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Effect of Shading on Accumulation of Flavonoid Compounds in (*Vitis vinifera* L.) Pinot Noir Fruit and Extraction in a Model System

JESSICA M. CORTELL AND JAMES A. KENNEDY*

Department of Food Science and Technology, Oregon State University, Corvallis, Oregon, 97331

Accumulation and compositional changes of flavonols, proanthocyanidins, and anthocyanins were measured in *Vitis vinifera* L. cv. Pinot noir in shaded and exposed treatments. In addition, extraction of these compounds into a model wine solution was measured. The study was conducted in a commercial vineyard within a uniform zone of relatively low vigor vines. Light exclusion boxes were installed on pairs of clusters on the same shoot (shaded treatment), and a second set of clusters on an adjacent shoot were labeled as the exposed treatment. Fruit samples were harvested at the onset of ripening (véraison) and at commercial harvest. Cluster shading resulted in a substantial decrease in mg/berry accumulation of flavonols and skin proanthocyanidins and minimal differences in anthocyanins. In analyzing seed proanthocyanidins by phloroglucinolysis, shaded and exposed treatments were similar at véraison; however, by harvest, the shaded treatment had higher extension and terminal subunits (nmol/seed) as compared to the exposed treatment. For skin proanthocyanidins, shaded fruit was lower for all subunits (nmol/berry) at both véraison and harvest. Shading caused an increase in the proportion of (−)-epicatechin and a decrease in (−)-epigallocatechin at harvest in skin extension subunits. Seed proanthocyanidins in shaded fruit contained a lower proportion of (+)-catechin and a higher proportion of (−)-epicatechin-3-*O*-gallate in extension subunits and a lower proportion of (+)-catechin and (−)-epicatechin-3-*O*-gallate and a higher proportion of (−)-epicatechin in terminal subunits. For anthocyanins, the shaded treatment had a proportional reduction in delphinidin, cyanidin, petunidin, and malvidin and a large increase in peonidin glucosides. The model extractions from the two treatments paralleled differences in the fruit with a lower concentration of flavonols, anthocyanins, and proanthocyanidins in the shaded treatment. The skin proanthocyanidin percent extraction was found to be ∼17% higher in the exposed model extraction than the shaded treatment.

KEYWORDS: Shading; UV exposure; flavonoids; flavonols; anthocyanins; proanthocyanidins; flavan-3ol monomers; HPLC; GPC; model wine extraction

INTRODUCTION

Flavonoid compounds provide a range of functions in plants such as attracting pollinators and seed dispersers, providing UV light protection, and resisting pathogens and herbivores (*1*). Three major classes of flavonoid compounds found in grapes (*Vitis vinifera* L.) include proanthocyanidins (condensed tannins), anthocyanins, and flavonols (*2*) (**Figure 1**). Flavonoids are important in wine because of their color, astringency, and potential role in human health (*3*).

Flavonols are found in grape skins as glycosides of kaempferol, quercetin, myricetin, and isorhamnetin (**Figure 1a**). Grape seed flavan-3-ols include (+)-catechin (C), (−)-epicatechin (EC), and (−)-epicatechin-3-*O*-gallate (ECG), which exist as both monomers and/or polymeric proanthocyanidins (**Figure 1b**) (*4*−*6*). Skin flavan-3-ols differ from those found in seeds in that skins contain a low concentration of flavan-3-ol monomers the proanthocyanidins contain (−)-epigallocatechin (EGC), have a higher degree of polymerization and a lower proportion of ECG (**Figure 1b**). Anthocyanins exist as 3-*O*-monoglucosides and their acylated derivatives. Pinot noir fruit is distinct in having no acylation (**Figure 1c**) (*7*).

Grapes are a nonclimacteric fruit and have two stages of berry growth separated by a lag phase (*8*). Flavonols, flavan-3-ol monomers, and proanthocyanidins are biosynthesized during the first phase of berry growth, whereas anthocyanins are biosynthesized during fruit ripening (*9*−*13*; **Figure 2**). Flavonoid accumulation can also respond to external factors such as UV radiation, drought, and cold temperatures (*14*, *15*). Two possible mechanisms have been proposed for plant response to UV stress including the biosynthesis of UV absorbing compounds and scavengers of active oxygen species (*16*−*18*).

* To whom correspondence should be addressed. Tel: +1-541-737-9150. Fax: +1-541-737-1877. E-mail: james.kennedy@oregonstate.edu.

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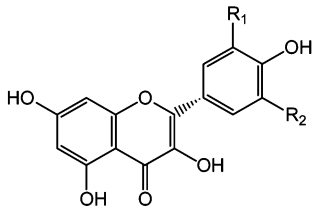
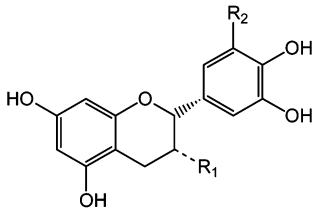
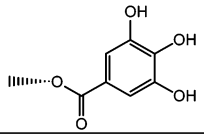
a



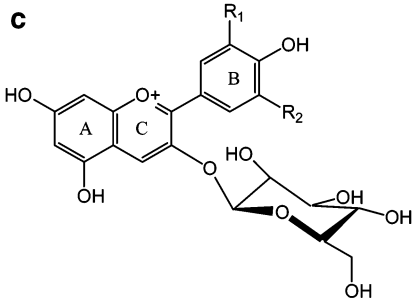
Flavonol	R_1	R_2
kaempferol	H	H
quercetin	OH	H
myricetin	OH	OH

b



Flavan-3-ol Monomer	R_1	R_2
(+)-catechin		H
(-)-epicatechin		H
(-)-epigallocatechin		OH
(-)-epicatechin-3-*O*-gallate		H

c



Anthocyanidin	R_1	R_2
cyanidin	OH	H
peonidin	OCH_3	H
delphinidin	OH	OH
petunidin	OCH_3	OH
malvidin	OCH_3	OCH_3

Figure 1. Structures of flavonols (**a**), flavan-3-ol monomers (**b**), and anthocyanins (**c**) based on substitution patterns commonly found in *V. vinifera*.

Flavonols are highly responsive to light exposure and appear to function as UV protectants (*1*, *19*−*22*). The total proanthocyanidin amount has been observed to decrease slightly with respect to exposure (*22*) and vine water status (*23*). However, an increase in the skin proanthocyanidin amount with a reduction in vine vigor has also been reported (*24*). Anthocyanins have been found to have a variable response to light exposure (*20*, *21*, *23*−*28*). Various viticultural practices also influence anthocyanin accumulation (*29*−*33*).

Compositional shifts in response to UV-B have been found in flavonol biosynthesis in Arabidopsis (*34*). Anthocyanins change toward a higher proportion of B ring trihydroxylation in response to UV light (*22*, *34*, *35*). Recent results on grape skin proanthocyanidins suggest that light exposure can also result in higher B ring trihydroxylation (*22*, *24*).

In addition to the flavonoid amount in the fruit, the rate of extraction is an important parameter that determines wine flavonoid concentration. Fruit ripeness, ethanol content (*36*), and perhaps berry size have been reported to influence the extraction of flavonoids (*37*). An increase in skin proanthocyanidin extraction with a reduction in vine vigor has been reported (*24*).

