AN ABSTRACT OF THE DISSERTATION OF

Jessica M. Cortell for the degree of Doctor of Philosophy in Food Science and Technology, presented on September 5, 2006.
Title: Influence of Vine Vigor and Shading in Pinot noir (*Vitis vinifera* L.) on the Concentration and Composition of Phenolic Compounds in Grapes and Wine.

Abstract approved: _____
James A. Kennedy

In 2003 and 2004 vigor zones were delineated in two commercial vineyard sites based on vine growth variation to assess fruit and wine phenolic chemistry. In 2004, the effects of shading on the accumulation of phenolic compounds were also investigated. Model extractions were done from the shading experiment and the vigor zones in 2004. Wine and extracts were analyzed by HPLC and spectrophotometry.

Berry weight, dry skin weight, °Brix, pH and anthocyanin content were higher and titratable acidity and the proportion of malvidin-3-*O*-glucoside was lower in 2003 compared to 2004. High vigor zones had lower °Brix and higher titratable acidity and a trend for lower anthocyanin content per berry in both years. Site A had proportionally higher peonidin and lower malvidin than site B. While there were minimal differences in seed proanthocyanidin, large increases were found in low vigor zones for skin proanthocyanidin, proportion of (-)-epigallocatechin, and pigmented polymer content in fruit. In 2004, the shade treatment had lower

accumulation of flavonols, lower skin proanthocyanidin, minimal differences in anthocyanins, a large proportional increase in peonidin glucosides, and proanthocyanidin compositional differences. The model extractions from the shade experiment paralleled treatment differences in the fruit except that skin proanthocyanidin percent extraction was found to be ~ 17% higher in the exposed treatment.

For the vigor zone model extractions, there were no differences in pomace weight. Site A model extracts tended to have a higher anthocyanin concentration and a lower proportion of malvidin-3-*O*-glucoside than those from site B. The 2003 wines had a higher anthocyanin concentration and a lower proportion of malvidin-3-*O*-glucoside than in 2004. The same response was seen in the fruit. The medium vigor zone wines had higher anthocyanin concentrations than either high or low vigor zones. In both years, there were higher proportions of delphinidin and petunidin glucosides in wines made from low vigor zone fruit. Low vigor zone wines had ~ a two-fold increase in pigmented polymer concentration, a large increase in the proportion of skin proanthocyanidin, greater sulfite resistant pigments, higher color density and lower flavan-3-ol monomer concentration. Differences found in the wines magnified variation in the fruit.

Influence of Vine Vigor and Shading in Pinot noir (*Vitis vinifera* L.) on the Concentration and Composition of Phenolic Compounds in Grapes and Wine.

by
Jessica M. Cortell

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented September 5, 2006
Commencement June 2007

Doctor of Philosophy dissertation of Jessica M. Cortell presented on September 5, 2006.

APPROVED:

Major Professor, representing Food Science & Technology

Head of the Department of Food Science & Technology

Dean of the Graduate School

I understand that my dissertation will become a part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Jessica M. Cortell, Author

ACKNOWLEDGEMENTS

The author wishes to express appreciation for the support of my major professor Dr. James A. Kennedy and committee members including Dr. Ronald Wrolstad, Dr. Barbara Bond, Dr. Timothy Righetti and Dr. Larry Burt. The author acknowledges the contributions of project collaborators Dr. Timothy Righetti, Dr. Andrew Gallagher, Dr. Paul Schreiner, Michael Halbleib, and Anne Connelly. The author acknowledges the participation and contributions of the cooperating wineries, Archery Summit and Chehalem and the personnel Leigh Bartholomew, Anna Matzinger, Harry Peterson Nedry, Mike Eyres and Michael Davies. In addition, the author would like to acknowledge the hard work of students Jose Pastor del Rio, Fiorella Cerpa, Carmen Boone, Jason Bell, and Chris Zielinski.

CONTRIBUTION OF AUTHORS

Dr. Timothy Righetti helped with the use of global positioning systems (GPS) and data analysis. Michael Halbleib provided aerial photography and surface maps of data. Dr. Andrew Gallagher provided support for vineyard soil sampling and analysis.

TABLE OF CONTENTS

Page

Introduction 1

IMPORTANCE OF PHENOLIC COMPOUNDS 1

Description of anthocyanins 1

Description of proanthocyanidins 4

Description of flavonols 5

BERRY DEVELOPMENT 9

BIOSYNTHESIS OF PHENOLIC COMPOUNDS 11

Accumulation of anthocyanins 11

Accumulation of proanthocyanidins in grape skins and seeds 13

Accumulation of flavonols 15

INFLUENCES ON ACCUMULATION OF PHENOLIC COMPOUNDS 16

INFLUENCES ON COMPOSITION OF PHENOLIC COMPOUNDS 22

EXTRACTION OF PHENOLIC COMPOUNDS INTO WINE 24

FORMATION OF PIGMENTED POLYMERS 27

WINE ASTRINGENCY AND BITTERNESS 31

RESEARCH JUSTIFICATION AND OBJECTIVES 33

TABLE OF CONTENTS (Continued)

Influence of Vine vigor on Grape (*Vitis vinifera* L. Cv. Pinot noir) and wine proanthocyanidins 35

ABSTRACT 36

INTRODUCTION 37

MATERIALS AND METHODS 41

RESULTS AND DISCUSSION 47

ABBREVIATIONS USED 73

ACKNOWLEDGMENTS 73

LITERATURE CITED 74

Influence of Vine Vigor on Grape (*Vitis vinifera* L. Cv. Pinot noir) Anthocyanins: Part I. Anthocyanin Concentration and Composition in Fruit 79

ABSTRACT 80

INTRODUCTION 81

MATERIALS AND METHODS 84

RESULTS AND DISCUSSION 89

ABBREVIATIONS USED 116

ACKNOWLEDGMENT 116

LITERATURE CITED 117

TABLE OF CONTENTS (Continued)

