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Influence of Vine Vigor on Grape (*Vitis vinifera* L. Cv. Pinot Noir) and Wine Proanthocyanidins

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The relationships between variations in grapevine (*Vitis vinifera* L. cv. Pinot noir) growth and resulting fruit and wine phenolic composition were investigated. The study was conducted in a commercial vineyard consisting of the same clone, rootstock, age, and vineyard management practices. The experimental design involved monitoring soil, vine growth, yield components, and fruit composition (soluble solids, flavan-3-ol monomers, proanthocyanidins, and pigmented polymers) on a georeferenced grid pattern to assess patterns in growth and development. Vine vigor parameters (trunk cross-sectional area, average shoot length, and leaf chlorophyll) were used to delineate zones within both blocks to produce research wines to investigate the vine−fruit−wine continuum. There was no significant influence of vine vigor on the amount of proanthocyanidin per seed and only minimal differences in seed proanthocyanidin composition. However, significant increases were found in skin proanthocyanidin (mg/berry), proportion of (−)-epigallocatechin, average molecular mass of proanthocyanidins, and pigmented polymer content in fruit from zones with a reduction in vine vigor. In the wines produced from low-vigor zones, there was a large increase in the proportion of skin tannin extracted into the wine, whereas little change occurred in seed proanthocyanidin extraction. The level of pigmented polymers and proanthocyanidin molecular mass were higher in wines made from low-vigor fruit compared to wines made from high-vigor fruit, whereas the flavan-3-ol monomer concentration was lower.

KEYWORDS: Wine; phenolic; flavan-3-ols; proanthocyanidins; precision agriculture; precision viticulture; extraction; trunk cross-sectional area; shoot length; leaf chlorophyll

INTRODUCTION

Fruit composition plays a critical role in the quality of wines. Proanthocyanidins are grape-derived flavonoid compounds specifically important to red wine quality due to their astringent properties (*1*) and their role in long-term color stability (*2*). There is also increasing interest in the potential role that proanthocyanidins have in human health (*3*). Grape-based proanthocyanidins contain the flavan-3-ol subunits (+)-catechin (C), (−)-epicatechin (EC), (−)-epicatechin-3-*O*-gallate (ECG), and (−)-epigallocatechin (EGC) (*4*−*7*) (**Figure 1**). Skin proanthocyanidins differ from those found in seeds in that skins contain prodelphinidins (EGC) and have a higher degree of polymerization and a lower proportion of galloylated subunits (*8*).

Given the complex nature of plant growth, it can be difficult to separate the specific factors that cause changes in fruit composition. Although the relationship between environmental factors and grape composition has been investigated (*9*−*15*), examples are limited (*16*, *17*) in which the essential components that might affect fruit composition have been individually manipulated (i.e., light, heat, water relations, and nutrient content). Specific studies focused on grape seed and skin proanthocyanidin indicate that proanthocyanidins can significantly change in the developing berry (*18*−*21*), yet little is understood about the effect of environmental factors.

A fundamental goal of plant science is to "tease" out the effect that individual environmental factors have on fruit composition so that new and novel approaches to plant improvement can be developed. Although these types of experiments are critical to our understanding, they do not address the complexity in a vineyard where multiple influences exist. Progress in this systems approach to plant improvement has accelerated with the use of precision agriculture tools (*22*, *23*).

Precision agriculture is a production approach that is being used to manage spatial variation in agricultural crops resulting from site environment differences. This approach to crop management uses technologies such as global positioning systems (GPS), remote sensing, and geographical information

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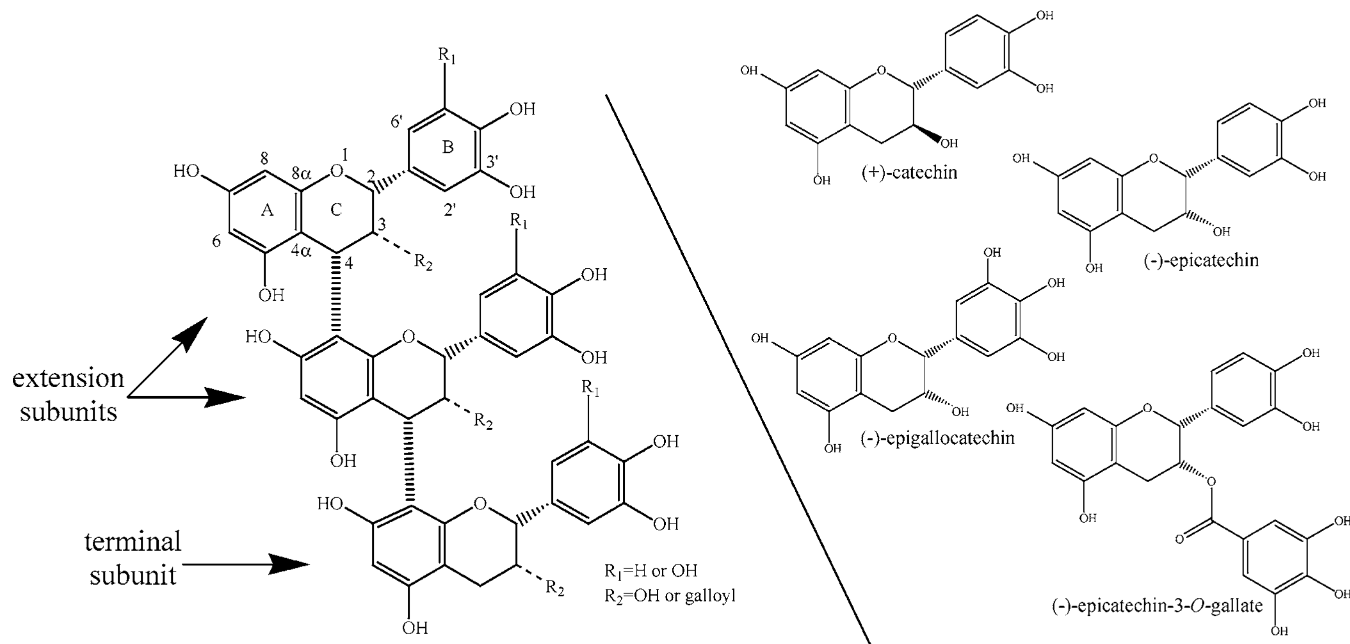

Figure 1. Generalized proanthocyanidin structure and grape-based proanthocyanidin subunits.

systems (GIS) to link novel and traditional on-site measurements (physical, chemical, and biological) to specific locations within a vineyard. With this management approach, crop production decisions become much more focused and targeted. In vineyards, spatial variations in topography, climatic conditions, physical and chemical characteristics of the soil, and pests and diseases have been associated with spatial variations in yield and fruit soluble solids (*22*, *23*). Previous research has found a relationship between canopy structure and sunlight exposure and subsequent fruit phenolics (*24*). In addition, a relationship between variations in vine growth and differences in total phenolic levels (measured as absorbance at 280 nm) has been observed using remotely sensed images (*25*). The assumption in our study was that vigor differences would influence fruit and wine proanthocyanidin chemistry.

