines with reduced vigor (**Table 13**). In 2003, at both sites, percent red pigment was significantly higher in wines made from low-vigor vines. Percent red pigment was higher in the medium-vigor zone of site A compared to the other zones in 2004. High pigmented polymer concentration was associated with a lower hue and a higher percentage of red pigments. This agrees with other

Table 10. Mean and SEM of Percent Composition of Anthocyanins Including Vitisin A in Wines from Sites A and B (Weighted Average of Sites) in 2003 and 2004

site	year	delphinidin[a] (%)	cyanidin (%)	petunidin (%)	peonidin (%)	malvidin (%)	vitisin A (%)
A	2003	3.1	0.66	5.6	11.0	73.6	6.0
B	2003	2.9	0.53	5.0	9.0	64.4	18.2
A	2004	1.8	0.66	3.81	11.6	80.1	2.1
B	2004	1.6	0.65	3.5	10.1	81.7	2.5
SEM		0.1	0.05	0.1	0.3	1.3	1.3
site *p* value[b]		0.1244	0.2008	0.0002	<0.0001	0.0070	<0.0001
year *p* value[b]		<0.0001	0.2743	<0.0001	0.0108	<0.0001	<0.0001
site × year *p* value[b]		0.5256	0.2537	0.1336	0.4083	0.0003	<0.0001

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

Table 11. Mean and SEM of Percent Composition of Anthocyanins Including Vitisin A in Wines Made from Vine Vigor Zones in 2003 and 2004

site	year	vigor zone	delphinidin[a] (%)	cyanidin[a] (%)	petunidin[a] (%)	peonidin[a] (%)	malvidin[a] (%)	vitisin A[b] (%)
A	2003	high	2.37c	0.37a	4.79b	9.86c	82.62a	3.50c
		medium	3.19b	0.68a	6.01a	11.66b	78.46b	4.62b
		low	4.39a	1.08a	6.66a	13.31a	74.57c	12.77a
SEM			0.29	0.22	0.23	0.23	0.68	0.15
p value[c]			0.0040	0.1027	0.0047	0.0012	0.0012	<0.0001
B	2003	high	3.24b	0.69b	5.77b	11.05b	79.24a	7.38c
		medium	3.39b	0.51c	6.13ab	10.61d	79.36a	21.15b
		low	3.98a	0.88a	6.46a	11.69d	77.01b	23.74a
SEM			0.14	0.04	0.13	0.19	0.29	0.54
p value[c]			0.0401	0.0035	0.0548	0.0366	0.0075	<0.0001
A	2004	high	1.57b	0.52c	3.47c	9.55c	84.89a	1.68c
		medium	1.72b	0.68b	3.77b	11.82b	82.01b	1.98b
		low	2.19a	0.77a	4.45a	13.39a	79.20c	2.84a
SEM			0.05	0.01	0.07	0.14	0.23	0.05
p value[c]			0.0019	<0.0001	<0.0001	0.0001	0.0002	0.0003
B	2004	high	1.58b	0.62b	3.40b	9.31c	85.09a	2.15c
		medium	1.59b	0.65b	3.41b	9.95b	84.40b	2.43b
		low	1.97a	0.76a	4.04a	12.06a	81.16c	2.81a
SEM			0.03	0.03	0.05	0.05	0.10	0.06
p value[c]			0.0020	0.0119	0.0011	<0.0001	<0.0001	0.0034

[a] 3-*O*-Glucosides of individual anthocyanidins; percent calculated on total of native anthocyanins excluding vitisin A. [b] Percent calculated on total anthocyanins including vitisin A. [c] ANOVA to compare data (*p* indicated), $n = 3$: values sharing the same letter within each column are not significantly different at $p \geq 0.05$.