Influence of Vine Vigor on Grape (*Vitis vinifera* L. Cv. Pinot noir) Anthocyanins: Part II. Anthocyanins and Pigmented Polymers in Wine.....123

ABSTRACT.....124

INTRODUCTION.....125

MATERIALS AND METHODS.....128

RESULTS AND DISCUSSION.....131

ABBREVIATIONS USED.....172

ACKNOWLEDGMENT.....172

LITERATURE CITED.....172

Effect of shading on accumulation of flavonoid compounds in (*Vitis vinifera* L.) Pinot noir fruit and extraction in a model system.....178

ABSTRACT.....179

INTRODUCTION.....180

MATERIALS AND METHODS.....185

RESULTS.....189

DISCUSSION.....204

ABBREVIATIONS USED.....220

ACKNOWLEDGMENT.....220

LITERATURE CITED.....220

TABLE OF CONTENTS (Continued)

Influence of Vine Vigor on Pinot noir Fruit Composition,
Wine Chemical Analysis and Wine Sensory Attributes.....227

ABSTRACT 228

INTRODUCTION.....229

VINEYARD AND CHEMICAL ANALYSES
MATERIAL AND METHODS 232

SENSORY ANALYSIS MATERIALS AND
METHODS.....236

RESULTS 240

DISCUSSION.....254

ABBREVIATIONS USED.....259

ACKNOWLEDGMENT 259

LITERATURE CITED 260

Conclusions 264

Bibliography 270

LIST OF FIGURES

Page

Figure 1.1. Structure of anthocyanins found in Pinot noir grapes. 2

Figure 1.2. Equilibrium among the four structure groups of anthocyanins. 3

Figure 1.3. Structures of flavan-3-ols found in grape seed and skins 6

Figure 1.4. Structure of flavan-3-ol monomers and condensed proanthocyanidins 7

Figure 1.5. Structures of common flavonols found in grape skins. 9

Figure 1.6. Diagram of berry development showing relative size and color of berries from flowering through ripeness, periods when compounds accumulate, the levels of juice °brix, rate of xylem and phloem inflow. Illustrated by Jordan Koutroumandis, Winetitles. 10

Figure 1.7. 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, 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; MT, methyltransferase 13

Figure 1.8. Diagram showing relationships among vineyard site environment and management practices on fruit composition and wine quality (Jackson and Lombard 1993). 17

Figure 1.9. Structure of pigment formed by addition of pyruvic acid to malvidin 3-O-glucoside 28

Figure 1.10. Structure showing an ethyl bridge 29

Figure 1.11. A-T pigmented polymer formed through direct condensation of malvidin-3-O-glucoside and flavan-3-ol subunits. 30

LIST OF FIGURES (Continued)

Figure 2.1. Generalized proanthocyanidin structure and grape-based proanthocyanidin subunits. 38

Figure 2.2. High resolution image with delineation of wine production vigor zones. 44

Figure 2.3. High resolution image of the study site with block A (west) and block B (east) highlighted (a) and vine vigor index variation (b). 50

Figure 2.4. Grape skin proanthocyanidin chemistry including concentration of grape skin proanthocyanidins (a) and % (-)-epigallocatechin extension subunits (b) determined by phloroglucinolysis. 58

Figure 2.4 cont. Grape skin proanthocyanidin chemistry including incorporation of 520 nm absorbing material or pigmented polymer (c) determined by GPC. 59

Figure 2.5. Surface map of total (seed + skin) proanthocyanidin (mg/kg) in fruit at harvest. 61

Figure 2.6. Fractional 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). 68

Figure 2.7 Concentration of proanthocyanidins in seed (a), skin (b) and skin + seed (c) in grapes at harvest and in the corresponding wine, and with error bars indicating ±SEM (n=3). 70

Figure 2.8. Surface map of soil depth (a) and corresponding water holding capacity (b) for block 72

Figure 3.1. Structures of anthocyanin-3-O-glucoside monomers based on substitution pattern commonly found in V. vinifera. 81

Figure 3.2. Monthly average of daily maximum temperatures in °C for 2003 and 2004 from March through September. 90

LIST OF FIGURES (Continued)

Figure 3.3. Correlation between Pinot noir leaf chlorophyll (SPAD units) and leaf area (cm^2) representing a range of leaf sizes from low, medium and high vigor zones 92

Figure 3.4. Surface map of spatial variation in total anthocyanin accumulation in mg per berry in 2004. 98

Figure 4.1. Site A anthocyanin evolution during fermentation for a) delphinidin, b) cyanidin, c) petunidin, d) peonidin, e) malvidin and f) total glucosides from the start of fermentation (Day 2) through pressing (Day 7). Post cold-soak data not included (Day 1). Analyzed by reversed-phase HPLC. 154

Figure 4.2. Site B anthocyanin evolution during fermentation for a) delphinidin, b) cyanidin, c) petunidin, d) peonidin, e) malvidin and f) total glucosides from the start of fermentation (Day 2) through pressing (Day 7). Post cold-soak data not included (Day 1). Analyzed by reversed-phase HPLC. 155

Figure 4.3. GPC chromatogram showing monomeric and polymeric peaks after cold soak and after pressing in a medium vigor zone wine. 158

Figure 4.4. Site A monomeric 520nm absorbing material (mg/L) evolution during fermentation quantified as malvidin equivalents. Analyzed by GPC. 159

Figure 4.5. Site B monomeric 520nm absorbing material (mg/L) evolution during fermentation quantified as malvidin equivalents. Analyzed by GPC. 159

Figure 4.6. Site A pigmented polymer (mg/L) evolution during fermentation quantified as malvidin equivalents at 520nm. Analyzed by GPC. 160