The purpose of this study was to investigate proanthocyanidin compositional differences in grapes as influenced by site environment, using georeferenced data to establish a link between the vineyard, fruit composition, and wine. Specifically, there was interest in measuring proanthocyanidin variation in grapes and wine across two vineyard blocks, A and B (**Figure 2**), known to produce wines with distinctly different price points (U.S. $38.00/bottle versus U.S. $75.00/bottle, respectively). These vineyard blocks were in close proximity, under similar management, and the winemaking personnel considered that a significant reason for the price point variation was due to the phenolic composition of the wines. More importantly, this study was designed to investigate how vine vigor influenced proanthocyanidin amount and composition in grapes and wine within a commercial vineyard.

MATERIALS AND METHODS

Vineyard. This study was conducted 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. Vines were planted at a spacing of 1 m (within row) × 2.8 m (between rows) with ~5113 vines/ha (**Figure 2**). The training system was a vertical shoot position with each vine pruned to 10−12 buds. Two vineyard blocks (A and B) were selected for the study and were 1.28 and 0.21 ha, respectively. These blocks were under similar management practices. The vineyard received minimal irrigation post véraison (<150 mm). This research was initiated in April 2003, starting with budbreak.

Soil Measurements. Soil pedons were collected on a grid pattern from three horizons with a sampling density of ~25 soil cores/ha. Horizon descriptions included thickness, structure, texture, color (Munsell color chart), and other pertinent soil morphology. Soils were classified to the soil series level. Available water-holding capacity (AWHC) for each soil pedon was estimated on the basis of soil texture, structure, coarse fragments, and depth to rock. Soil morphology for each horizon was compared to water retention data from the National Soil Survey Laboratory (NSSL) database. The AWHC reported by NSSL is the volumetric difference of water retention between field capacity (−0.033 MPa matric potential) and the permanent wilting point (−1.5 MPa matric potential). Bulk densities from similar pedons in the NSSL database were used. The estimated AWHC for each pedon was calculated as a weighted average in millimeters of water.

Vine Measurements. Data vines were established on a grid pattern in each block (consisting of every 15th vine in every other row, ~220 vines/ha). The location was recorded by both vine and row coordinate and with a GPS, which had a measurement accuracy of ±1 m. The goal was to collect vine growth data between budbreak and véraison in order to divide the blocks into relative vine vigor zones during véraison so that research wines could be produced. Data on average shoot length (June, prior to hedging), estimated leaf chlorophyll content (SPAD-502 m, Minolta) (1 month prior to véraison), and cross-sectional trunk area were collected. A vigor index was calculated using a percent Rank (MS Excel) function on the raw data; next the rank for the three variables was averaged for each data vine, and then a percent Rank function was performed on the average to give a vigor index value for each data vine. Due to a lack of specific information pertaining to this vineyard, these factors were weighted equally. Zones were delineated on the basis of variation in the vigor index and ease of management.

Surface Maps. Surface maps were made using ESRI software (Redlands, CA) with the ordinary kriging utility. Data were originally collected as point data with the spatial attribute for that location recorded along with its central coordinates. A continuous surface map was created by applying the mathematical approach of kriging to interpolate a surface derived from the collected point feature field data. This technique, common to precision agriculture, has been previously applied in vineyards (*22*, *23*). With respect to the kriging, no trend removal was applied, a spherical search radius was used, and the neighborhood was set to include all of the data points.

Figure 2. High-resolution image of the study site with block A (west) and block B (east) highlighted (**a**) and vine vigor index variation (**b**).

Fruit Sampling and Extraction. Fruit samples were collected from the same grid spacing as was used for soil sampling. An additional sample was collected across each vigor zone (three replicates per zone) to reflect the fruit used for wine production. Harvest date was determined by the cooperating winery. Fruit samples were frozen and stored at −35 °C until processed. Frozen berries were removed from the rachis, and samples of 150 berries were randomly collected, weighed, and then processed as previously described (*20*).

Winemaking. Triplicate wines were produced from each vigor zone. For each replicate, 35 kg of fruit was destemmed with a Velo DPC 40 stemmer/crusher operated without the crusher, subjected to a 2.5 day prefermentation cold maceration (10 °C), and then inoculated with Lalvin RC 212 yeast according to the manufacturer's guidelines. On day 2 of fermentation, wines were transferred to a water bath maintained at 32 °C. Wines were punched down two times per day and pressed 6 days after inoculation (bladder-type press, Wilmes, Germany), to a maximum pressure of 2 bar. Wines were transferred into 5-gal carboys. At dryness, wines were inoculated with malolactic bacteria (OSU-1 strain, Lalvin) according to the manufacturer's guidelines. Upon completion of malolactic fermentation, wines were racked and 35 ppm of SO_2 was added, which was followed by 4 weeks of cold stabilization, after which the wines were bottled. The same time/temperature profile was maintained during all fermentations to reflect vineyard-derived differences.

Chemicals. All solvents were of HPLC grade. Acetonitrile, methanol, ethanol, glacial acetic acid, ascorbic acid, potassium metabisulfite, and potassium hydroxide were purchased from J. T. Baker (Phillipsburg, NJ). Phloroglucinol, (+)-catechin, and (−)-epicatechin were purchased from Sigma (St. Louis, MO). Ammonium phosphate monobasic and orthophosphoric acid were purchased from Fisher Scientific (Santa Clara, CA). Hydrochloric acid and sodium acetate anhydrous were purchased from E. M. Science (Gibbstown, NJ) and Mallinckrodt (Phillipsburg, NJ), respectively.

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

Reversed-Phase HPLC of Flavan-3-ol Monomers. Total flavan-3-ol monomer content in grape seed and wine was measured by reversed-phase HPLC using a previously described method (*28*). Aqueous extracts and wines were filtered using Teflon filters (0.45

μm, Acrodisc CR13) before injection. Eluting flavan-3-ol monomers were identified and quantified using C and EC standards.

Phloroglucinolysis. Proanthocyanidin isolates were characterized by acid-catalysis in the presence of excess phloroglucinol followed by reversed-phase HPLC (phloroglucinolysis) using a previously described method (*29*) under modified HPLC conditions (*30*). Phloroglucinolysis provided information on subunit composition, conversion yield, and mean degree of polymerization (mDP). To prepare seed and skin extracts for analysis, 3 mL of aqueous extract was freeze-dried and then dissolved in 5 mL (seed) or 2 mL (skin) of methanol. Equal volumes of the methanolic extracts were combined with the phloroglucinolysis reagent (double strength) before reaction.

For wine proanthocyanidin analysis, an 8-mL wine sample was concentrated under reduced pressure and 40 °C, dissolved in 6 mL of water, and then applied to a C18-SPE column (1-g Alltech) after activation with 10 mL of methanol followed by 15 mL of water. After the sample was applied, the column was washed with 15 mL of water and eluted with 10 mL of methanol. The methanolic solution was divided into two 5-mL samples. One sample was prepared for phloroglucinolysis and the other for gel permeation chromatography (GPC). For phloroglucinolysis, the methanolic sample was evaporated under reduced pressure and 40 °C, reconstituted into 1 mL of methanol, and then treated as described above for seed and skin extracts.