In an initial study assessing the impact of vine vigor on flavonoid accumulation, substantial differences were found in skin proanthocyanidin accumulation and composition (*24*). Because vine vigor modifies the canopy structure, we were interested in determining whether differences were due to variations in light exposure or other vigor-related factors (e.g., water stress and nutrient uptake). Our first objective was to investigate the relative importance of sunlight exposure on fruit flavonoid accumulation and composition. The second objective was to determine if fruit exposure influenced flavonoid extractability in a model wine system.

MATERIALS AND METHODS

Vineyard. This study was conducted in 2004 within an 8 year old commercial *V. vinifera* L. cv. Pinot noir vineyard (clone Dijon 777

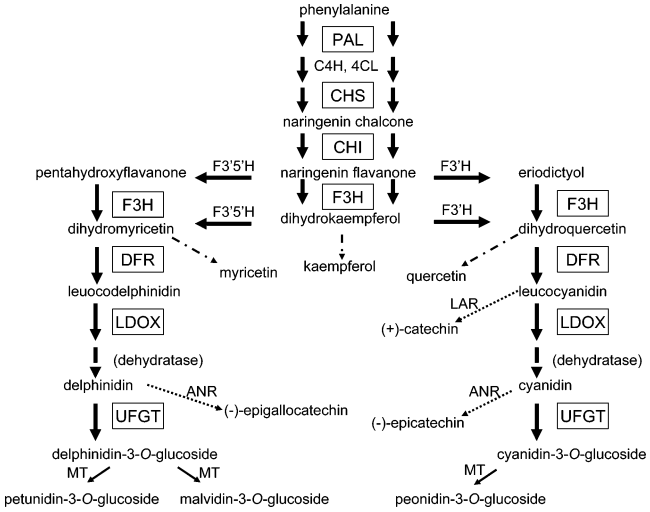


Figure 2. Simplified flavonoid biosynthetic pathway showing products from flavonoid 3′-hydroxylase (F3′H) and flavonoid 3′,5′-hydroxylase (F3′5′H) activity: flavonols, dashed arrows; flavan-3-ols, dotted arrows; and anthocyanins, solid arrows. Abbreviations: PAL, phenylalanine ammonia lyase; CHS, chalcone synthase; CHI, chalcone isomerase; DFR, dihydroflavonol-4-reductase; LDOX, leucoanthocyanidin dioxygenase; UFGT, UDP glucose:flavonoid-3-*O*-glucosyltransferase; LAR, leucoanthocyanidin reductase; ANR, anthocyanin reductase; and MT, methyltransferase.

Figure 3. Light exclusion boxes installed over fruit clusters in the vineyard.

grafted onto *Riparia gloire* rootstock) located in the Willamette Valley in Oregon. Vines were planted at a spacing of 1 m × 2.8 m with ~5113 vines per hectare. The training system was vertical shoot position with each vine pruned to 10−12 nodes. Vine vigor zones within the study block were determined as previously described (*24*). For this experiment, data vines were randomly selected within the low vigor zone in block A. The goal was to use a zone of uniform vine vigor to investigate the influence of light exposure specifically.

On each randomly selected data vine, clusters were selected on two shoots. Two clusters on one shoot were enclosed in opaque boxes (**Figure 3**) (shaded treatment), and two clusters on an adjacent shoot were labeled as the exposed treatment. Boxes were identical to those used in previous research on Shiraz in Australia (*22*). The temperature within the box was found to be within 0.5 °C of the ambient canopy temperature (*22*). In this experiment, the temperature was also monitored with dataloggers (Onset; Bourne, MA) and the variation was similar (data not shown). With the exception of exposure, cluster management was identical for both treatments. Boxes were applied when berries were approximately 2 mm in diameter (June 18).

Ten replicates (1−2 clusters each) of both treatments (shaded, exposed) were collected at véraison (August 6). Random numbers were used to determine whether to collect the number one- or two-positioned cluster on the shoot for each set (shaded, exposed). Remaining clusters were collected 1 day prior to commercial harvest (September 9). Harvested clusters were randomly divided into subsamples for juice composition (soluble solids, titratable acidity, and pH), high-performance liquid chromatography (HPLC) analysis, and model extraction. For juice composition (5−6 replicates) and HPLC analysis (8−10 replicates), a replicate consisted of all of the berries from 1 to 2 clusters. For HPLC analysis, frozen berries were removed from the rachis and prepared as previously described (*38*).

Chemicals. All solvents were HPLC grade. Acetonitrile, methanol, ethanol, glacial acetic acid, ascorbic acid, potassium metabisulfite, and potassium hydroxide were purchased from J.T. Baker (Phillipsburg, NJ). *N*,*N*-Dimethylforamide (DMF) was purchased from Burdick and Jackson (Muskegon, MI). Phloroglucinol, C, EC, and quercetin were purchased from Sigma (St. Louis, MO). Ammonium phosphate monobasic and orthophosphoric acid were purchased from Fisher Scientific (Santa Clara, CA). Hydrochloric acid was purchased from E.M. Science (Gibbstown, NJ). Sodium acetate anhydrous and lithium chloride were purchased from Mallinckrodt (Phillipsburg, NJ). Malvidin-3-*O*-glucoside (Mv) was purchased from Extrasynthése (Genay, France).

Model Extraction. For the model extraction, 10 replicates (~six clusters per replicate) of each treatment were processed. Clusters were randomly assigned to treatment replicates. Berries were carefully removed from the rachis in order to avoid losing juice. Berries from the six cluster replicate were mixed, and then, a 300 g sample was taken. 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. A 300 mL amount of the ethanol solution was added to the 300 g berry sample resulting in an approximate 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, the musts were pressed using a buchner 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.

HPLC Analysis. 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.

Total flavan-3-ol monomers, flavonols, and anthocyanins in grape seeds, skins, and model extracts were measured by reversed-phase HPLC (*39*). Aqueous extracts were filtered using Teflon filters (0.45 μm; Acrodisc CR13) before injection. C, quercetin, and Mv were used as quantitative standards for flavan-3-ols, flavonols, and anthocyanins, respectively.

Proanthocyanidin isolates were characterized by phloroglucinolysis (*40*) under modified HPLC conditions (*41*). Phloroglucinolysis provided information on subunit composition, conversion yield, and mean degree of polymerization (mDP). Seed and skin extracts were prepared as previously described (*24*). Skin and seed proanthocyanidin extraction into model extracts was calculated as described (*42*).

Gel permeation chromatography (GPC) was used to analyze proanthocyanidins while still intact (*41*). Proanthocyanidins were considered to be 280 nm absorbing material over 500 molecular weight units. Samples were prepared as previously described (*24*); however, after freeze drying, samples were dissolved in GPC mobile phase. C and Mv were used as quantitative standards at 280 and 520 nm, respectively.

Statistical analysis of data was performed using two-way analysis of variance (ANOVA) to determine statistically different values at a significance level of $\alpha = 0.05$ or less. All statistical analyses were performed using SAS version 8.2.