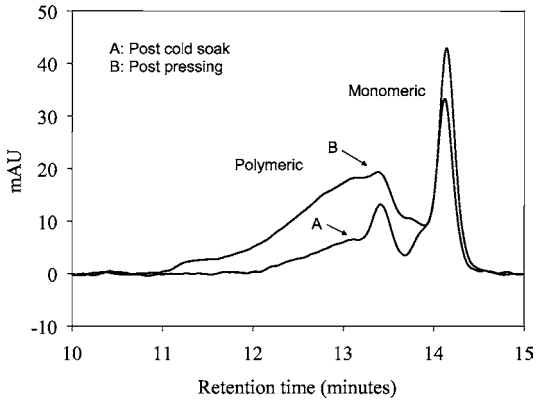

Figure 3. GPC chromatogram showing pigment chromatography after cold soak and after pressing in a medium-vigor-zone wine.

findings of wines with lower levels of pigmented polymers reportedly being slightly less reddish (*14*).

In wines with a lower proanthocyanidin to anthocyanin ratio, it is likely that an increase in proportion of derived pigments that do not involve proanthocyanidins will be observed (*52*). These pigments could be pyranoanthocyanins such as vitisin A that are more orange and bisulfite resistant than anthocyanin proanthocyanidin adducts. In this study, there were small differences in percent bisulfite-resistant pigments in the wines, and the low-vigor zones had a greater formation of vitisin A. However, the high-vigor-zone wines had a lower proanthocyanidin concentration and a substantially lower concentration of pigmented polymer formation than wines from low-vigor zones, so the proportion of orange compounds in the wine contributing to color could be greater. Although pyranoanthocyanins are thought to contribute very little to observed wine color (*56*), this explanation coupled with the lower overall concentration of anthocyanins relative to other phenolic compounds might explain the higher hue values and reduction in the percent red color in the high-vigor wines. On the other hand, as seen in **Figure 4**, there was a strong correlation between the proanthocyanidin concentration (milligrams per liter) in wine and the formation of pigmented polymers. Wines from the low-vigor zones had substantially higher skin proanthocyanidin (**Table 9**) and pigmented polymer concentration than wines made from high-vigor zones. High concentrations of proanthocyanidins would be expected to encourage proanthocyanidin−anthocyanin (T−A) adducts, which have color properties similar to those of anthocyanins (*57*). Initial concentrations of reaction products can influence reaction rates, but the role of yeast and production

Table 12. Mean and SEM of Color Density, Hue, Red Pigment, and Bisulfite-Resistant Pigments of Wines from Sites A and B (Weighted Average of Sites) in 2003 and 2004

site	year	color density (420 nm + 520 nm)	hue (420 nm/520 nm)	red pigment (%)	bisulfite-resistant pigments (%)	pigmented polymer (mg/L)
A	2003	6.2	0.75	26.7	38.3	865
B	2003	9.8	0.64	35.3	41.2	1223
A	2004	7.2	0.55	33.9	10.8	391
B	2004	7.3	0.54	23.3	9.3	360
SEM		0.4	0.02	1.5	1.0	41
site *p* value[a]		<0.0001	0.0004	0.5233	0.4911	0.0004
site × year *p* value[a]		<0.0001	0.0031	<0.0001	0.0393	<0.0001

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

Table 13. Mean and SEM of Color Density, Hue, Red Pigment, and Bisulfite-Resistant Pigments of Wines Made from Vine Vigor Zones in 2003 and 2004

site	year	vigor zone	color density (420 nm + 520 nm)	hue (420 nm/520 nm)	red pigment (%)	bisulfite-resistant pigments (%)	pigmented polymer (mg/L)
A	2003	high	4.54a	0.79a	26.39b	36.86b	632c
		medium	6.00b	0.77a	25.37b	37.70b	844b
		low	8.24c	0.68b	31.95a	41.63a	1090a
SEM			0.13	0.01	0.60	0.86	14.8
p value[a]			<0.0001	0.0082	0.0014	0.0341	<0.0001
B	2003	high	7.95c	0.67a	29.79b	33.32b	989c
		medium	9.60b	0.64ab	35.11b	43.74a	1223b
		low	12.07a	0.62b	41.21a	44.31a	1459a
SEM			0.24	0.01	1.73	0.80	46.0
p value[a]			0.0007	0.1244	0.0140	0.0007	0.0050
A		high	6.07c	0.75a	25.73b	6.21c	227c
		medium	7.11b	0.53b	37.44a	11.16b	399b
		low	8.49a	0.48b	24.82b	12.04a	425a
SEM			0.25	0.02	2.22	0.22	6.0
p value[a]			0.0023	0.0005	0.0277	0.0001	0.0005
B	2004	high	6.62b	0.58a	20.83b	7.29c	286b
		medium	7.69a	0.53b	24.45a	9.31b	386a
		low	7.25ab	0.52b	23.81ab	11.35a	388a
SEM			0.21	0.01	0.97	0.47	8.7