The proportion of seed and skin proanthocyanidin extracted into wine was calculated using a previously described method (*31*). The percent skin proanthocyanidin extracted from the fruit into the wine was calculated on the basis of the ratio of EGC to EC in the fruit and wine for each vigor zone/replicate combination.

GPC. GPC was used to analyze intact tannins (i.e.: intact proanthocyanidins and pigmented polymers). By using GPC, information on the size distribution as well as pigment content (in the case of skin and wine material) could be obtained. The GPC method used has been described previously (*30*). Samples were prepared as described above; however, after drying, they were dissolved in mobile phase. Malvidin-3-glucoside was obtained from Polyphenols Labs (Sandness, Norway) and was used as a standard for GPC analysis at 520 nm, whereas (+)-catechin was used as the quantitative standard at 280 nm.

Bisulfite Bleaching of Wines. Wines were subjected to bisulfite bleaching with SO_2 using a previously described method (*32*).

Statistical Analyses. Statistical analysis of data 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 vine growth, data vines within vigor zones were treated as independent samples. Tukey's adjusted *p* value was used for all specific comparisons and for data with unequal sample sizes. All statistical analyses were performed using SAS version 8.2.

RESULTS AND DISCUSSION

Vine Growth and Yield. Geospatial maps of vine vigor or photosynthetically active biomass (PAB), based upon a relative index (**Figure 2b**) of average shoot length, trunk circumference, and leaf chlorophyll content, were used to delineate high-, medium-, and low-vigor zones within each block so that research wines could be produced (**Figure 3**; **Table 1**). The use of multiple growth measurements based upon a combination of vine−leaf biomass and leaf chlorophyll content has been used to characterize canopy size, density, and vigor (*22*). A multi-parameter approach was also used in this study and included cross-sectional trunk area, which was designed to measure long-term growth response to the site (*26*, *27*).

Average shoot length and leaf chlorophyll content (SPAD units) decreased with decreasing vigor in both blocks. Trunk cross-sectional areas were similar between high- and medium-vigor zones in both blocks, whereas trunks in the low-vigor zone were significantly smaller. The vigor index was significant in separating different levels of vigor in both vineyard blocks (**Figure 2b**; **Table 1**). The vigor index ranged from a high of 0.82 in the A-high zone to a low of 0.09 in the B-low zone (**Table 1**). When each block was delineated into zones representing high, medium, and low vigor, there was a continuum in the vigor index from A-high to B-low. In other studies, relationships have been found between vine measurements in the vineyard (i.e., leaf biomass and leaf chlorophyll content) and fruit yield and composition such as total phenolics and color (*22*−*24*).

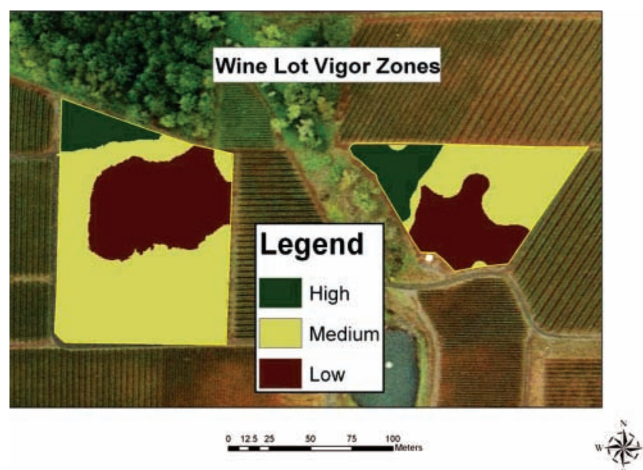

Figure 3. High-resolution image with delineation of wine production vigor zones.

Table 1. Vine Growth and Yield between Blocks and Vigor Zones[a]

block	zone	yield[b] (kg/vine)	length[c] (cm)	CSA[d] (cm^2)	leaf chlorophyll SPAD units[e]	vigor index[f]
A	high	1.07b	122.3a	8.6a	45.4a	0.82a
	med	1.22ab	108.1b	8.9a	41.6b	0.64b
	low	1.36a	98.5c	7.3b	40.1b	0.44cd
B	high	1.08b	108.0b	7.2b	40.3b	0.49c
	med	1.27a	90.9c	7.2b	38.6c	0.35d
	low	0.80c	72.9d	5.0c	34.2d	0.09e
p value		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[a] ANOVA to compare data (*p* indicated): values sharing the same letter within each column are not significantly different at $p \geq 0.05$. [b] Fruit yield. [c] Average shoot length. [d] Trunk cross-sectional area. [f] Combined influence of shoot length, trunk cross-sectional area, and leaf chlorophyll and weighted equally.

Yield variations of up to 10-fold within a vineyard have been associated with fruit composition differences (*23*). In our study, there was a 40% reduction in yield (A-low vs B-low, **Table 1**); however, vigor variation did not vary linearly with yield. The medium-vigor zones had the highest yield, whereas vigor extremes were lower yielding (**Table 1**). B-low had a significant yield reduction, and on the basis of the observed stunted shoot growth and basal leaf senescence, it appeared that stress contributed to yield reduction. The yield reduction in A-high may have been due to reduced bud fruitfulness and/or reduced fruitset.

In general, berry weight increased with vigor (**Table 2**). The only significant difference in berry weight, however, was in comparing A-high with B-low. where B-low was 0.21 g/berry lower [Tukey adjusted $p = 0.0053$ (CI; 0.063, 0.35)]. On the basis of the surface area-to-volume ratio, berry weight is generally thought to influence wine phenolic concentration (*33*), although phenolic concentration has been shown to vary independently of berry size (*34*).

Analysis of Seed. In general, there were greater numbers of seeds per berry in low-vigor zones compared to high-vigor zones (**Table 2**). Dry seed mass per berry showed a similar trend (data not shown).

Table 2. Average Berry Weight, Soluble Solids, and Seeds per Berry between Vine Vigor Zones[a]

block	zone	av berry wt (g)	soluble solids (°Brix)	seeds per berry
A	high	0.99a	23.5d	1.31c
	med	0.91ab	24.3a	1.37bc
	low	0.87bc	24.1b	1.56a
B	high	0.82bc	23.7c	1.45abc
	med	0.87bc	24.0b	1.50ab
	low	0.78c	24.4a	1.59a
p value		0.0079	<0.0001	0.0040

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

Although there was an overall reduction in total flavan-3-ol monomers per seed with a reduction in vigor (**Table 3**), there was no significant difference when calculated on a per berry basis. This is due to the higher number of seeds per berry in the low-vigor zones (**Table 2**). The seed flavan-3-ol monomers observed included C and EC, with approximately twice as much C as EC. There were also differences in the proportion of C and EC with respect to vigor, where C increased proportionally with decreasing vigor (**Table 3**). It has been observed that during fruit ripening the amount of flavan-3-ol monomer declines and the proportion of C declines (*19*−*21*). That the vigor zones with the lowest overall flavan-3-ol monomer amounts generally had a higher proportion of C suggests that differences in flavan-3-ol monomer were not ripening related.