RESULTS

Berry Composition. No treatment differences were found in average cluster weight or average berry weight at véraison or harvest (**Table 1**). Average seeds per berry were the same at véraison; however, at harvest, the exposed treatment was higher

Table 1. Mean (±SEM) Average Cluster Weight (g), Average Berry Weight (g), Average Seeds Per Berry, Dry Average Seed Weight (mg), Dry Average Skin Weight (mg), Fresh Seed (%), Fresh Skin (%), Fresh Pulp (%), Soluble Solids (°Brix), Titratable Acidity (g/L), and pH of Shaded and Exposed Treatments at Véraison and Commercial Harvest

parameter	sample time	shaded	exposed	*p* value[a]
cluster weight (g)	véraison	38.6 ± 4.3	36.4 ± 4.25	0.6652
	harvest	54.1 ± 2.0	57.1 ± 2.02	0.1458
berry weight (g)	véraison	0.46 ± 0.02	0.47 ± 0.02	0.8532
	harvest	0.64 ± 0.10	0.71 ± 0.11	0.1799
seeds per berry	véraison	1.14 ± 0.03	1.13 ± 0.03	0.6476
	harvest	0.99 ± 0.04	1.08 ± 0.04	0.0223
dry seed weight (mg)	véraison	16.4 ± 0.8	15.1 ± 0.8	0.2933
	harvest	16.7 ± 0.8	17.0 ± 0.7	0.6044
dry skin weight (mg)	véraison	7.1 ± 0.3	8.6 ± 0.3	0.0043
	harvest	12.9 ± 1.4	16.2 ± 1.4	0.1127
fresh seed (%)	harvest	4.0 ± 0.12	3.8 ± 0.11	0.3134
fresh skin (%)	harvest	10.6 ± 0.57	9.8 ± 0.54	0.3254
fresh pulp (%)	harvest	85.6 ± 0.58	86.2 ± 0.55	0.4355
soluble solids (°Brix)[b]	harvest	23.9 ± 0.45	23.6 ± 0.47	0.1516
titratable acidity (g/L)[b]	harvest	7.2 ± 0.27	8.2 ± 0.27	0.0679
pH[b]	harvest	3.08 ± 0.03	3.22 ± 0.04	0.0412

[a] ANOVA to compare data (*P* indicated); $n = 8–10$. [b] $n = 5–6$.

than the shaded treatment. No differences were observed for average dry seed or skin weight (mg) in ripe fruit although at véraison, the shaded treatment had a lower dry skin weight compared to the exposed treatment. Shaded and exposed treatments had similar proportions of seed, skin, and pulp. Soluble solids (°Brix) were similar at harvest for shaded and exposed treatments while titratable acidity (g/L) and pH were slightly higher for exposed treatment.

Skin Flavonols. In all analyses, quercetin derivatives were the most abundant flavonols. At véraison, the shaded treatment flavonol concentration was ~5.5 times lower than the exposed treatment (0.009 vs 0.049 mg/berry, $p = 0.0001$). By harvest, the shaded treatment concentration was slightly more than eight times lower than the exposed treatment (0.012 vs 0.10 mg/berry, $p = 0.0002$). Because of low HPLC peak areas in the shaded treatment, it was not possible to assess compositional changes.

Seed Flavan-3-ols. Seed flavan-3-ol monomers included C and EC (**Table 2a**). The total seed monomer amount was similar between treatments at véraison and harvest. In both treatments, the amount decreased slightly from véraison to harvest. The shaded treatment had a higher proportion of EC than in the exposed treatment at both sample dates. The flavan-3-ol monomer proportion of C increased ~2%, and EC had a similar decrease in both treatments between véraison and harvest.

The proanthocyanidin amount was determined by GPC and phloroglucinolysis. By GPC, a higher seed proanthocyanidin concentration of 5.08 ± 0.54 mg/seed was found in the shaded treatment as compared to 3.27 ± 0.51 mg/seed for the exposed treatment at harvest ($p = 0.0435$). At véraison, the proanthocyanidin amount for the shaded treatment (5.37 ± 0.81 mg/seed) and the exposed treatment (3.83 ± 0.77 mg/seed) were similar ($p = 0.2030$). When seed proanthocyanidin data were expressed on a per berry basis (data not shown), there were no differences between treatments at véraison ($p = 0.2063$) or harvest ($p = 0.2863$). Overall, GPC values were consistent although higher than phloroglucinol results.

By phloroglucinolysis, no differences in proanthocyanidin extension or terminal subunit amount per seed were observed at véraison (**Table 2b)**. The shaded treatment extension proanthocyanidin subunits decreased ~7% while the exposed treatment decreased ~15% from véraison to harvest. The terminal proanthocyanidin subunits were similar for the shaded treatment between véraison and harvest and decreased by ~8% in the exposed treatment during the same time period. The shaded treatment had a higher seed mDP at véraison, but by harvest, the treatments were similar.

Table 2. Mean (±SEM) (**a**) Seed Flavan-3-ol Monomer Concentration (nmol/Seed) and Molar Proportion, (**b**) Extension, Terminal, and Total Subunit Concentration (nmol/Seed) and mDP, and (**c**) Proanthocyanidin Molar Extension and Terminal Subunit Proportions by Phloroglucinolysis from Shaded and Exposed Treatments at Véraison and Commercial Harvest

(**a**) Flavan-3-ol Monomer Concentration and Composition

treatment	time	monomer (nmol/seed)	C (%)	EC (%)
shaded	véraison	1846 ± 132	57.9 ± 0.56	42.1 ± 0.56
exposed	véraison	1787 ± 132	65.6 ± 0.56	34.4 ± 0.56
p value[a]		0.7609	<0.0001	<0.0001
box	harvest	1621 ± 133	59.5 ± 1.20	40.5 ± 1.20
exposed	harvest	1424 ± 113	67.5 ± 1.09	32.5 ± 1.09
p value[a]		0.2102	0.0003	0.0003

(**b**) Proanthocyanidin Concentration

treatment	time	extension (nmol/seed)	terminal (nmol/seed)	mDP	total (nmol/seed)
shaded	véraison	6377 ± 228	2801 ± 161	14.5 ± 0.8	9178 ± 382
exposed	véraison	5914 ± 228	2651 ± 161	11.9 ± 0.8	8565 ± 382
p value[a]		0.1853	0.5272	0.0341	0.2857
shaded	harvest	5927 ± 240	2818 ± 190	7.0 ± 0.3	8718 ± 408
exposed	harvest	5015 ± 750	2442 ± 165	6.6 ± 0.2	7457 ± 368
p value[a]		0.0380	0.0502	0.3081	0.0407

(**c**) Proanthocyanidin Composition

		extension			terminal		
treatment	time	C (%)	EC (%)	ECG (%)	C (%)	EC (%)	ECG (%)
shaded	véraison	12.6 ± 0.3	71.3 ± 0.4	16.2 ± 0.3	51.9 ± 0.7	29.9 ± 0.5	18.2 ± 0.6
exposed	véraison	16.0 ± 0.3	72.1 ± 0.4	11.9 ± 0.3	59.5 ± 0.7	26.7 ± 0.5	13.8 ± 0.6
p value[a]		<.0001	0.1553	<0.0001	<0.0001	0.0005	0.0004
shaded	harvest	12.7 ± 0.6	71.9 ± 0.5	15.5 ± 0.3	53.0 ± 0.7	34.2 ± 0.8	13.0 ± 0.4
exposed	harvest	16.7 ± 0.5	72.3 ± 0.5	11.0 ± 0.3	60.6 ± 0.6	29.3 ± 0.7	10.2 ± 0.4
p value[a]		0.0010	0.3880	<0.0001	0.0002	0.0062	0.0012

[a] ANOVA to compare data (*P* indicated); $n = 8–10$.