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

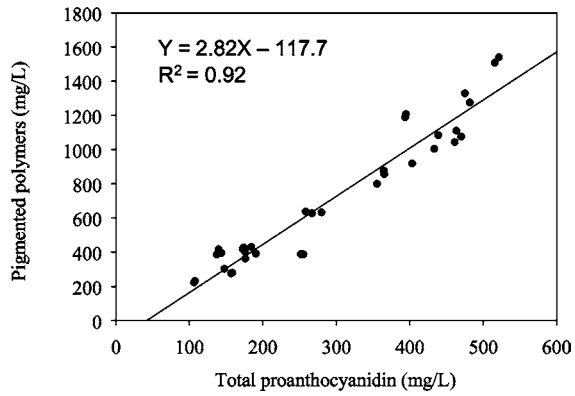

Figure 4. Relationship between pigmented polymer concentration in wine (mg/L) determined by GPC and total proanthocyanidin in wine (mg/L) determined by phloroglucinolysis.

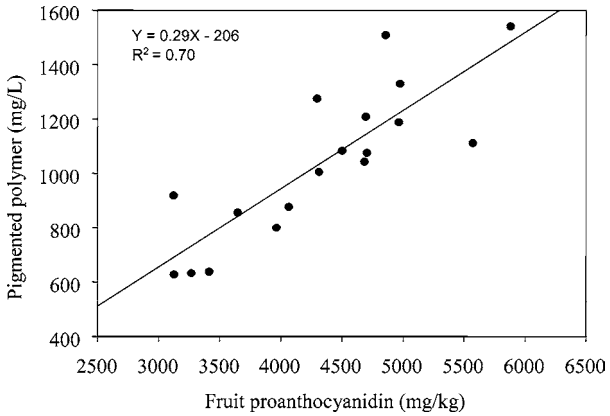

Figure 5. Relationship in 2003 between pigmented polymer concentration (mg/L) determined by GPC and total proanthocyanidin in fruit (mg/kg) determined by phloroglucinolysis.

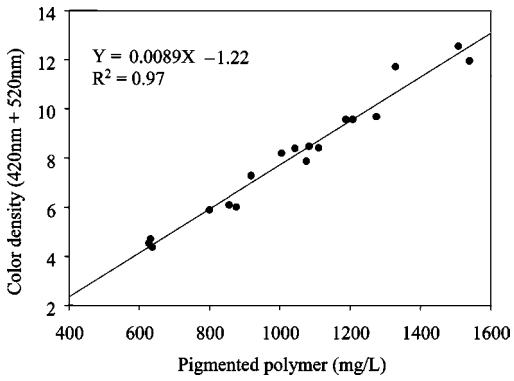

Figure 6. Relationship between wine color density (420 nm + 520 nm) determined by UV–vis spectrophotometry in 2003 and pigmented polymer in wine (mg/L) determined by GPC.

of secondary metabolites in wine also needs to be considered. The concentration of pyruvic acid and acetaldehyde generated during fermentation is likely to influence the formation of pigments such as vitisins A and B (*58*).

In summary, although there was variation in anthocyanin accumulation in fruit in response to variations in vine vigor within a site, the variation in the fruit appears to be magnified in wines due to variations in berry size, differences in percent extraction of anthocyanins, and also the formation of pigmented polymers in wine. In this study, lower vine vigor produced wines with higher concentrations of anthocyanins and bisulfite-resistant pigments (e.g., pigmented polymers, vitisin A), greater diversity of anthocyanins, greater color density, higher percentage of red pigments, and reduced hue. Differences in anthocyanins in the fruit and wine from different vine vigor zones may be due to improved sun exposure in the canopy, as supported by our results in a shading experiment (*59*), rather than directly from vine vigor. The high variability in anthocyanin content in berries and differences in extraction make it difficult to use fruit anthocyanin content alone as a predictor of red wine color. In this study, there was a stronger positive correlation between the formation of pigmented polymers in wine and the proanthocyanidin concentration than between pigmented polymers and

the anthocyanin concentration. Further investigation is needed to determine factors that influence anthocyanin extraction from the berry and how proanthocyanidin concentration and composition influence pigmented polymer formation.