Figure 4.7. Site B pigmented polymer (mg/L) evolution during fermentation quantified as malvidin equivalents at 520nm. Analyzed by GPC. 160

LIST OF FIGURES (Continued)

Figure 4.8. Relationship between total proanthocyanidin in wine (mg/L) determined by phloroglucinolysis against pigmented polymer concentration (mg/L) determined by GPC. 167

Figure 4.9. Relationship in 2003 between total proanthocyanidin in fruit (mg/kg) determined by phloroglucinolysis against pigmented polymer concentration (mg/L) determined by GPC. 167

Figure 4.10. Relationship between pigmented polymer in wine (mg/L) analyzed by GPC against wine color density (420nm + 520nm)) determined by spectrophotometry in 2003. 169

Figure 5.1a. Structures of flavonols based on substitution pattern commonly found in V. vinifera. 181

Figure 5.1b. Structures of anthocyanins based on substitution pattern commonly found in V. vinifera cv. Pinot noir 181

Figure 5.1c. Structures of flavan-3-ol monomers based on substitution pattern commonly found in V. vinifera. 182

Figure 5.2. 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, 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; MT, methyltransferase 184

Figure 5.3. Light exclusion boxes installed over fruit clusters in the vineyard. 186

Figure 5.4a. 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 proanthocyanidin were determined by phloroglucinol. 199

LIST OF FIGURES (Continued)

Figure 5.4b. Total accumulation of seed flavan-3-ol monomers, seed proanthocyanidins, skin proanthocyanidins, skin flavonols and skin anthocyanins (mg/berry) in shaded and exposed fruit at commercial harvest (n=8-9). Seed and skin proanthocyanidin were determined by phloroglucinol.....199

Figure 6.1. High resolution image with delineation of wine production vigor zones. 234

Figure 6.2. Canonical variate plot of the wines. Circles indicate 95% confidence intervals.....248

Figure 6.3. Cobweb showing variation in attribute intensity for all significant attributes for the means of vine vigor zone wines.....249

Figure 6.4. Correlation loadings of fruit characteristics for wine sensory attributes. 252

Figure 6.5. Correlation loadings of fruit characteristics for wine stringency.....252

Figure 6.6. Relationship between fruit total tannin (mg/kg) and astringency in vigor zone wines. 253

Figure 6.7. PLS prediction of wine astringency based on fruit parameters including number of seeds, berry monomers, skin tannin, berry weight, seed tannin and total tannin. 253

LIST OF TABLES

Table 2.1. Vine Growth and Yield between Blocks and Vigor Zones[a] 48

Table 2.2. Average Berry Weight, Soluble Solids, and Seeds per Berry between Vine Vigor Zones[a]. 49

Table 2.3. Seed Flavan-3-ol Monomer and Proanthocyanidin Concentration and Percent Composition Analysis by Phloroglucinolysis. 53

Table 2.4. Skin Proanthocyanidin Concentration and Percent Composition Analysis by Phloroglucinolysis. 56

Table 2.5. Skin Proanthocyanidin Analysis by Gel Permeation Chromatography 57

Table 2.6. Wine flavan-3-ol monomer and proanthocyanidin concentration and composition by phloroglucinolysis. 64

Table 2.7. Extraction of Skin and Seed Proanthocyanidin into Wine as Determined by Phloroglucinolysis. 65

Table 2.8. Wine Tannin Analysis by Gel Permeation Chromatography and Sulfite Bleaching. 66

Table 3.1. Temperature data (°C) from 2004 showing variation by block, month and time period. 87

Table 3.2. Temperature data (°C) from 2004 showing variation by block, vigor zone, month and time period 88

Table 3.2 cont. Temperature data (°C) from 2004 showing variation by block, vigor zone, month and time period. 89

Table 3.3. Weighted mean (± SEM) of average berry weight (g), dry average skin weight (mg), soluble solids (°Brix), titratable acidity (g/L) and pH in vineyard sites in 2003 and 2004.. 94

LIST OF TABLES (Continued)

Table 3.4. Mean (± SEM) of average berry weight (gm), dry average skin weight (mg), soluble solids (°Brix), titratable acidity (g/L) and pH in vigor zones in 2003.....95

Table 3.5. Mean (± SEM) of average berry weight (gm), dry average skin weight (mg), soluble solids (°Brix), titratable acidity (g/L) and pH in vigor zones in 2004.....96

Table 3.6. Mean and SEM of total and individual anthocyanins in mg/berry for vine vigor zones in 2004. Fruit samples were used for the spatial surface map and model extractions (Part II). 99

Table 3.7. .Mean and SEM of total anthocyanin content in mg/berry and mg/kg and in mg/berry for delphinidin, cyanidin, petunidin, peonidin, and malvidin anthocyanin glucosides in 2003 and 2004. 102

Table 3.8. Mean and SEM of percent composition for delphinidin, cyanidin, petunidin, peonidin, malvidin, dioxygenated and trioxygenatedanthocyanin glucosides for site A and B in 2003 and 2004. 103

Table 3.9. Mean and SEM of total anthocyanin content (mg/berry and mg/kg), delphinidin, cyanidin, petunidin, peonidin, and malvidin glucoside content in mg/berry for vine vigor zones in 2003. 106

Table 3.10. Mean and SEM of total anthocyanin content (mg/berry and mg/kg), delphinidin, cyanidin, petunidin, peonidin, and malvidin glucoside content in mg/berry for vine vigor zones in 2004. 107

Table 3.11. Mean and SEM of percent composition for delphinidin, cyanidin, petunidin, peonidin, malvidin, dioxygenated, and trioxygenated anthocyanin glucosides for vine vigor zones in 2003.....108

LIST OF TABLES (Continued)

Table 3.12. Mean and SEM of percent composition for delphinidin, cyanidin, petunidin, peonidin, malvidin, dioxygenated, and trioxygenated anthocyanin glucosides for vine vigor zones in 2004. 109