The composition of proanthocyanidins was determined by phloroglucinolysis (**Table 2c**). At véraison and harvest, no treatment differences in the proportion of EC extension subunits were observed. For the shaded treatment at harvest, the extension subunit proportion was lower for C and higher for ECG as compared to the exposed treatment. For terminal subunits at both véraison and harvest, the shaded treatment had a lower proportion of C and higher EC and ECG. In both treatments, the terminal subunit proportion at harvest remained constant for C, increased for EC, and decreased for ECG when compared to the values at véraison.

Skin Flavan-3-ols. The total proanthocyanidin amount was determined by GPC and phloroglucinolysis. By GPC, the shaded treatment had 0.64 ± 0.15 mg/berry skin proanthocyanidin, which was 0.95 ± 0.15 (mg/berry) lower than the exposed treatment at harvest ($p = 0.0038$). The difference was also apparent at véraison where the shaded treatment had 0.74 ± 0.10 mg/berry as compared to 1.20 ± 0.10 mg/berry skin proanthocyanidin in the exposed treatment ($p = 0.0116$).

By phloroglucinolysis, the skin proanthocyanidin amount (mg/berry) was substantially lower in the shaded treatment at both véraison and harvest. At harvest, the extension subunits concentration for the exposed treatment was ~77% higher than

Table 3. Mean (±SEM) (**a**) Skin Proanthocyanidin Extension, Terminal, and Total Subunit Concentration (nmol/berry) and mDP and (**b**) Extension Subunit Molar Proportions by Phloroglucinolysis from Shaded and Exposed Treatments at Véraison and Commercial Harvest

(**a**) Proanthocyanidin Concentration

treatment	time	extension (nmol/berry)	terminal (nmol/berry)	mDP	total (nmol/berry)
shaded	véraison	1650 ± 192	61.4 ± 6.7	28.6 ± 1.8	1712 ± 198
exposed	véraison	3267 ± 192	94.6 ± 6.7	36.7 ± 1.8	3362 ± 198
p value[a]		0.0002	0.0044	0.0159	0.0002
shaded	harvest	1346 ± 139	207 ± 17	7.5 ± 1.7	1553 ± 142
exposed	harvest	2378 ± 131	147 ± 16	18.9 ± 1.6	2525 ± 134
p value[a]		0.0010	0.0349	0.0012	0.0016

(**b**) Proanthocyanidin Composition

treatment	time	C (%)	EC (%)	EGC (%)	ECG (%)	3′4′-OH (%)	3′4′5′-OH (%)
shaded	véraison	0.8 ± 0.2	75.0 ± 1.1	20.5 ± 1.2	3.7 ± 0.2	79.5 ± 1.1	20.5 ± 1.2
exposed	véraison	2.3 ± 0.2	60.5 ± 1.1	35.7 ± 1.2	1.5 ± 0.2	64.3 ± 1.1	35.7 ± 1.2
p value[a]		0.0007	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
shaded	harvest	1.6 ± 0.3	78.3 ± 1.0	19.2 ± 1.0	0.9 ± 0.1	80.8 ± 1.0	19.2 ± 1.0
exposed	harvest	2.4 ± 0.3	61.9 ± 0.9	34.7 ± 1.0	0.9 ± 0.1	65.3 ± 1.0	34.7 ± 1.0
p value[a]		0.0920	<0.0001	<0.0001	0.9342	<0.0001	<0.0001

[a] ANOVA to compare data (*P* indicated); $n = 8–10$.

the shaded treatment (**Table 3a**). The terminal subunit concentration for the shaded treatment was lower than the exposed treatment at véraison, whereas at harvest, the terminal subunit concentration for the shaded treatment was substantially higher than the exposed treatment. The shaded treatment had a lower skin proanthocyanidin mDP as compared to the exposed treatment at both véraison and harvest. In comparing véraison to harvest, both treatments had a reduction in proanthocyanidin mDP, although there was a much greater reduction for the shaded treatment as compared to the exposed treatment.

Skin proanthocyanidin extension subunits consisted of C, EC, ECG, and EGC (**Table 3b**). C was the only terminal subunit observed, and it was not differentiated from C monomers. At véraison, the proanthocyanidin proportion for the shaded treatment was higher for EC and ECG and lower for C and EGC when compared to the exposed treatment. At harvest, the relative proportions between treatments were similar to those at véraison with the exception of ECG. At harvest, EGC extension subunits were ~16% higher in the exposed treatment as compared to the shaded treatment indicating an increase in B ring trihydroxylation. At véraison and harvest, shading consistently had a lower proportion of trihydroxylated proanthocyanidin extension subunits as compared to the exposed treatment.

Skin Anthocyanins. On a per berry basis, there was a trend toward a reduced anthocyanin concentration in the shaded treatment of ~32% (**Table 4**). On a berry weight basis, there was a minimal trend observed ($p = 0.1166$, data not included). Shading resulted in lower proportions of delphinidin-3-*O*-glucoside (Dp), cyanidin-3-*O*-glucoside (Cy), petunidin-3-*O*-glucoside (Pt), and Mv with only an increase in the proportion of peonidin-3-*O*-glucoside (Pn). The proportion of Pn in the shaded treatment was double the proportion found in the exposed treatment. In comparing the B ring substitution pattern, the shaded treatment had a lower proportion of trihydroxylated anthocyanins than the exposed treatment.

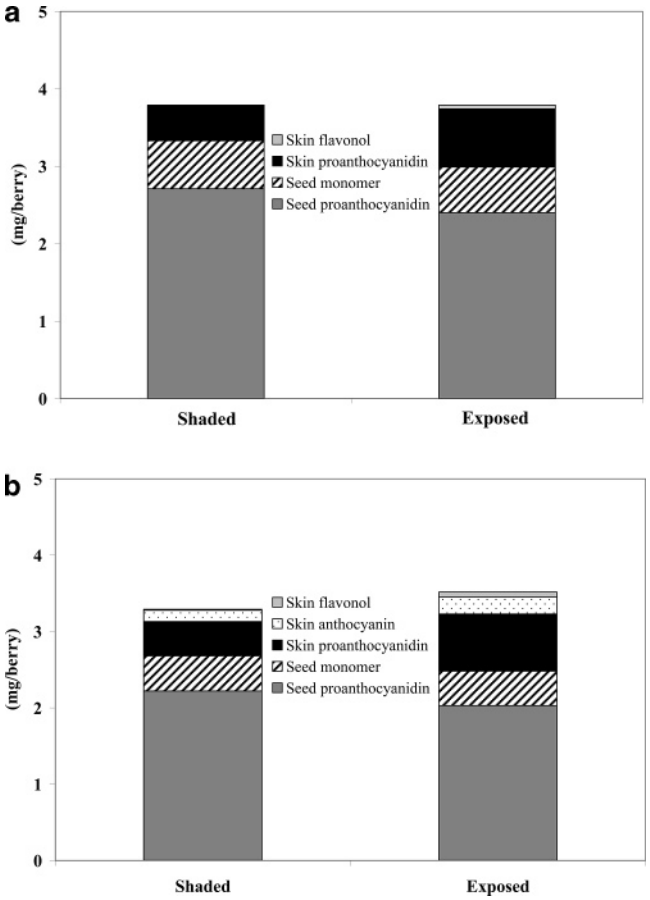


Figure 4. (**a**) Total accumulation of seed flavan-3-ol monomers, seed proanthocyanidins, skin proanthocyanidins, and skin flavonols (mg/berry) in shaded and exposed fruit at véraison ($n = 10$). Seed and skin proanthocyanidins were determined by phloroglucinol. (**b**) Total accumulation of seed flavan-3-ol monomers, seed proanthocyanidins, skin proanthocyanidins, skin flavonols, and skin anthocyanins (mg/berry) in shaded and exposed fruits at commercial harvest ($n = 8–9$). Seed and skin proanthocyanidins were determined by phloroglucinol.