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; SEM, standard error of the mean.

ACKNOWLEDGMENT

We thank Archery Summit Winery for the use of their vineyards and fruit for wine production as well as Leigh Bartholomew and Anna Matzinger specifically for their support. In addition, we thank students Jason Bell, Fiorella Cerpa, Jose Luis Pastor del Rio, and Chris Zielinski for their help.

LITERATURE CITED

(1) Wang, H.; Cao, G.; Prior, R. L. Oxygen radical absorbing capacity of anthocyanins. *J. Agric. Food Chem.* **1997**, *45*, 304−309.

(2) Muñoz-Espada, A. C.; Wood, K. V.; Bordelon, B.; Watkins, B. A. Anthocyanin quantification and radical scavenging capacity of Concord, Norton, and Marechal Foch grapes and wines. *J. Agric. Food Chem.* **2004**, *52*, 6779−6786.

(3) Wulf, L. W.; Nagel, C. W. High pressure liquid chromatographic separation of anthocyanins of *Vitis vinifera*. *Am. J. Enol. Vitic.* **1976**, *29*, 42−49.

(4) Fong, R. A.; Kepner, R. E.; Webb, A. D. Acetic-acid-acylated anthocyanin pigments in the grape skins of a number of varieties of *Vitis vinifera*. *Am. J. Enol. Vitic.* **1971**, *22*, 150−155.

(5) Matthews, M. A.; Anderson, M. M. Fruit ripening in *Vitis vinifera* L.: response to seasonal water deficits. *Am. J. Enol. Vitic.* **1988**, *39*, 313−320.

(6) Roby, G.; Harbertson, J. F.; Adams, D. O.; Matthews, M. A. Berry size and vine water deficits as factors in winegrape composition: anthocyanins and tannins. *Aust. J. Grape Wine Res.* **2004**, *10*, 100−107.

(7) Walker, R. R.; Blackmore, D. H.; Clingeleffer, P. R.; Kerridge, G. H.; Rühl, E. H.; Nicholas, P. R. Shiraz berry size in relation to seed number and implications for juice and wine composition. *Aust. J. Grape Wine Res.* **2005**, *11*, 2−8.

(8) Sims, C. A.; Bates, R. P. Effect of skin fermentation time on the phenols, anthocyanins, ellagic acid sediment, and sensory characteristics of a red *Vitis rotundifolia* wine. *Am. J. Enol. Vitic.* **1994**, *45*, 56−62.

(9) Pérez-Magarino, S.; González-San José, M. L. Evolution of flavanols, anthocyanins, and their derivatives during the aging of red wines elaborated from grapes harvested at different stages of ripening. *J. Agric. Food Chem.* **2004**, *52*, 1181−1189.

(10) Canals, R.; Llaudy, M. C.; Vallis, J.; Canals, J. M.; Zamora, F. Influence of ethanol concentration on the extraction of color and phenolic compounds from the skins and seeds of Tempranillo grapes at different stages of ripening. *J. Agric. Food Chem.* **2005**, *53*, 4019−4025.

(11) Sacchi, K. L.; Bison, L. F.; Adams, D. O. A review of the effect of winemaking techniques on phenolic extraction in red wines. *Am. J. Enol. Vitic.* **2005**, *56*, 197−206.

(12) Nagel, C. W.; Wulf, L. W. Changes in anthocyanins, flavonoids, and hydroxycinnamic acid esters during fermentation and aging of Merlot and Cabernet Sauvignon. *Am. J. Enol. Vitic.* **1979**, *30*, 111−116.

(13) Watson, B. T.; Price, S. F.; Valladao, M. Effect of fermentation practices on anthocyanin and phenolic composition of Pinot noir wines. *Am. J. Enol. Vitic.* **1995**, *46*, 404 (Abstract).