A slight increase in per-seed proanthocyanidin was seen in A-high and A-medium zones (**Table 3**); however, when calculated on a per-berry basis, there were no significant differences. Overall, environmental factors have been found to have limited influence on seed proanthocyanidin amount. This includes vine water status (*20*, *34*) and light exclusion (*17*). In another study comparing seed proanthocyanidins in three varieties (Cabernet Sauvignon, Syrah, and Pinot noir), the major contributing factor to the difference in total seed proanthocyanidin per berry was the number of seeds rather than the amount of proanthocyanidin per seed (*35*). Our results agree with previous research and suggest that seed proanthocyanidin accumulation is not highly responsive to environmental influences.

For proanthocyanidin composition, there was no apparent pattern in proportion of C, EC, and ECG terminal subunits. However differences were found in the proportion of extension subunits, where C increased in proportion with a reduction in vigor and EC and ECG decreased. Overall, the results of this study indicate that the amount of grape seed proanthocyanidins was independent of vine vigor, whereas differences in composition were found.

Analysis of Skins. Very little research has been done on skin proanthocyanidins in comparison to seed proanthocyanidins as they are generally more difficult to analyze due to the presence of interfering sugars and other phenolics. Due to low flavan-3-ol monomer concentrations observed in this study as well as others (*36*, *38*), these components were not quantified.

By phloroglucinolysis, per-berry (**Figure 4a**; **Table 4**) and per-berry-weight proanthocyanidin amount increased substantially in skins with decreasing vine vigor. B-low had an increase of 71% in total extension subunits compared to A-high ($p = 0.0014$, $n = 3$). In comparing A-high with B-low, there was an ∼23% increase in terminal subunits ($p = 0.0003$, $n = 3$). The increase in extension subunits relative to terminal subunits suggests a corresponding increase in molecular weight.

By GPC (**Table 5**) a significant increase was also found in total tannin amount (mg/berry) in both blocks with decreasing vine vigor, consistent with results observed by phloroglucinolysis (**Table 4**). The greatest increase was between A-high and B-low, where there was an ∼69% increase in total tannin (mg/berry) (**Table 5**). Small differences in total tannin amount at harvest have been observed with respect to light exposure (*17*) and vine water status (*37*).

Skin extension subunits consisted of C, EC, ECG, and EGC, in agreement with others (*5*, *21*, *36*). EC and EGC were the

Table 3. Seed Flavan-3-ol Monomer and Proanthocyanidin Concentration and Percent Composition Analysis by Phloroglucinolysis[a]

Flavan-3-ol Monomers

block	zone	monomer[b] (nmol/seed)	C[c] %	EC[c] %
A	high	1613.3a	62.3b	37.7a
	med	1440.9ab	63.1b	36.9a
	low	1300.7b	67.2ab	32.8ab
B	high	1366.5ab	66.4ab	33.6ab
	med	1494.6ab	71.6a	28.5b
	low	1288.7b	71.5a	28.5b
p value		0.15	0.0134	0.0134

Procyanidin

		concentration[b]			extension[c]			terminal[c]		
block	zone	extension (nmol/seed)	terminal (nmol/seed)	total (nmol/seed)	C %	EC %	ECG %	C %	EC %	ECG %
A	high	6205.4a	1733.5a	7938.9a	12.0c	76.2a	11.7a	53.2ab	33.9a	12.9b
	med	6268.5a	1516.7a	7785.1a	13.8bc	75.2ab	11.0ab	43.9b	39.0a	17.1a
	low	6027.9ab	1637.8a	7665.6ab	15.2ab	74.3ab	10.5bc	54.9ab	31.8a	13.3ab
B	high	5200.7b	1288.1a	6488.9b	15.5ab	74.4ab	10.1bc	54.6ab	32.0a	13.4ab
	med	5940.2ab	1712.9a	7653.1ab	16.5a	73.4b	10.1c	58.5a	30.2a	11.4b
	low	5601.7ab	1480.6a	7082.3ab	16.5a	73.8b	9.7c	57.9ab	29.5a	12.6b
p value		0.1611	0.2146	0.1441	0.0031	0.0976	0.006	0.2608	0.373	0.1316

[a] ANOVA to compare data (*p* indicated): values sharing the same letter within each column are not significantly different at $p \geq 0.05$. [b] Flavan-3-ol monomer or procyanidin concentration. [c] Molar proportion, and with the following subunit abbreviations: C, (+)-catechin; EC, (−)-epicatechin; ECG, (−)-epicatechin-3-*O*-gallate.

Table 4. Skin Proanthocyanidin Concentration and Percent Composition Analysis by Phloroglucinolysis[a]

block	zone	extension[b] (nmol/berry)	terminal[c] (nmol/berry)	mDP	total[d] (nmol/berry)	C[e] %	EC %	EGC %	ECG %
A	high	2002.9c	74.4c	27.95c	2077.3c	2.2a	71.9a	24.4c	1.6a
	med	2453.6cb	69.4c	36.23ab	2523.0cb	2.2a	68.9b	27.2b	1.7a
	low	3439.0a	107.3a	33.04b	3546.3a	2.2a	66.8bc	29.2ab	1.8a
B	high	2892.4ab	83.0bc	35.71ab	2975.4ab	1.7b	66.6bc	30.0a	1.7a
	med	3331.5a	95.9ab	35.78ab	3427.3a	2.1a	65.7c	30.7a	1.6a
	low	3459.5a	91.8b	38.71a	3551.3a	2.3a	65.1c	30.8a	1.8a
p value		0.0014	0.0003	0.0028	0.0014	0.0374	0.0018	0.0017	0.448

[a] ANOVA to compare data (*p* indicated): values sharing the same letter within each column are not significantly different at $p \geq 0.05$. [b] Proanthocyanidin extension subunit concentration. [c] Proanthocyanidin terminal subunit concentration. [d] Proanthocyanidin concentration. [e] Extension subunit molar proportion, and with the following subunit abbreviations: C, (+)-catechin; EC, (–)-epicatechin; EGC, (–)-epigallocatechin; ECG, (–)-epicatechin-3-*O*-gallate.