At véraison and harvest, the individual flavonoid classes were compared on a per berry basis. Although the total amount of flavonoids was similar at véraison ($p = 0.9300$), skin flavonoids (skin proanthocyanidins and flavonols) were higher in the exposed treatment as compared to the shaded treatment (**Figure 4a**; $p = 0.0004$). Similarly, at harvest, the total flavonoid amount for the three classes was similar on a per berry basis ($p = 0.1549$); however, the relative accumulation of specific classes

Table 4. Mean (±SEM) Skin Anthocyanin Amount (mg/Berry) Calculated in Malvidin Equivalents, Proportional Analysis, and Oxygenation Pattern in Shaded and Exposed Treatments at Commercial Harvest

treatment	time	total (mg/berry)	delphinidin (%)	cyanidin (%)	petunidin (%)	peonidin (%)	malvidin (%)	3′4′-OH (%)	3′4′5′-OH (%)
shaded	harvest	0.15 ± 0.03	3.0 ± 0.4	2.2 ± 0.1	4.0 ± 0.3	43.2 ± 1.0	47.5 ± 1.2	45.5 ± 1.0	54.5 ± 1.0
exposed	harvest	0.22 ± 0.03	7.9 ± 0.4	2.5 ± 0.1	7.0 ± 0.3	18.7 ± 0.9	63.8 ± 1.1	21.2 ± 1.0	78.8 ± 1.0
p value[a]		0.0931	<0.0001	0.0982	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a] ANOVA to compare data (*P* indicated); $n = 8–10$. 3′,4′-OH, cyanidin and peonidin; 3′,4′,5′-OH, delphinidin, petunidin, and malvidin.

Table 5. Mean (±SEM) for Number of Berries in 300 (g), Average Berry Weight (g), Pomace Weight (g), and Juice Volume (mL) from Shaded and Exposed Model Extractions

parameter	shaded	exposed	*p* value
no. of berries in 300 (g)	458 ± 19	419 ± 19	0.0140
berry weight (g)	0.66 ± 0.03	0.73 ± 0.03	0.0138
pomace weight (g)	118 ± 2	116 ± 2	0.3977
extract volume (mL)	450 ± 2	454 ± 2	0.1679

[a] ANOVA to compare data (*P* indicated); $n = 8–10$.

differed between treatments (**Figure 4b**). The shaded treatment had a similar proportion of seed flavonoids (monomers and proanthocyanidins) and a reduction of 0.49 mg/berry ± 0.09 ($p = 0.0035$) in accumulation of skin flavonoid compounds compared to the exposed treatment. In comparing the percent skin flavonoid content per berry, at véraison, the shaded treatment contained 12% and the exposed had 21% skin flavonoids ($p = <0.0001$). With the additional accumulation of anthocyanins at harvest, the shaded treatment was 17% and the exposed treatment was 30% skin flavonoids on a per berry basis ($p = 0.0017$).

Model Extracts. An additional goal of this project was to conduct model extractions in order to better understand the relationship between light exposure and flavonoid extraction. A model system was used due to low fruit quantities. The average berry weight for the 150 berry fruit sample was similar to the 300 g berry sample used for the model extracts although the 150 g berry sample was not statistically significant at $p \leq 0.05$ (**Tables 1** and **5**). Pomace weight (g) and juice volume (mL) did not differ between treatments.

Model Extract Flavonols. The total flavonol concentration in the shaded treatment of 45 ± 7 mg/L was ∼2.5 times lower than the exposed treatment of 111 ± 7 mg/L ($p = <0.0001$). The treatment difference in model extracts was less than that for the fruit skin extracts (∼2.5× vs ∼8×, respectively).

Model Extract Flavan-3-ols. A higher extract concentration of flavan-3-ol monomers was found in the shaded treatment compared to the exposed treatment (**Table 6a**). There was a higher proportion of C flavan-3-ol monomers in the exposed extract than for the shaded treatment.

By GPC, the shaded treatment proanthocyanidin concentration of 245 ± 6 mg/L was 147 ± 6 mg/L ($p = <0.0001$) lower than the exposed treatment. By phloroglucinolysis, the shaded treatment total subunit proanthocyanidin concentration was ∼29% less than the exposed treatment (**Table 6b**).

The proanthocyanidin composition of model extracts (**Table 6c**) indicated that the shaded treatment had a proportion that was similar in C, higher in EC and ECG, and lower in EGC compared to the exposed treatment. The composition of ECG extension subunits in model extracts was similar to the proportional differences between treatments found in the seeds (**Table 2b**). The C and EC terminal subunit ratio in model extracts was similar to the relationship found between treatments in seed terminal units (**Table 2b**). For EC and EGC, the variations seen in the model extracts (**Table 6c**) are consistent with the treatment differences seen in the skin extension subunits (**Table 3b**). The mDP for the shaded treatment proanthocyanidins was lower than the exposed treatment although only slightly so. The exposed treatment had a higher concentration of skin proanthocyanidin (**Table 7**). In addition, there was a greater percent of skin proanthocyanidin extraction in the exposed as compared to the shaded extracts. However, no differences were observed between treatments for seed proanthocyanidin concentration in the extracts.

Table 6. Mean (±SEM) (**a**) Seed Flavan-3-ol Monomer Concentration (μmol/L) and Molar Proportion, (**b**) Proanthocyanidin Extension, Terminal, Total Subunit Concentration (μmol/L), and mDP, and (**c**) Proanthocyanidin Extension and Terminal Subunit Molar Proportions by Phloroglucinolysis from Shaded and Exposed Model Extractions

(**a**) Flavan-3-ol Monomer Concentration and Composition

treatment	monomer (μmol/L)	C (%)	EC (%)
shaded	60.4 ± 2.8	53.0 ± 0.8	47.0 ± 0.8
exposed	50.2 ± 2.8	66.2 ± 0.8	33.8 ± 0.8
p value[a]	0.0004	<0.0001	<0.0001

(**b**) Proanthocyanidin Concentration

treatment	extension units (μmol/L)	terminal units (μmol/L)	total subunits (μmol/L)	mDP
shaded	58.1 ± 3.4	19.2 ± 0.8	77.4 ± 3.9	11.37 ± 0.5
exposed	88.9 ± 3.4	20.1 ± 0.8	109.1 ± 3.9	12.56 ± 0.5
p value[a]	<0.0001	0.3100	<0.0001	0.0826

(**c**) Proanthocyanidin Composition

	extension subunits				terminal subunits	
treatment	C (%)	EC (%)	EGC (%)	ECG (%)	C (%)	EC (%)
shaded	3.7 ± 0.2	81.2 ± 0.4	9.3 ± 0.4	5.8 ± 0.1	73.2 ± 0.6	26.8 ± 0.6
exposed	3.4 ± 0.2	71.5 ± 0.4	22.5 ± 0.4	2.6 ± 0.1	76.5 ± 0.6	23.5 ± 0.6
p value[a]	0.2743	<0.0001	<0.0001	<0.0001	0.0036	0.0036

[a] ANOVA to compare data (*P* indicated); $n = 8–10$.