(14) Gao, L.; Girard, G.; Mazza, G.; Reynolds, A. G. Changes in anthocyanins and color characteristics of Pinot noir wines during different vinification processes. *J. Agric. Food Chem.* **1997**, *45*, 2003−2008.

(15) Brouillard, R.; Delaport, B. Chemistry of anthocyanin pigments. 2. Kinetic and thermodynamic study of proton transfer, hydration, and tautomeric reactions of malvidin-3-glucoside. *J Am. Chem. Soc.* **1977**, *99*, 8461−8468.

(16) Boulton, R. The copigmentation of anthocyanins and its role in the color of red wine: a critical review. *Am. J. Enol. Vitic.* **2001**, *52*, 67−87.

(17) Salas, E.; Montserreat, D.; Schwarz, M.; Winterhalter, P.; Cheynier, V.; Fulcrand, H. Characterization of pigments from different high speed countercurrent chromatography wine fractions. *J. Agric. Food Chem.* **2005**, *53*, 4536−4546.

(18) Bakker, J.; Timberlake, C. F. Isolation, identification, and characterization of new color stable anthocyanins occurring in some red wines. *J. Agric. Food Chem.* **1997**, *45*, 35−43.

(19) Fulcrand, H.; Benabdeljalil, C.; Rigaud, J.; Cheynier, V.; Moutounet, M. A new class of wine pigments generated by reaction between pyruvic acid and grape anthocyanins. *Phytochemistry* **1998**, *47*, 1401−1407.

(20) Lee, D. F.; Ewald, E. S.; Asenstorfer, R. E.; Jones, G. P. Factors affecting the formation of red wine pigments. In *Red Wine Color: Revealing the Mysteries*; Waterhouse, A. L., Kennedy, J., Eds.; ACS Symposium Series 886; American Chemical Society: Washington, DC, 2004; pp 125−143.

(21) Dallas, C.; Ricardo-Da-Silva, M. J.; Laureano, O. Products formed in model wine solutions involving anthocyanins, procyanidin B2, and acetaldehyde. *J. Agric. Food Chem.* **1996**, *44*, 2402−2407.

(22) Fulcrand, H.; Atanasova, V.; Salas, E.; Cheynier, V. The fate of anthocyanins in wine: are there determining factors? In *Red Wine Color: Revealing the Mysteries*; Waterhouse, A. L., Kennedy, J., Eds.; ACS Symposium Series 886; American Chemical Society: Washington, DC, 2004; pp 68−88.

(23) Es-Safi, N.; Cheynier, V. Flavanols and anthocyanins as potent compounds in the formation of new pigments during storage and aging of red wine. In *Red Wine Color: Revealing the Mysteries*; Waterhouse, A. L., Kennedy, J., Eds.; ACS Symposium Series 886; American Chemical Society: Washington, DC, 2004; pp 143−159.

(24) Remy, S.; Fulcrand, H.; Labarbe, B.; Cheynier, V.; Moutounet, M. First confirmation in red wine of products resulting from direct anthocyanin−tannin reactions. *J. Sci. Food Agric.* **2000**, *80*, 745−751.

(25) Ribéreau-Gayon, P.; Sudraud, P. P.; Milhe, J. C.; Canbas, A. Recherches technologiques sur les composés phénoliques des vin rouges. *Connaiss. Vigne Vin* **1970**, *4*, 133−144.

(26) Somers, T. C. Polymeric nature of wine pigments. *Phytochemistry* **1971**, *10*, 2175−2186.

(27) Salas, E.; Fulcrand, H.; Meudec, E.; Cheynier, V. Reactions of anthocyanins and tannins in model solutions. *J. Sci. Food Agric.* **2003**, *51*, 7951−7961.

(28) Hayasaka, Y.; Kennedy, J. A. Mass spectrometric evidence for the formation of pigmented polymers in red wine. *Aust. J. Grape Wine Res.* **2003**, *9*, 1−11.

(29) Gómez-Cordovés, C. New pigments in red wines via different enological processes. In *Red Wine Color: Revealing the Mysteries*; Waterhouse, A. L., Kennedy, J., Eds.; ACS Symposium Series 886; American Chemical Society: Washington, DC, 2004; pp 89−124.