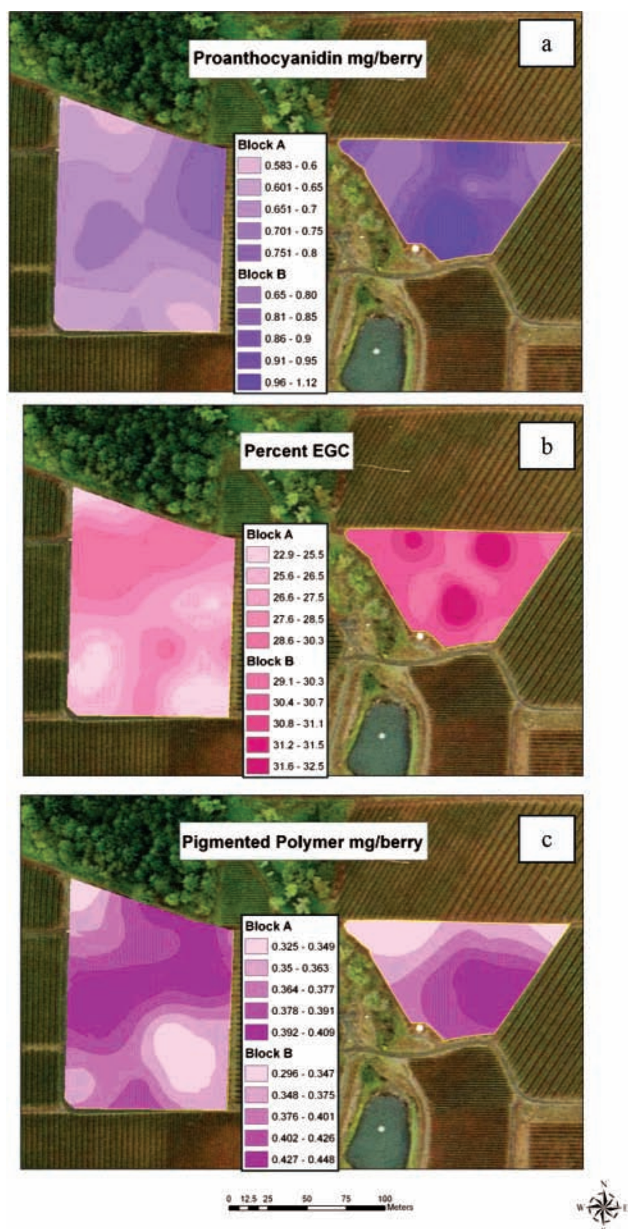

Figure 4. Grape skin proanthocyanidin chemistry including concentration of grape skin proanthocyanidins (**a**), and percent (–)-epigallocatechin extension subunits (**b**), by phloroglucinolysis, as well as incorporation of 520 nm absorbing material or pigmented polymer by GPC (**c**).

primary extension subunits, also in agreement with other studies (*5*, *21*, *36*, *37*). C was the only terminal unit observed, and it was not differentiated from possible C monomers. No difference in the response of C or ECG proportion to vigor was observed. However, the proportion of EGC increased and EC proportion decreased with a reduction in vine vigor (**Table 4**). A similar pattern was observed in the surface map of percent EGC (**Figure 4b**). When the extremes in vine vigor zones were compared, there was an EGC increase of 6.4% [Tukey adjusted $p = 0.0023$ (CI; 2.50, 10.73)] in B-low compared to A-high. Calculated on a nanomoles per berry basis instead of percent, this was an ~2-fold increase (from A-high to B-low) in EGC containing a trihydroxylated B-ring. In previous research, a shift was found toward a decrease in trihydroxylation compared to dihydroxylation of the B-ring with cluster shading (*17*). This suggests the substitution pattern on the B-ring may be influenced by differences in fruit sun exposure. In addition to environmental factors (*17*, *37*), fruit maturity appears to have an influence on EGC proportion (*21*, *36*). In this study, it could not be determined if the differences observed in proanthocyanidin proportion were due to maturity, the environment, or a combination of these effects.

Table 5. Skin Tannin Analysis by Gel Permeation Chromatography[a]

block	zone	molecular mass 50% (g/mol)	tannin (mg/berry)	pigmented polymer (mg/berry)
A	high	9915d	1.15d	0.32d
	med	10680c	1.34cd	0.44bc
	low	11183bc	1.79ab	0.48abc
B	high	12224a	1.52bc	0.39cd
	med	11258b	1.69ab	0.49ab
	low	11517b	1.94a	0.56a
p value		<0.0001	0.0026	0.010

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

By phloroglucinolysis, the mDP for A-high was lower than that for B-low by 10.76 [Tukey adjusted $p = 0.002$ (CI; 4.19, 16.88)]. The difference between the extremes was more obvious than for the intermediate levels of vigor. In other work, the molecular weight of skin proanthocyanidin has been found to increase with maturity (*5*, *36*, *37*). Skin mDP has been observed to increase during the early phase of berry development but then decrease after véraison (*21*). Downey et al. (*17*) found a decrease in skin proanthocyanidin mDP in shaded fruit. The observation of greater sun exposure in the fruiting zones of low-vigor vines could explain the increase in mDP in these geographical regions in the vineyard. Another possible explanation is that the apparent mDP increase in fruit from low-vigor

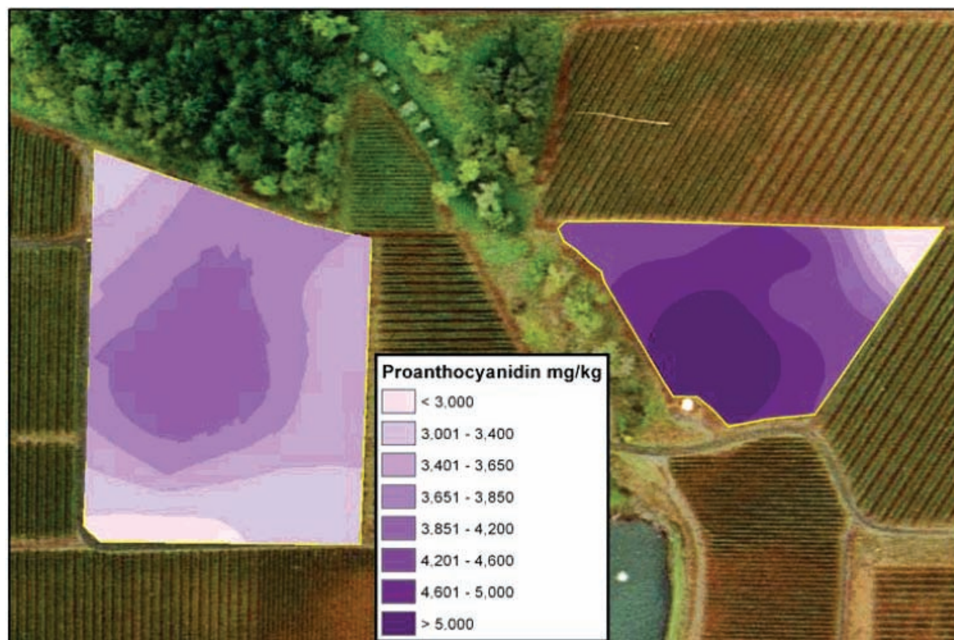

Figure 5. Surface map of total (seed + skin) proanthocyanidin (mg/kg) in fruit at harvest by phloroglucinolysis.

vines is related to differences in ripening. This explanation seems less likely given the minimal differences in soluble solids (**Table 2**).