Table 7. Mean (±SEM) of Total, Skin, and Seed Proanthocyanidin (mg/L) and Percent Skin Extraction Determined by Phloroglucinol from Shaded and Exposed Model Extractions

treatment	total (mg/L)	skin extracted (%)	skin (mg/L)	seed (mg/L)
shaded	105.6 ± 6.1	54.1 ± 2.9	56.6 ± 6.8	46.4 ± 4.5
exposed	146.5 ± 5.8	71.2 ± 2.8	105.0 ± 6.4	41.50 ± 4.3
p value[a]	0.0004	0.0017	0.0012	0.2607

[a] ANOVA to compare data (*P* indicated); $n = 8–10$.

Model Extract Anthocyanins. The anthocyanin concentration (mg/L) was higher in the exposed treatment model extract than the shaded treatment (**Table 8**). The extract treatment difference in anthocyanin concentration was ∼67%, which was greater than the treatment difference observed in the fruit (**Table 4**). The exposed treatment also had a higher concentration of pigmented polymers (data not shown).

There were substantial compositional differences between shaded and exposed model extracts for all anthocyanins. With the exception of Pn, the shaded treatment extract had a lower proportion of all other anthocyanins (**Table 8**). The anthocyanin compositional treatment differences found in the fruit (**Table 4**) were reflected in the model extracts (**Table 8**). However, in both treatments, there was a reduction in the proportion of Dp and Pt in model extracts as compared to the proportions found in the fruit.

DISCUSSION

Under conditions of low water and nutrient availability, plants can reduce growth and shift carbon into producing more secondary plant metabolites (*43*). Ultraviolet radiation and water stress have been shown in numerous studies to be the most relevant factors in the induction of flavonoid biosynthesis (*43*). In a previous paper, we investigated how variations in vine vigor, related to differences in available soil water and nutrients, influenced the accumulation and composition of flavonoid compounds in Pinot noir grapes (*24*). In this study, we compare

Table 8. Mean (±SEM) Anthocyanin Concentration (mg/L) Calculated in Malvidin Equivalents and Proportional Analysis in Shaded and Exposed Model Extractions

treatment	time	total (mg/L)	delphinidin (%)	cyanidin (%)	petunidin (%)	peonidin (%)	malvidin (%)
shaded	harvest	129.8 ± 5.1	1.3 ± 0.08	1.4 ± 0.05	2.8 ± 0.08	40.2 ± 0.47	54.4 ± 0.53
exposed	harvest	216.4 ± 5.1	3.4 ± 0.08	1.8 ± 0.05	5.3 ± 0.08	21.8 ± 0.47	67.5 ± 0.53
p value[a]		<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001

[a] ANOVA to compare data (*P* indicated); $n = 8–10$.

the results of a shading treatment in low vigor vines to our findings in high vigor vines that inherently had higher available water and nutrients and also greater shading in the fruiting zone.

Berry Composition. The lack of differences observed in soluble solids accumulation (**Table 1**) minimizes possible impacts of shading on maturity and improves the ability to focus on the influence of light exposure. In previous light exposure studies, a reduction in sugar accumulation has been observed (*28*) while others found no effect from shading (*21*, *22*). In this experiment, the average number of seeds per berry was determined to be similar at véraison but was lower in the shaded treatment at harvest (**Table 1**). It is possible that this was due to high sample variability. Upon the basis of previous work (*44*), the number of seeds per berry was the major contributing factor to the amount of proanthocyanidin per berry rather than the concentration per seed.

Environmental influences such as water deficit (*45*) can affect average berry size and subsequent skin, seed, and pulp proportions (*46*). In this experiment, berry size was similar and no differences were observed in percent skin, seed, or pulp (**Table 1**). A higher skin tissue mass has been found in berries from vines grown under a low vine water status (*47*).

Skin Flavonols. The flavonoid pathway involves a number of enzymes some of which are shared and others which are specific to the production of flavonols, proanthocyanidins, and anthocyanins (**Figure 2**). Flavonol synthase (FLS) is involved in flavonol biosynthesis, and there are two periods of synthesis with the first occurring around flowering and the second during berry ripening (*48*, *49*). As anticipated, flavonols were minimal in the shaded treatment at véraison and harvest. The exposed treatment was about eight times higher than the shaded treatment in skin flavonol concentration at harvest. Shading has been shown to cause significant reductions in flavonol concentration in grapes (*24*, *21*, *22*) and apple (*50*), and our results are consistent with these observations.

Seed Flavan-3-ols. Proanthocyanidins in seeds are thought to provide protection from early feeding of unripe fruits (*51*) and also to protect developing fruit from fungal pathogens (*52*). Bogs et al. (*10*) found that the two LAR genes involved in proanthocyanidin biosynthesis had different patterns of expression in seeds and skins, which effect the concentration and composition of proanthocyanidins. Our results are consistent with different patterns of expression in tissues as we saw different responses in seeds and skins.

Although the proanthocyanidin concentration was higher on a per seed basis in the shaded treatment, there were no differences on a per berry basis when analyzed by either phloroglucinolysis or GPC possibly due to the higher number of seeds per berry in the exposed treatment at harvest (**Table 1**). These results are similar to what was observed in the vine vigor study where there were no differences when calculated on a berry basis since fruit from low vigor vines had more seeds per berry than from high vigor vines (*24*). Other studies on the influence of environmental factors on seed proanthocyanidin accumulation and composition have been somewhat hard to interpret and have in general shown minimal influence from vine water status (*38*, *47*) and light exclusion (*22*).

The general pattern of flavan-3-ol monomer accumulation was shown to involve a rapid increase near or 1−2 weeks after véraison followed by a decline leading to harvest (*49*, *53*). In the present experiment, total flavan-3-ol monomer concentrations were similar between treatments at véraison and harvest **Table 2a**). Downey et al. (*22*) found higher levels of monomers in exposed clusters at véraison but no differences between shaded and exposed fruits at harvest. In other results, total flavan-3-ol monomers were found to be lower in minimally irrigated Cabernet Sauvignon vines (*38*) and in low vigor vines (*24*). These results are different from another study in that the amount of monomers per seed was lower than previously reported in Pinot noir and there was relatively little change between the amount at véraison and harvest (*54*). This may have been due to the early fruit harvest (based upon sugar concentration).

There were differences in the flavan-3-ol monomer proportions of EC and C (**Table 2a**). The shaded treatment had a higher proportion of EC compared to the exposed treatment, and this pattern was consistent at both véraison and harvest. In our previous research, C was proportionally higher than EC in fruit from low vigor vines (*24*). The patterns in this exposure study are similar to the observations in the vine vigor study suggesting that the response is caused by differences in sun exposure. In other studies on ripening, the EC:C ratio was found to change as fruit matured resulting in ripe fruit having more EC than C at harvest (*38*, *49*, *53*). This differs from the present study in that C increased about 2% in both treatments from véraison to harvest. The proportions of C and EC in the shaded fruit at harvest in this study are similar to values previously reported in Pinot noir (*54*).