(30) Cortell, J. M.; Halbleib, M.; Gallagher, A. V.; Righetti, T.; Kennedy, J. A. Influence of vine vigor on grape (*Vitis vinifera* L. cv. Pinot Noir) anthocyanins. 1. Anthocyanin concentration and composition in fruit. *J. Agric. Food Chem.* **2007**, *55*, 6575−6584.

(31) Cortell, J. M.; Halbleib, M.; Gallagher, A. V.; Righetti, T.; Kennedy, J. A. Influence of vine vigor on grape (*Vitis vinifera* L. cv. Pinot Noir) and wine proanthocyanidins. *J. Agric. Food Chem.* **2005**, *53*, 5798−5808.

(32) Lamuela-Raventos, R. M.; Waterhouse, A. L. A direct HPLC separation of wine phenolics. *Am. J. Enol. Vitic.* **1994**, *45*, 1−5.
(33) Kennedy, J. A.; Taylor, A. W. Analysis of proanthocyanidins by high-performance gel permeation chromatography. *J. Chromatogr., A* **2003**, *995*, 99−107.
(34) Somers, T. C.; Evans, M. E. Spectral evaluation of young red wines: anthocyanin equilibria, total phenolics, free and molecular SO_2, 'chemical age'. *J. Sci. Food Agric.* **1977**, *28*, 42−54.
(35) Ojeda, H.; Andary, C.; Kraeva, E.; Carbonneau, A.; Deloire, A. Influence of pre- and post-veraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. *Am. J. Enol. Vitic.* **2002**, *53*, 261−267.
(36) Hardie, W. J.; Considine, J. A. Response of grapes to water-deficit stress in particular stages of development. *Am. J. Enol. Vitic.* **1976**, *27*, 55−61.
(37) Ribéreau-Gayon, P. Évolution des composés phénoliques au cours de la maturation du raisin. II. Discussion des resultants obtenus en 1969, 1970 et 1971. *Connaiss. Vigne Vin* **1972**, *6*, 161−175.
(38) McCloskey, L. P.; Yengoyan, L. S. Analysis of anthocyanins in *Vitis vinifera* wines and red color versus aging by HPLC and spectrophotometry. *Am. J. Enol. Vitic.* **1981**, *32*, 257−261.
(39) Price, S. F.; Breen, P. J.; Valladao, M.; Watson, B. T. Cluster exposure and quercetin in Pinot noir grapes and wine. *Am. J. Enol. Vitic.* **1995**, *46*, 187−194.
(40) Mazza, G.; Fukumoto, L.; Delaquis, P.; Girard, B.; Ewert, B. Anthocyanins, phenolics, and color of Cabernet franc, Merlot, and Pinot noir wines from British Columbia. *J. Agric. Food Chem.* **1999**, *47*, 4009−4017.
(41) Reynolds, A. G.; Yerle, S.; Watson, B.; Price, S. F.; Wardle, D. A. Fruit environment and crop level effects on Pinot noir. III. Composition and descriptive analysis of Oregon and British Columbia wines. *Am. J. Enol. Vitic.* **1996**, *47*, 329−339.
(42) Parley, A.; Vanhanen, L.; Heatherbell, D. Effects of pre-fermentation enzyme maceration on extraction and colour stability in Pinot noir wine. *Aust. J. Grape Wine Res.* **2001**, *7*, 146−152.
(43) Schwarz, M.; Picazo-Bacete, J. J.; Winterhalter, P.; Hermosín-Gutiérrez, I. Effects of copigments and grape cultivar on the color of red wine fermented after the addition of copigments. *J. Agric. Food Chem.* **2005**, *53*, 8372−8381.
(44) Lorenzo, C.; Pardo, F.; Zalacain, A.; Alonso, L. G.; Salinas, M. R. Effect of red grapes co-winemaking in polyphenols and color of wines. *J. Agric. Food Chem.* **2005**, *53*, 7609−7616.
(45) Liao, H.; Cai, Y.; Haslam, E. Polyphenolic interactions. Anthocyanins: copigmentation and colour changes in red wines. *J. Sci. Food Agric.* **1992**, *59*, 299−305.