By GPC, the molecular mass at 50% elution increased with a reduction in vigor (**Table 5**). This is consistent with the difference in mDP by phloroglucinolysis. Using a similar analytical procedure, the proportion of high molecular weight material has been observed to increase with berry development (*36*).

GPC was used to determine pigmented polymer content in grape skins (**Figure 4c**; **Table 5**). In this experiment, there was an ~75% increase in per-berry pigmented polymer content from A-high to B-low ($p = 0.001$, $n = 3$). Although the presence of pigmented polymer in the grape has been observed previously (*36*, *37*), its origin is not clear. Recent evidence suggests that pigmented polymers may include oligomeric anthocyanins (*39*). However, the presence of pigmented compounds could also be an artifact of sample preparation and extraction.

Summary of Grape Phenolics. In this study, seed phenolics were minimally affected by changes in vine vigor. Hence, only geospatial maps for skin proanthocyanidin composition are included (**Figure 4**). An apparent relationship was observed between vine vigor (**Figure 2b**) and the concentration of skin proanthocyanidin (mg/berry, **Figure 4a**), percent skin EGC extension subunits (**Figure 4b**), and pigmented polymers (mg/berry, **Figure 4c**). The findings of this study, showing minimal differences in seed proanthocyanidin while there were substantial variations in skin proanthocyanidin in response to vine vigor, agree with previous findings on a differential response between seed and skin proanthocyanidins (*17*). In this particular study, that used light exclusion boxes in cv. Shiraz (*17*), much smaller differences were found in grape seed relative to skin proanthocyanidins.

Total skin and seed proanthocyanidin (mg/kg) was determined to assess the potential proanthocyanidin available for extraction into wine (**Figure 5**). In this study, total (skin plus seed) proanthocyanidin (mg/kg) increased in response to reduced vine vigor, as can be seen in the surface map where zones containing low-vigor vines had higher proanthocyanidin amounts than higher vigor regions (**Figure 5**). Total proanthocyanidin amount in the fruit increased ~50% when A-high was compared with B-low ($p = 0.0063$, $n = 3$), thus indicating an apparent relationship between vine growth parameters and the accumulation of proanthocyanidins in the fruit in this study. In addition to the initial amount of proanthocyanidin present in the fruit, conditions during winemaking are also important in determining the eventual amount of skin and seed proanthocyanidin extracted into wine.

Analysis of Wines. A major objective of this study was to focus on the effect of vine vigor on wine proanthocyanidin amount and composition and, therefore, every attempt was made to maintain consistent fermentation conditions across all wines (similar maceration time, temperature, and pressing).

An increase in flavan-3-ol monomers in wines was observed with an increase in grapevine vigor (**Table 6**). In a comparison of A-high to B-low, A-high had a 0.06 mM [Tukey adjusted $p = 0$ 0.0001 (CI; 0.03, 0.09)] increase in monomer concentration compared to B-low. The proportion of C and EC was similar to the relationship found in seeds (**Table 3**), although there was an ~10−20% increase in catechin compared to epicatechin in the wine (**Table 6**). This increase in the proportion of C in wine relative to seed has been observed in other studies (*40*, *41*). Potential explanations for this observation include differences in localization in seed tissue, differential extraction, and reactivity (i.e., rate of flavan-3-ol monomer epimerization and proanthocyanidin hydrolysis). The majority of flavan-3-ol monomers are likely to come from the seeds due to the low amounts found in the skin. In terms of relative importance in wine, the monomer fraction accounted for only between 7% (B-low) and 20% (A-high) of the total flavan-3-ol fraction (**Table 6**). This is similar to other research in which the polymeric fraction in wines represented 75−81% of total flavan-3-ols in seeds and 94−98% in skins (*38*). The presence of low molecular weight flavanols may be important in terms of increasing the perception of bitterness in wine (*8*).

Overall, there appeared to be a relationship between the total proanthocyanidin (skin plus seed) concentration in the fruit expressed by weight (**Figure 5**) and the proanthocyanidin concentration in the wines (**Table 8**). This is of interest because winemakers in general would like to develop a means to predict

Table 6. Wine Flavan-3-ol Monomer and Proanthocyanidin Concentration and Composition by Phloroglucinolysis[a]

Flavan-3-ol Monomers

block	zone	monomer (mM)	C %	EC %
A	high	0.18a	77.3c	22.7a
	med	0.17ab	75.7c	24.3a
	low	0.16ab	77.6c	22.4a
B	high	0.13c	84.0b	16.1b
	med	0.12c	86.6a	13.4c
	low	0.12c	88.0a	12.0c
p value		<0.0001	<0.0001	<0.0001

Proanthocyanidins

		concentration			extension				terminal	
block	zone	extension (mM)	terminal (mM)	total (mM)	C %	EC %	ECG %	EGC %	C %	EC %
A	high	0.64d	0.08c	0.72d	5.0ab	78.2a	4.8a	12.0d	73.0a	27.0a
	med	0.95c	0.09bc	1.05c	3.9b	76.1b	3.1b	16.9c	86.9a	13.1a
	low	1.24b	0.15a	1.39b	4.1ab	74.4bc	2.1c	19.4b	78.2a	21.8a
B	high	1.20b	0.13ab	1.33b	5.3a	73.6cd	0.9d	20.3b	74.1a	25.9a
	med	1.20b	0.11abc	1.31b	4.6ab	72.1ed	0.7d	22.7a	66.3a	33.7a
	low	1.46a	0.13ab	1.59a	4.2ab	71.3e	0.7d	23.8a	67.4a	32.6a
p value		<0.0001	0.0358	<0.0001	0.2006	0.0001	<0.0001	<0.0001	0.3772	0.3772

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

Table 7. Extraction of Skin and Seed Proanthocyanidin into Wine As Determined by Phloroglucinolysis[a]

block	zone	total proantho-cyanidin (mg/L)	skin extracted (%)	skin proantho-cyanidin (mg/L)	seed proantho-cyanidin (mg/L)
A	high	268.6d	52.8d	142.0c	127.04ab
	med	361.9c	64.3c	232.9b	129.0ab
	low	457.6ab	68.0bc	311.1a	146.5a
B	high	432.8b	70.0ab	319.5a	113.3b
	med	423.5b	75.3ab	307.4a	116.1ab
	low	504.3a	77.9a	387.9a	116.3b
p value		<0.0001	<0.0001	<0.0001	0.1885

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

Table 8. Wine Tannin Analysis by Gel Permeation Chromatography and Bisulfite Bleaching[a]

block	zone	tannin (mg/L)	mol mass 50% elution (g/mol)	pigmented polymer (mg/L)	sulfite-resistant pigment
A	high	1040e	1146.5c	632e	0.94f
	med	1340d	1235.5c	844d	1.28e
	low	1586c	1506.2b	1090b	2.04d
B	high	1611c	1478.3b	989c	1.59c
	med	1792b	1751.3a	1223b	2.56b
	low	2051a	1778.4a	1459a	3.30a
p value		<0.0001	<0.0001	<0.0001	<0.0001

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

wine tannin amount and composition from fruit analysis. There was a 120% increase in wine total proanthocyanidin subunit concentration from A-high to B-low ($P = <0.0001$, $n = 3$, **Table 6**). The observed increase in extension subunit concentration was greater than the increase in terminal subunits, suggesting an increase in proanthocyanidin average molecular weight in wine with a reduction in vine vigor. There was a slightly greater than 3-fold increase in galloylated derivatives between the A-high and B-low wines on a molar basis. In a study investigating the effect of fruit ripeness on wines, an increase in galloylation was found in wines made from grapes that were harvested last (*42*).