Table 4.1. Mean and SEM of fruit composition in the model extraction in 2004 including number of berries in 300 g, pomace weight (g), and juice volume (mL). 132

Table 4.2. Mean and SEM of total, delphinidin, cyanidin, petunidin, peonidin and malvidin anthocyanin concentrations (mg/L) in model extractions in 2004 (average of vigor zones) 135

Table 4.3. Mean and SEM of total anthocyanin, delphinidin, cyanidin, petunidin, peonidin and malvidin concentrations (mg/L) in the model extractions in 2004. 136

Table 4.4. Mean and SEM of anthocyanin amount (mg) in the 300 g. of fruit, amount in the extract volume (mg) and percent extraction in the model extraction experiment. 138

Table 4.5. Mean and SEM of total anthocyanin, delphinidin, cyanidin, petunidin, peonidin and malvidin concentrations (mg/L) in wines for site A and B (average of vigor zones). 139

Table 4.6. Mean and SEM of total anthocyanin, delphinidin, cyanidin, petunidin, peonidin, malvidin and vitisin A concentration (mg/L) in wines made from vine vigor zones in 2003. 141

Table 4.7. Mean and SEM of total anthocyanin, delphinidin, cyanidin, petunidin, peonidin, malvidin and vitisin A concentration (mg/L) in wines made from vine vigor zones in 2004. 142

Table 4.8. Mean and SEM of percent composition for delphinidin, cyanidin, petunidin, peonidin and malvidin in model extractions in 2004 in site A and B (average of vigor zones). 145

LIST OF TABLES (Continued)

Table 4.9. Mean and SEM of percent anthocyanin composition for delphinidin, cyanidin, petunidin, peonidin and malvidin in the model extractions for vigor zones in site A and B in 2004. 146

Table 4.10. Weighted mean and SEM of percent composition for delphinidin, cyanidin, petunidin, peonidin, malvidin and vitisin A in wines from site A and B in 2003 and 2004. 148

Table 4.11. Mean and SEM of percent composition for delphinidin, cyanidin, petunidin, peonidin, malvidin and vitisin A in wines made from vine vigor zones in 2003. 151

Table 4.12. Mean and SEM of percent composition for delphinidin, cyanidin, petunidin, peonidin, malvidin and vitisin A in wines made from vine vigor zones in 2004. 152

Table 4.13. Mean and SEM of color density (420nm + 520nm), hue (420/520), red pigment (%) and sulfite resistant pigments (%) of wines averaged for Site A and B. 163

Table 4.14. Mean and SEM of color density (420nm + 520nm), hue (420/520), red pigment (%) and sulfite resistant pigments (%) of wines made from vine vigor zones in 2003. 164

Table 4.15. Mean and SEM of color density (420nm + 520nm), hue (420/520), red pigment (%) and sulfite resistant pigments (%) of wines made from vine vigor zones in 2004. 165

Table 5.1. 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. 190

Table 5.2. Mean (± SEM) a) seed flavan-3-ol monomer concentration (nmol/seed) and molar proportion and b) extension, terminal and total subunit concentration (nmol/seed) and mDP by phloroglucinolysis from shaded and exposed treatments at véraison and commercial harvest. 193

LIST OF TABLES (Continued)

Table 5.2 (continued). Mean (± SEM) c) proanthocyanidin molar extension and terminal subunit proportions by phloroglucinolysis from shaded and exposed treatments at véraison and commercial harvest. 194

Table 5.3. 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. 196

Table 5.4. Mean (± SEM) skin anthocyanin amount (mg/berry) calculated in malvidin equivalents, proportional analysis and oxygenation pattern in shaded and exposed treatments at commercial harvest. 198

Table 5.5. Mean (± SEM) for number of berries in 300 (g), average berry weight (g), pomace weight (g), juice volume (ml) and solids (%) from shaded and exposed model extractions. 201

Table 5.6. 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. 203

Table 5.7. Mean (± SEM) of total, skin and seed proanthocyanidin (mg/L) and percent skin extraction determined by phloroglucinol from shaded and exposed model extractions. 205

Table 5.8. Mean (± SEM) anthocyanin concentration (mg/L) calculated in malvidin equivalents and proportional analysis in shaded and exposed model extractions. 205

Table 6.1. List of wines, site, zone, replicate, and codes used in the sensory experiment. 237

Table 6.2: Sensory attributes, their definitions and respective standards. All standards were added the base wine: “05 CS Base HGH” – 30 mL VE-wine cellar. 239

LIST OF TABLES (Continued)

Table 6.2 (continued): Sensory attributes, their definitions and respective standards. All standards were added the base wine: "05 CS Base HGH" – 30 mL VE-wine cellar. 239

Table 6.3. Vigor zone ratings and vine vigor zone fruit composition. 242

Table 6.4. Chemical analyses of the fruit from the vine vigor zones. 243

Table 6.5. Chemical analyses of the wines from the vine vigor zones. 244

Table 6.6. Wine intensity of sensory attributes for all wines included in the sensory study. 245

Table 6.7. Wine intensity of sensory attributes for the means of wines from each vigor zone included in the sensory study. 246

Table 6.8. Pearson correlation coefficients and p-values for fruit and wine chemical analyses and sensory attributes. 247

Influence of Vine Vigor and Shading in Pinot noir (*Vitis vinifera* L.) on the Concentration and Composition of Phenolic Compounds in Grapes and Wine.