(46) Morata, A.; Gómez-Cordovés, M. C.; Suberviola, J.; Bartolomé, B.; Colomo, B.; Suárez, J. A. Adsorption of anthocyanins by yeast cell walls during the fermentation of red wines. *J. Agric. Food Chem.* **2003**, *51*, 4084−4088.
(47) Romero, C.; Bakker, J. Interactions between grape anthocyanins and pyruvic acid with effect of pH and acid concentration on anthocyanin composition and color in model solutions. *J. Agric. Food Chem.* **1999**, *47*, 3130−3139.
(48) Alexandre, H.; Charpentier, C. Biochemical aspects of stuck and sluggish fermentation in grape must. *J. Ind. Microb. Biotechnol.* **1998**, *20*, 20−27.
(49) Mazza, G. Anthocyanins in grapes and grape products. *Crit. Rev. Food Sci. Nutr.* **1995**, *35*, 341−371.
(50) Peng, Z.; Iland, P. G.; Oberholzer, A.; Sefton, M. A.; Walters, E. J. Analysis of pigmented polymers in red wine by reverse phase HPLC. *Aust. J. Grape Wine Res.* **2002**, *8*, 70−75.
(51) Harbertson, J. F.; Picciotto, E. A.; Adams, D. O. Measurement of polymeric pigments in grape berry extracts and wine using a protein precipitation assay combined with bilsulfite bleaching. *Am. J. Enol. Vitic.* **2003**, *54*, 301−306.
(52) Morel-Salmi, C.; Souquet, J. M.; Magali, B.; Cheynier, V. Effect of flash release treatment on phenolic extraction and wine composition. *J. Agric. Food Chem.* **2006**, *54*, 4270−4276.
(53) Eglinton, J.; Griesser, M.; Henschke, P.; Kwiatkowski, M.; Parker, M.; Herderich, M. Yeast mediated formation of pigmented polymers in red wine. In *Red Wine Color: Revealing the Mysteries*; Waterhouse, A. L., Kennedy, J., Eds.; ACS Symposium Series 886; American Chemical Society: Washington, DC, 2004; pp 125−142.
(54) Mazza, G.; Miniata, E. Grapes. In *Anthocyanins in Fruits, Vegetables, and Grain*; Miniata, E., Ed.; CRC Press: Boca Raton, FL, 1993; pp 149−199.
(55) Brossaud, F.; Cheynier, V.; Asselin, C.; Moutounet, M. Flavonoid compositional differences of grapes among site test plantings of Cabernet franc. *Am. J. Enol. Vitic.* **1999**, *50*, 277−284.
(56) Schwarz, M; Quast, P.; von Baer, D.; Winterhalter, P. Vitisin A content in Chilean wines from *Vitis vinifera* cv. Cabernet Sauvignon and contribution to the color of aged red wines. *J. Agric. Food Chem.* **2003**, *51*, 6261−6267.
(57) Salas, E.; Le Guernevé, C.; Fulcrand, H.; Poncet-Legrand, C.; Cheynier, V. Structure determination and color properties of a newly synthesized direct-linked flavanol−anthocyanin dimer. *Tetrahedron Lett.* **2004**, *45*, 8725−8729.
(58) Morata, A.; Gómez-Cordovés, M. C.; Colomo, B.; Suárez, J. A. Pyruvic acid and acetaldehyde production by different strains of *Saccharomyces cerevisiae*: relationship with vitisin A and B formation in red wines. *J. Agric. Food Chem.* **2003**, *51*, 7402−7409.
(59) Cortell, J. M.; Kennedy, J. A. Effect of shading on accumulation of flavonoid compounds in *Vitis vinifera* L. Pinot noir fruit and extraction in a model system. *J. Agric. Food Chem.* **2006**, *54*, 8510−8520.

Received for review January 22, 2007. Revised manuscript received May 28, 2007. Accepted May 29, 2007. Funding for this project was provided by the USDA Northwest Center for Small Fruit Research and the American Society for Enology and Viticulture.

JF070196N