A direct relationship between the distribution of seed and skin proanthocyanidins in fruit and those in wine does not exist (*31*). Given the apparent differences in sensory properties between seed and skin proanthocyanidins in wine (*8*), it is of interest to better understand the relationship between fruit proanthocyanidin distribution in fruit and differential extraction into wine. On the basis of seed and skin subunit analysis, seed proanthocyanidin extraction into wine remained relatively constant with vigor (**Table 7**). The proportion and amount of EGC in wine increased, indicating that skin proanthocyanidin extraction increased dramatically with a decrease in grapevine vigor (**Table 7**; **Figure 6**). Vigor zone B-low had 246 mg/L more skin proanthocyanidin than A-high [Tukey adjusted $p = <0.0001$ (CI; 205, 287)]. This agrees with previously reported results that the subunit composition of wine proanthocyanidins resembled the profile found in skins more than that of the seeds, particularly due to the presence of EGC extension subunits (*38*). In wine there was a trend toward an increase in mDP in block B with decreasing vigor but not in block A (**Table 8**). However, molecular mass at 50% elution determined by GPC showed an increase in wine proanthocyanidin molecular size with decreasing vigor (**Table 8**).

There was a strong relationship between the vigor index, proanthocyanidin production in the grape, and resulting proanthocyanidin concentration in the wines (**Figure 7**). There was almost a 2-fold increase in total proanthocyanidin in wines made from the B-low-vigor zone compared to wines made from the A-high-vigor zone (**Table 7**). Although there was a strong relationship overall (**Figure 7c**), the relationship appears to be driven by the skin proanthocyanidins (**Figure 7b**) as opposed to the seed procyanidins (**Figure 7a**). In total, ∼9% of total proanthocyanidins were extracted from the grape across all vigor zones ($r^2 = 0.87$). When only skin proanthocyanidins were

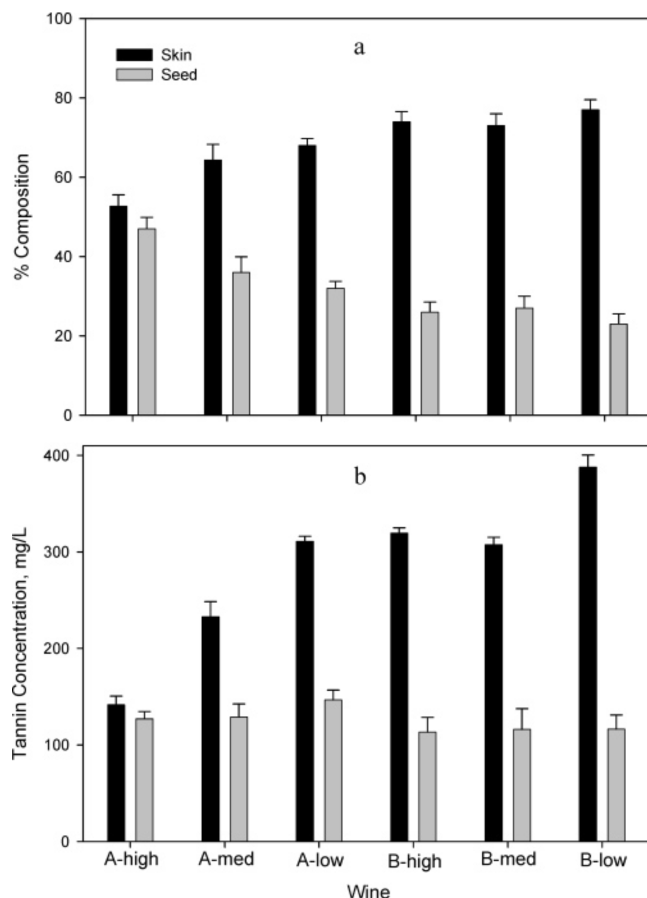

Figure 6. Percent composition (**a**) and concentration (**b**) of skin and seed proanthocyanidins in red wines made from grapes sourced from different vigor zones, and with error bars indicating ±SEM ($n = 3$).

considered, extraction increased (22−29%) with a stronger correlation ($r^2 = 0.90$). Overall, these data suggest that wine proanthocyanidin composition is driven by the amount of proanthocyanidin material present in the fruit (assuming constant winemaking), and that the overall extraction of skin proanthocyanidins increased with a reduction in vigor (22% and 29% extraction for A-high and B-low, respectively).

The pigmented polymer concentration in wine was determined by GPC (**Table 8**). As described previously, there was an ∼75% increase in pigmented polymers in the grape skins with decreasing vigor (A-high to B-low, **Table 5**). In wine, this difference was greater (than found in skins) with a 2-fold increase when A-high was compared to B-low (**Table 8**). Overall, these quantities seemed to be quite high compared with previous work (*43*), and upon comparison of the results with those by reversed-phase HPLC, it was realized that the response of the standard to the GPC conditions was different from that for the pigmented polymer. Specifically, the flavylium form of malvidin-3-glucoside was less stable in DMF than the pigmented polymer; consequently, the quantity of pigmented polymer was overestimated. Nevertheless, the trends across vigor zones were similar when GPC results were compared with bisulfite-resistant pigments (**Table 8**). Furthermore, the strong relationship between pigmented polymer by GPC and bisulfite-resistant pigment ($r^2 = 0.97$) suggests that although questions remain with regard to the nature and source of pigmented polymers in grapes (artifact or not), evidence from different analytical approaches is consistent and therefore at least predictive in understanding the relationship between vine vigor and pigmented polymer in this study.