For proanthocyanidin analysis by phloroglucinolysis, no differences in extension or terminal subunit concentrations per seed were observed at véraison; however, by harvest, the shaded treatment was higher for both of these variables (**Table 2b**). This treatment response at harvest was greater than in our previous study where there was only a minimal trend toward higher total and extension subunits in zones containing high vigor vines (*24*). In other studies, extension proanthocyanidin subunits were highest at véraison (*38*, *54*) or 2 weeks postvéraison (*49*) and then declined leading to harvest. These results agree with our findings in both treatments; however, there was a greater reduction in extension proanthocyanidin subunits from véraison to harvest in the exposed treatment. The seed mDP values found in this study were higher than reported values in Pinot noir at véraison and were similar at harvest (*54*).

Shading resulted in a lower proportion of C and a higher proportion of ECG in the seed extension subunits at véraison and harvest (**Table 2c**). For terminal subunits, the shaded treatment had lower C and higher EC and ECG proportions as compared to the exposed treatment. For both treatments, at

véraison and harvest, the proportion of C increased slightly, EC increased, and ECG decreased. Kennedy et al. (*38*) found a similar pattern in Cabernet Sauvignon. High vigor vines with a shadier, more vigorous canopy were found to have a lower proportion of C and higher EC and ECG extension subunits while no differences in terminal subunit proportions were found (*24*). Consequently, the results for the extension subunits in high vigor vines are in agreement with our data for the shaded treatment in the present experiment.

Skin Proanthocyanidins. Skin proanthocyanidins are difficult to study due to the presence of anthocyanins and flavonols also found in skins and covalent or noncovalent associations with anthocyanins (*55*). To date, it is still unclear whether pigmented proanthocyanidins (*56*) are formed in the grape skin or are an artifact of processing. In the present study, we focused on skin proanthocyanidins rather than pigmented polymers. Previous studies have shown that the skin proanthocyanidin concentration peaked near véraison and then declined with increasing maturity (*23*, *49*, *54*). Recently, we found vines with low vigor had a substantially higher skin proanthocyanidin concentration than high vigor vines (*24*). Therefore, we were interested in investigating the relationship between sun exposure and skin proanthocyanidin accumulation.

Although there were minimal differences in skin dry weight (**Table 1**), the skin proanthocyanidin concentration was much higher in the skins of the exposed treatment by both phloroglucinolysis and GPC. In one study, vines grown under water deficits had a greater dry weight of skin in addition to a higher concentration of skin proanthocyanidins (*23*). It is not possible to assess whether the higher concentration of skin proanthocyanidins was directly related to vine water status or to variations in light exposure in the fruiting zone. In another light exclusion study, no differences were found in skin proanthocyanidin levels (mg/berry) at harvest although exposed fruit had a maximum level of twice as many extension subunits at véraison (*22*). Previously, we reported an increase of about 42% in total extension subunits when comparing low to high vigor vines and an increase of 69% when the total proanthocyanidin concentration (mg/berry) was analyzed by GPC (*24*). This increase in skin proanthocyanidin content may have been in response to differences in exposure in the fruiting zone rather than vigor per se. Thicker skins may have some benefit in a water deficit situation while an increase in skin proanthocyanidin concentration may play an, as of yet, undetermined role.

The average molecular weight of skin proanthocyanidin in Shiraz grapes was found to increase with berry development (*54*) while Downey et al. (*49*) reported that skin mDP increased during the early phase of berry development and then decreased after véraison. Our observations in both treatments agree with a reduction in skin mDP between véraison and harvest (**Table 3a**). The mDP values found in the present study at véraison were consistent with reported values in Pinot noir; however, our harvest values were lower (*54*). Downey et al. (*22*) found a similar reduction in skin mDP with shading. Previously, a higher mDP with a reduction in irrigation (*23*) and in fruit from low vigor vines was reported (*24*). It is possible that the reported responses could have been from greater sun exposure in the fruiting zone rather than specifically from water deficit.

The difference in percent EGC (15.7%) in this study (**Table 3)** was substantially greater than the increase of 6.4% previously reported in fruit from low vigor as compared to high vigor vines (*24*). This strong response with shading of low vigor vines in the present study suggests that the substantial decrease in EGC was due to fruit shading. This agrees with observations with Shiraz where EGC extension subunits were 13.2% higher in exposed clusters as compared to shaded clusters (*22*). In the present study, the proportion of EGC was similar at véraison and harvest; however, others have reported a reduction in EGC extension subunits from véraison to maturity (*51*, *55*). As EGC has the highest rate of degradation due to oxidation (*57*), it is possible that the differences seen between the maximal levels at véraison and at harvest are related to oxidation reactions.

Skin Anthocyanins. Beyond the enzymes required for flavan-3-ol biosynthesis, two additional enzymes (LDOX and UFGT) are required for anthocyanin biosynthesis (**Figure 2**) (*12*). For most grape varieties, UFGT is only found in red grape skins and is expressed at the time of anthocyanin accumulation (*13*). While many grape varieties have very complex anthocyanin profiles with up to 20 different anthocyanins (*58*), Pinot noir has only five anthocyanins: Dp, Cy, Pt, Pn, and Mv.

Shading reduced the anthocyanin content by about 32% at harvest although this difference was not significant at $p \leq 0.05$ even with 10 replicates of each treatment (**Table 4**). Downey et al. (*22*) did not find a difference in 2 out of 3 years for anthocyanin accumulation (with three replicates) in Shiraz using identical boxes for cluster shading. Price et al. (*19*) did not find sunlight exposure to have a significant effect on anthocyanin concentration in Pinot noir skin disks. However, in a number of other exposure studies, the anthocyanin content was found to be higher in exposed fruit (*21*, *25*−*27*).

For anthocyanin composition, shading resulted in lower proportions of Dp, Cy, Pt, and Mv with only an increase in Pn (**Table 4**). The proportion of Pn was approximately two times that found in the exposed treatment. In Reliance, a seedless *Vitis* hybrid, 95% shading resulted in a decrease in the percent Dp and Cy and an increase in Pn, Mv, and acylated Cy derivatives (*28*). In Shiraz, shading was found to have no effect on the proportion in the first season but showed a decrease in the relative proportions of Dp, Pt, and Mv while the proportion of Pn increased in the following two seasons (*22*). Our present results are consistent with the decrease in Dp and Cy and the increase in Pn found in Reliance and also with the Shiraz results.

In Merlot, a decrease in Dp and Cy and an increase in Mv derivatives with shading were observed (*21*). In the same study, the use of a UV barrier that blocks UV-B light showed a similar response. This shows a slightly different response, which might be variety specific; however, there was still a reduction in trihydroxylated Dp residues with shading (*21*). Interestingly, when sun-exposed fruit was cooled to the same temperatures as shaded fruit, the cooler temperature with the same sun exposure level resulted in an approximate 5% increase in Dp and a comparable decrease in Mv (*21*). Dp is more susceptible to oxidation than Mv (*59*). Mv has been previously reported to be less sensitive to light intensity as compared to the other four anthocyanins (*60*). Because of their phenolic B ring substitution, Pn and Mv are relatively stable and represent the major anthocyanin pools in mature grapes (*61*).