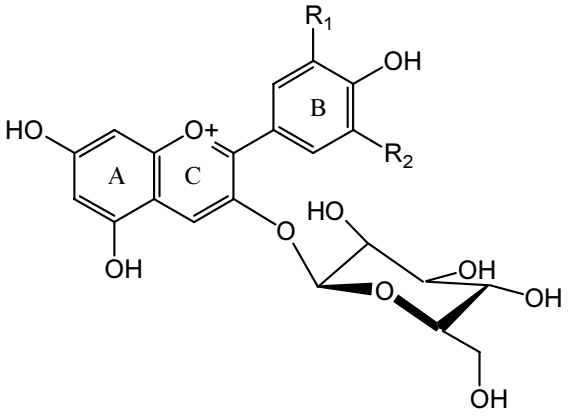
Introduction

IMPORTANCE OF PHENOLIC COMPOUNDS

Phenolic compounds include a wide variety of secondary plant metabolites that provide a range of functions including attracting pollinators, in seed dispersal, UV-light protection and protection against pathogens and herbivores (Winkel-Shirley, 2001). Phenolic compounds found in grapes and wine play an important role in wine quality and style by providing color, astringency and health benefits to wine. The three main classes of phenolic compounds found in grapes (*Vitis vinifera* L.) and wine include the anthocyanins, flavonols and the proanthocyanidins (also known as condensed tannins). In grapes, proanthocyanidins are present in the greatest concentration followed by anthocyanins then flavonols (Souqet et al, 1996). These three classes differ from each other due to the oxidation level of the C-ring oxygenated heterocycle (**Figure 1.1, 1.3, 1.5**).

Description of anthocyanins. Anthocyanins are pigmented compounds responsible for the red color of grapes and wine. Each grape variety has a unique compositional profile in terms of anthocyanin accumulation that is under genetic control of fruit ripening (Mazza and Miniata, 1993). In most grape varieties anthocyanins exist only in the skins and not in the flesh. Anthocyanins are identified by a flavylium C-ring nucleus with a positively charged oxygen oxonyium

(**Figure 1.1**). The flavylium is a stable oxonium cation that can exist in equilibrium between four structural groups dependent on pH. The color of the anthocyanin depends on the form (**Figure 1.2**) ranging from red for the flavylium, blue for the quinone base, colorless for the carbinol base, and very pale yellow for the chalcone form (Ribéreau et al. 2000). Anthocyanins are water soluble and are glycosylated most commonly at the C-3 position and the sugar can be acylated as well (Haslam 1977). The sugars that have been identified include glucose, galactose, xylose, rhamnose and arabinose (Francis 1989). Several of the most common acids involved in acylation include *p*-coumaric, caffeic, ferulic and *p*-hydroxy benzoic acids.



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 1.1. Structure of anthocyanins found in Pinot noir grapes.

The variation in hydroxylation on the B-ring of anthocyanins is important in terms of coloration, stability and antioxidant capacity. The hydroxylation pattern on the B-ring results in five anthocyanidin aglycons which include delphinidin, petunidin, peonidin, cyanidin and malvidin (**Figure 1.1**).