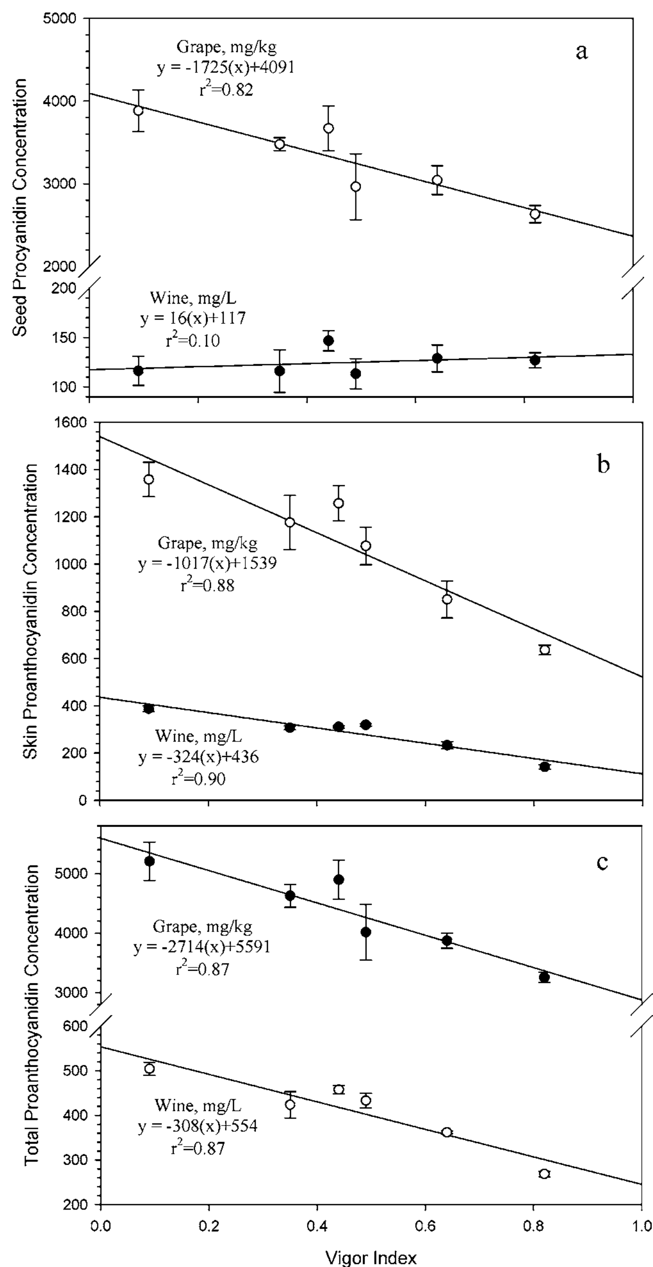

Figure 7. Concentration of proanthocyanidins in seed (**a**), skin (**b**), and skin plus seed (**c**) in grapes at harvest and in the corresponding wine, and with error bars indicating ±SEM ($n = 3$).

Summary. In this study, there was a much greater influence of vine vigor on skin proanthocyanidin accumulation compared to seed proanthocyanidins. In particular, the total amount of skin proanthocyanidin, proportion of EGC extension subunits, and pigmented polymer concentration significantly increased with decreasing vigor. It is possible these differences are related to an increase in light and/or heat exposure in the canopy or other environmental factors. Previous studies have shown an increase in total phenolics with an increase in light exposure; however, this is the first time proanthocyanidin compositional differences have been strongly connected to differences in vine vigor. Additional experiments are being conducted to investigate the influence of light on the compositional differences in skin and wine proanthocyanidins.

The use of georeferenced data was beneficial in developing our understanding of the link between the site environment, vine growth, fruit composition, and wine. The differences found in proanthocyanidin quantity and composition has possible rami-

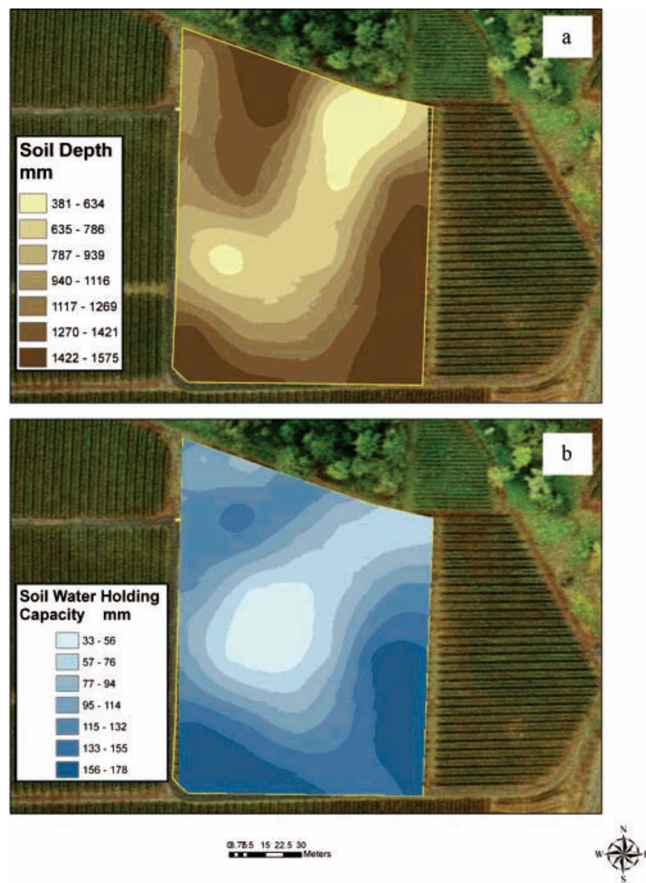

Figure 8. Surface map of soil depth (**a**) and corresponding water-holding capacity (**b**) for block A.

fications related to wine quality. For example, skin proanthocyanidins and pigmented polymers in wines are considered to have an affect on proanthocyanidin perception (*8*, *44*). However, proanthocyanidin composition is only one aspect of wine quality, and it is likely that differences in vine vigor can influence other factors as well.

In this study, it was possible to determine chemical compositional differences in proanthocyanidins from both the fruit and wine from two blocks that were considered by the winemaker to produce wine of differing qualities. This paper provides evidence for the importance of site environment related variations in fruit phenolic composition on wine chemistry. However, further research is necessary to develop the practical applications in vineyards. Future research goals include the following: (1) reducing the time needed to divide blocks by vine vigor and wine composition; (2) developing rapid vineyard fruit sampling assessment techniques; and (3) utilizing these results to modify vineyard practices to produce fruit to specification. To reduce analysis time, the use of high-resolution images is a preferred choice that is being investigated by several researchers (*22*−*24*). One possible goal would be to reduce variability; however, an understanding of the causal relationship between growing conditions and variation in vine vigor needs to be determined. In this study, soil analysis provides an explanation for the differences observed in vine vigor (**Figure 8**) in that a strong association between soil depth and corresponding water-holding capacity and vine vigor was observed. The relationship between soil water-holding capacity and vine growth is particularly important in vineyards receiving little or no irrigation. Differences in soil water-holding capacity can have a direct effect on vine vigor and an indirect effect on the vine microclimate in terms of sunlight exposure and temperature. These influences can, in turn, modify the accumulation of phenolic compounds in the fruit. In summary, this research improves our understanding of the relationships between vineyards and wine chemistry and provides justification for continued research toward understanding the differences in plant response to environment in terms of fruit-ripening biochemistry.

ABBREVIATIONS USED

C, (+)-catechin; EC, (−)-epicatechin; ECG, (−)-epicatechin-3-*O*-gallate; EGC, (−)-epigallocatechin; PA, precision agriculture; GPC, gel permeation chromatography; mDP, mean degree of polymerization; AWHC, available water-holding capacity; NSSL, National Soil Survey Laboratory; CI, 95% confidence interval.

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