When investigating anthocyanin F3′5′H products as compared to the F3′H products (**Figure 2**), the shaded treatment had a much lower proportion of trioxygenated (Dp, Pt, and Mv) anthocyanins (**Table 4**). The anthocyanin accumulation was consistent with increased F3′5′H activity. Downey et al. (*22*) found between a 3 and a 10% increase (depending on the year) in trioxygenated anthocyanins in exposed clusters as compared to shaded clusters. In Reliance, 95% shading resulted in an approximate 5% decrease in trioxygenated as compared to dioxygenated anthocyanins (*28*). However, in Merlot, no increases were noted in percent trioxygenated anthocyanins with

greater light exposure (*21*). Consequently, although there seems to be a pattern of an increased proportion of trioxygenated anthocyanins with greater exposure, the response may be variety specific or modified by temperature.

As seen in **Figure 4a**,**b**, there were tissue specific differences in accumulation of seed and skin proanthocyanidins at both véraison and harvest. This agrees with studies on gene expression (*10*) and makes sense in terms of the different roles proanthocyanidins play in ripening fruit. While there were no differences in seed proanthocyanidin on a per berry basis, skin flavonoids (flavonols, anthocyanins, and proanthocyanidins) were higher in the exposed treatment at harvest. The increase in skin flavonoids likely plays a role in UV protection. These differences in fruit composition seen between shaded and exposed treatments are also likely to affect flavonoid concentration in a wine system.

Model Extracts. The ratio of skin and seed material to pulp was thought to influence the concentration of flavonoids found in wine although a recent study suggests berry size may be of limited importance (*37*). Although there were no differences in percent fresh skin, seed, or pulp in the berry sample (**Table 1**) or in the pomace weight and juice volume (**Table 5**), the shaded model extraction had fewer berries of a smaller size. Generally, a smaller berry size is expected from low vigor fruit such as found in water deficit studies (*23*, *32*, *45*). However, reduced berry growth was reported when shading occurred in the initial stages of berry growth (*27*). In this experiment, the somewhat smaller berries in the shaded treatment did not appear to modify extraction as the shaded treatment was still lower in extraction of all flavonoids as compared to the exposed treatment.

As expected, flavonols were higher in the exposed model extraction than in the shaded extraction. However, there was not as much variation between treatments in the model extraction as was found in the fruit. The high concentration found in the exposed model extraction is in agreement with results in Pinot noir wines where much higher levels were found in wines made from exposed clusters (*19*). The amount found in the exposed model extractions was higher than the levels reported in Pinot noir (*19*) wine, and this could be due to the higher temperature or higher ethanol content.

Shading fruit reduced the skin proanthocyanidin concentration in both the fruit and the model extraction (**Tables 3a** and **6b**). The exposed model extract was substantially higher in proanthocyanidin, and the increase was associated with the higher amount of skin proanthocyanidin in the fruit. This result is similar to what we found when comparing wines made from high vigor and low vigor vines (*24*). Interestingly, the shaded treatment percent skin proanthocyanidin extraction of 54% (**Table 7**) was similar to the extraction in high vigor wines (53%), and the exposed treatment skin extraction of 71% was in the same range as the extraction in low vigor zones (70−78%) (*24*). Because of the higher concentration in the fruit, this may have resulted in a greater diffusion gradient in the model extraction although other factors could also play a role.

The anthocyanin concentration was much higher in the exposed model extraction (**Table 8**) than from the shaded treatment even though differences in the fruit were not apparent due to high variability (**Table 4**). In another study on Pinot noir, anthocyanin content was not affected by sun exposure while there was a 60% increase in anthocyanins in wines made from sun-exposed clusters as compared to shaded fruit (*19*). The authors suggested that the difference was related to berry size, which affects juice to skin ratios, and possibly lower accumulation in shaded fruit. In the present study, the exposed treatment had a higher average berry weight. Riper fruit has also been reported to improve extraction of flavonoid compounds into wine (*36*). In this case, there were no obvious differences in ripeness between treatments as determined by soluble solids (**Table 1**). This suggests that there was improved extractability of anthocyanins associated with the exposed treatment.

The anthocyanin composition in the model extractions (**Table 8**) was similar to the pattern found between treatments in the fruit (**Table 4**) although the proportion of Dp, Pt, and Pn decreased somewhat and Mv increased in the model extracts of both treatments. This pattern of change was reported in wine aging (*62*). The rate of reaction for pigmented polymers is related to both the concentration and the composition of anthocyanins, proanthocyanidins, and other cofactors (*63*). In this model extraction, the pigmented polymer concentration was substantially higher in the exposed treatment as compared to the shaded treatment. This may have been due to the higher concentration of proanthocyanidins in the exposed model extract.

Relationships between Vine Vigor, Sunlight Exposure, and Flavonoid Accumulation. Many of the response patterns to shading in this experiment were similar to our findings in the high vine vigor zone in our study on spatial variation although shading throughout the season with boxes may have been more extreme than the levels of shading found in high vigor vines (*24*). However, skin proanthocyanidin concentration and percent skin EGC were lower in the high vigor zone (characterized by a dense, shady canopy) as compared to low vigor zones. When shading was applied to low vigor vines, the same response was found. The variation found in this shading experiment in seed flavan-3-ol monomers was similar to fruit from high vigor vines, which had higher total flavan-3-ol monomers and also less C relative to EC. The pattern of lower anthocyanins and a reduction in the percent Dp in the shaded treatment in the present study are similar to what was observed in high vigor vines (unpublished data). This suggests that these responses in flavonoid accumulation are primarily due to changes in light exposure with limited influence from nutrient or water status.

In summary, the shading treatment in Pinot noir vines resulted in changes in the accumulation and composition of flavonols, skin proanthocyanidins, and anthocyanins. Apparently, there are adaptive advantages to the vine to induce changes in flavonoid biosynthesis particularly in skin tissues in response to UV exposure. Flavonols are likely to play a role in UV screening; however, the role of skin proanthocyanidins has yet to be determined. In addition to these compounds having value to the plant, they are important in wine quality in terms of color stability, astringency, and human health benefits. Skin proanthocyanidins are generally thought to provide an improved mouthfeel in wines as compared to seed-derived proanthocyanidins while flavan-3-ol monomers are reported to have a negative attribute of increasing the bitterness of wine (*64*). The concentration and composition of anthocyanins are important in color stability in wines, and flavonols also play a role in copigmentation (*65*). Increasing our understanding of how vines respond to environmental influences such as light add to our insight into plant secondary metabolite biochemistry and can also have practical applications in vineyard management and wine production.

ABBREVIATIONS USED

C, (+)-catechin; EC, (−)-epicatechin; ECG, (−)-epicatechin-3-*O*-gallate; EGC, (−)-epigallocatechin; DMF, *N*,*N*-dimethylforamide; GPC, gel permeation chromatography; CI, 95% confidence interval; mDP, mean degree of polymerization; Dp,

delphinidin-3-*O*-glucoside; Cy, cyanidin-3-*O*-glucoside; Pt, petunidin-3-*O*-glucoside; Mv, malvidin-3-*O*-glucoside; Pn, peonidin-3-*O*-glucoside; FLS, flavonol synthase.

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