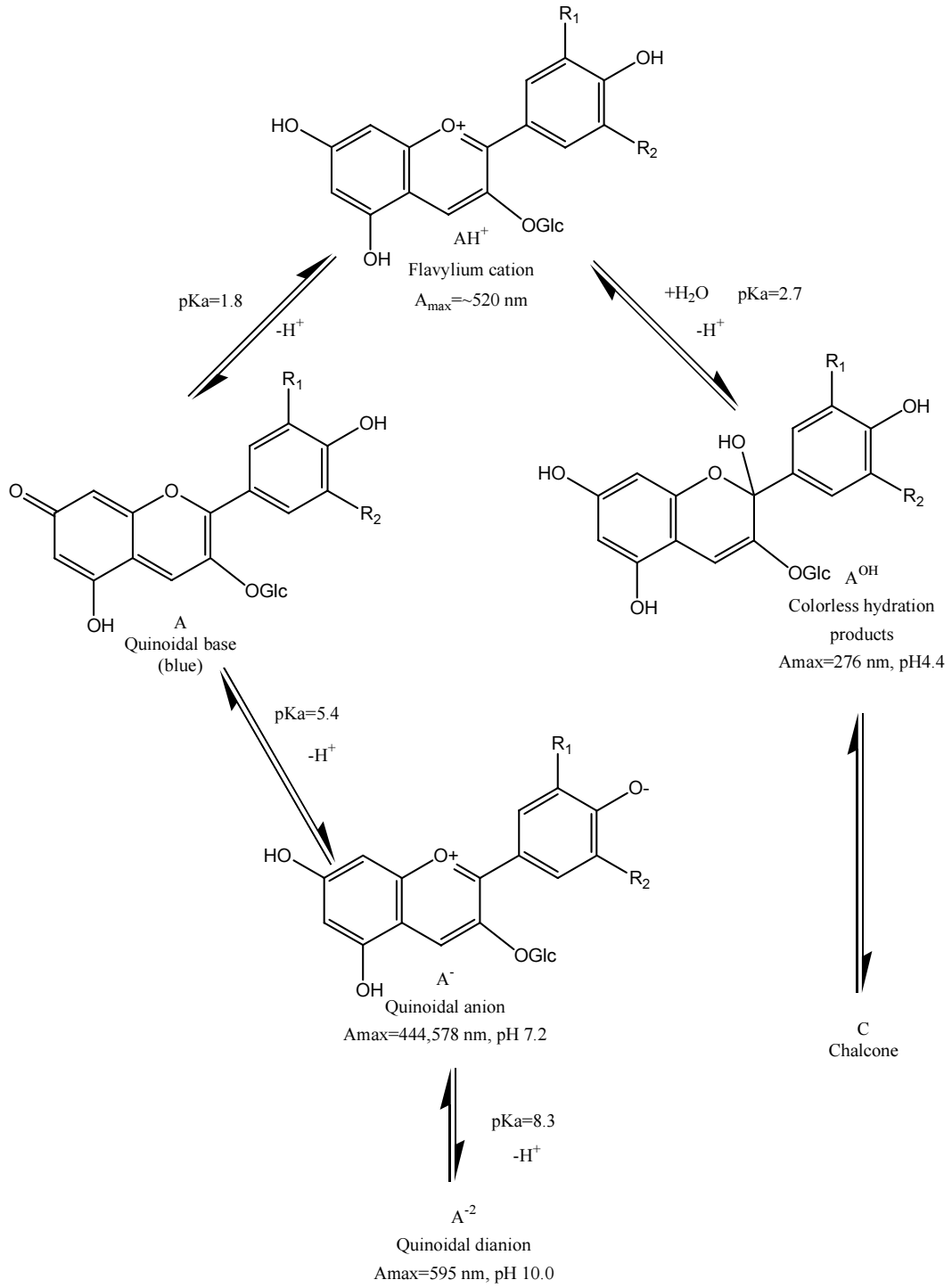


Figure 1.2. Equilibrium among the four structure groups of anthocyanins.

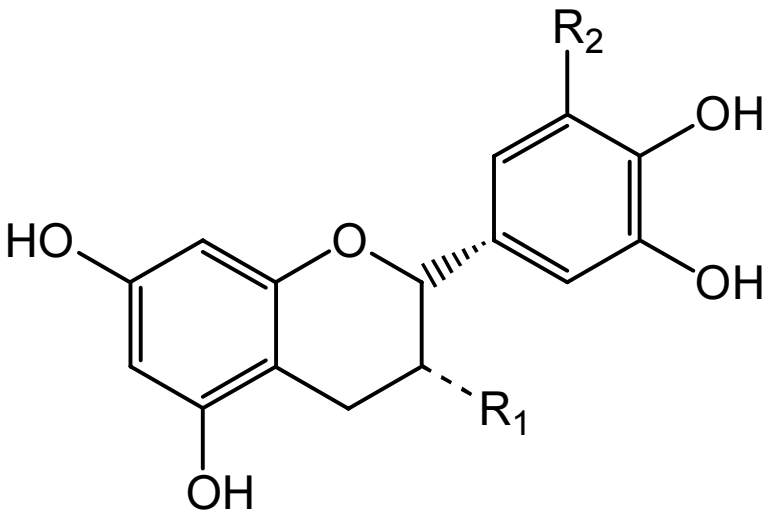
V. vinifera varieties usually produce 3-monoglucoside, 3-acetylglucoside and 3-*p*-coumarylglucoside derivatives of the aglycons. However, Pinot noir, a cool climate variety, only produces non-acylated forms (Fong et al., 1971). Consequently, it differs from other varieties in terms of its anthocyanin profile and in how this impacts color density and color stability of the wine. Malvidin derivatives are often the major forms present.

Description of proanthocyanidins. Proanthocyanidins in seeds are thought to provide protection from early feeding of unripe fruits (Harbourne 1997; Dixon et al. 2004) and also to protect developing fruit from fungal pathogens (Mercier 1987; Dixon et al. 2004). In grape skins, the role of proanthocyanidins is not as clear although they may play a role in UV protection. In wine, proanthocyanidins provide astringency and mouthfeel (Noble 1990; Gawel 1998). In addition they form pigmented polymers with anthocyanins that contribute to color stability (Somers 1971). Proanthocyanidins have been described as having molecular weights between 500 and 3000 and have the ability to precipitate proteins. The ability to precipitate salivary proteins is important in the tactile sensation of astringency in wine (Robichaud & Noble 1990). Proanthocyanidins also have antioxidant and other human health benefits (Santos-Buelga & Scalbert 2000; Dixon et al. 2004).

Proanthocyanidins differ from other classes of phenolic compounds in that the C-ring is a fully saturated oxygenated heterocycle (**Figure 1.3).** Variations in the B-ring hydroxylation pattern produce different flavan-3-ol monomers. Grape seeds contain (+)-catechin (C), (-)-epicatechin (EC), and (-)-epicatechin-3-*O*-gallate ECG

flavan-3-ol monomers (Prieur et al. 1994; Romeyer et al. 1986; Czochanska et al. 1979)**.** Skin proanthocyanidins contain a trihydroxylated flavan-3-ol monomer (-)-epigallocatechin (EGC) in addition to those found in seeds. Skins differ from seeds in that they have a low concentration of flavan-3-ol monomers, contain prodelphinidins, a higher degree of polymerization and a lower proportion of galloylated subunits. Proanthocyanidins are polymeric and consist of the same subunits connected by interflavonoid bonds (**Figure 1.4)**. These interflavonoid bonds usually between the C_4 in the upper flavan unit and C_6 or C_8 position in the lower unit. Proanthocyanidins can undergo acid catalyzed cleavage of the interflavonoid bond to release a flavan-3-ol unit from the lower part and a carbocation from the upper part (Ribéreau-Gayon et al. 2000). The reactive carbocation can be reacted with a nucleophile such as a thiol or phloroglucinol as a method to identify and quantify proanthocyanidin subunits (Haslam 1977; Kennedy & Jones 2001). In wine, these reactive carbocations can undergo other reactions some of which are further described under the formation of pigmented polymers.

Description of flavonols. Flavonols have many physiological functions in plants with the most widespread role being as UV protectants (Flint et al. 1985; Price et al. 1995; Smith & Markham 1998). A couple other important functions include free radical scavenging (Markham et al. 1998) and copigmentation with anthocyanins (Asen et al. 1972; Scheffeldt & Hrazdina 1978). Flavonols also act as co-pigments (Scheffeldt and Hrazdina, 1978) in wine. In terms of human health,



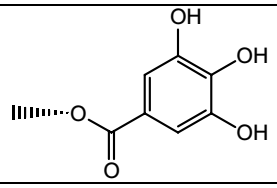
Flavan-3-ol Monomer	R_1	R_2
(+)-catechin	OH	H
(-)-epicatechin	OH	H
(-)-epigallocatechin	OH	OH
(-)-epicatechin-3-*O*-gallate		H

Figure 1.3. Structures of flavan-3-ols found in grape seed and skins.

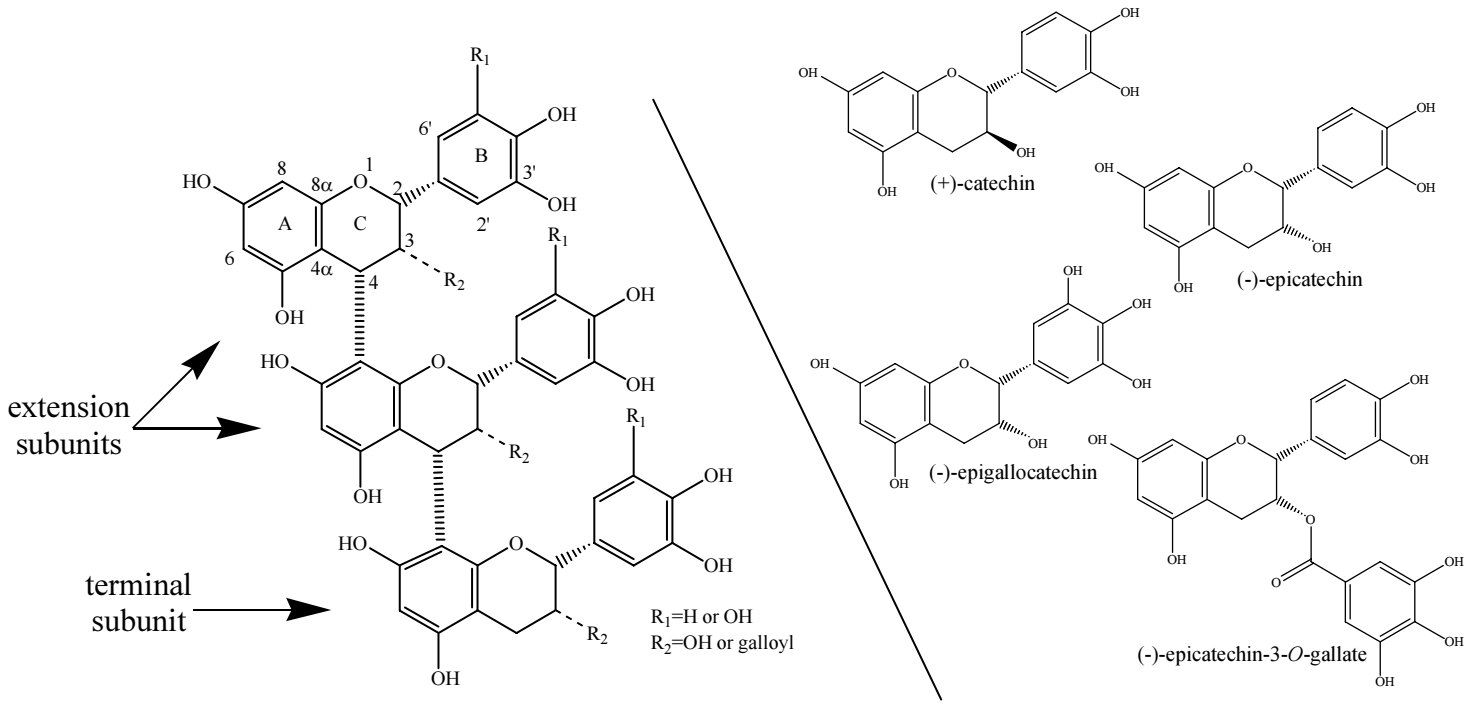
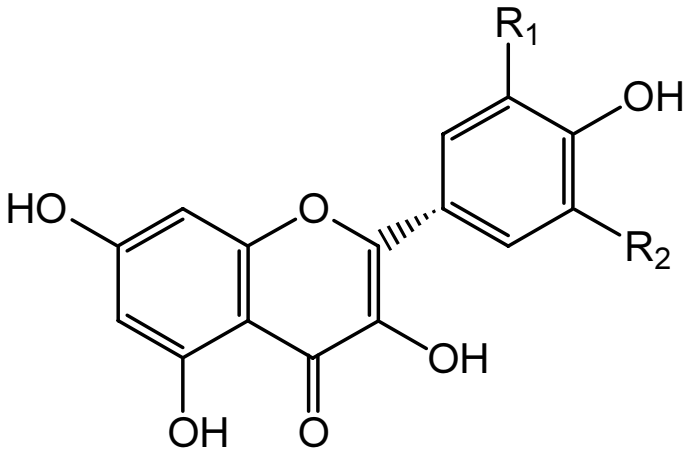


Figure 1.4. Structure of flavan-3-ol monomers and condensed proanthocyanidins

flavonols (mainly quercetin) have been found to inhibit cancer cell growth of tumors (Flamini 2003), protect LDL cholesterol against oxidation (Careri et al. 2003; Stecher et al. 2001) and have antioxidant and antihistamine properties (Stecher et al. 2001; Vuorinen et al 2000).

Flavonols are one of the major subclasses of phenolic compounds characterized by a C-ring structure with a double bond at the 2-3 position (**Figure 1.5**). Flavonols are found in grape skins and the substitution pattern on the B-ring produces kaempferol, quercetin, myricetin and isorhamnetin. Only kaempferol and quercetin based flavonols are produced in white grapes with a greater diversity being produced in red grapes (Ribereau-Gayon, 1964). Flavonols identified in grapes are usually conjugated in grape skins and in red grapes can include glycosides of myricetin, quercetin, kaempferol and isorhamnetin and glucuronides of myricetin, quercetin and kaempferol plus a few minor conjugates with other sugar derivatives (Makris et al. 2005). The most common flavonols found in grapes are quercetin-3-*O*-glucoside followed by quercetin-3-*O*-glucuronide (Cheynier & Rigaud 1986; Price et al. 1995). Flavonol levels were analyzed in 65 wines from different countries and the range was from 4.6 to 41.6 mg/L (Morag et al. 1998).



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

Figure 1.5. Structures of common flavonols found in grape skins.

BERRY DEVELOPMENT

Grapes are a non-climacteric fruit and berry growth follows a double sigmoid curve separated by a lag phase (**Figure 1.6**) (Coombe, 1976; Coombe and McCarthy, 2000). In the first phase of berry formation, rapid cell division occurs, seed embryos are produced, tartaric and malic acid accumulate, hydroxycinnamic acids are biosynthesized in addition to monomeric flavan-3ols and polymeric proanthocyanidins. Another class of non-flavonoid phenolic compounds, hydroxycinnamic acids are distributed in the flesh and skin of the berry and are

important because of their involvement in browning reactions, and because they are precursors to volatile phenols (Romeyer et al. 1983).

The lag phase is characterized by a slowing of growth, embryo development, beginning of chlorophyll loss from berries, softening of berries, and acidity reaching peak levels. Tartaric and malic acids are the main acids in grapes. Tartaric acid accumulates early during the first stage of berry development and malic acid peaks just before véraison (Hrazdina et al. 1984).

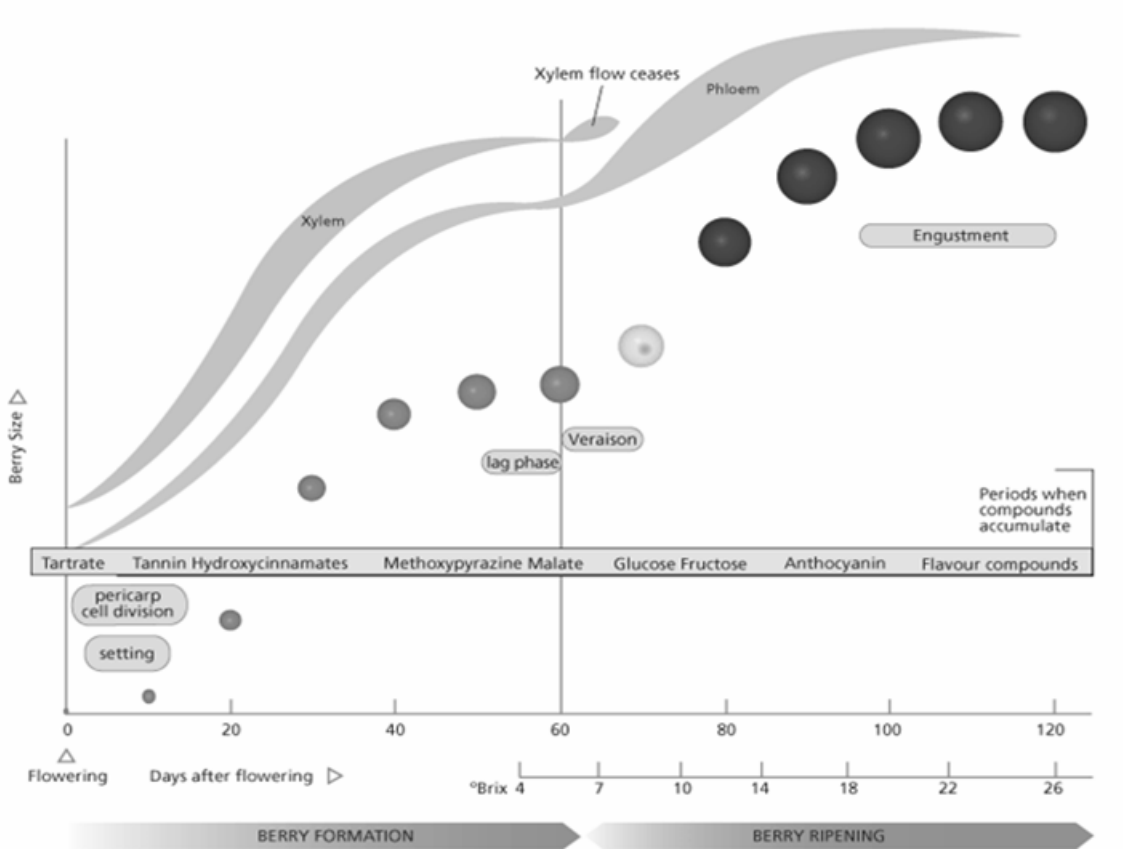


Figure 1.6. Diagram of berry development showing relative size and color of berries from flowering through ripeness, periods when compounds accumulate, the levels of juice °brix, rate of xylem and phloem inflow. Illustrated by Jordan Koutroumandis, Winetitles.

Ripening begins after the lag phase at a time termed véraison by viticulturalists. During ripening, there is a rapid influx of sugars, berry softening, anthocyanin accumulation (coloring), flavor and aroma development, reduction in acids, and a reduction and modification of proanthocyanidins. Sucrose is translocated to the fruit and is transported into the grape berry during fruit ripening. The sucrose is hydrolyzed into its constituent sugars glucose and fructose inside the berry. (Robinson & Davies 2000). Beginning at véraison, anthocyanins accumulate in the grape berry, and are correlated with increased sugar accumulation (Pirie & Mullins, 1980).

BIOSYNTHESIS OF PHENOLIC COMPOUNDS

Many of the structural and regulatory genes in the phenolic pathway (**Figure** 1.7) have been characterized in several plant species (Holton & Cornish, 1995) and also in grapes (Boss et al. 1996a, 1996b, Burger & Botha 2004; Bogs et al. 2005; Fujita et al. 2005; Bogs et al. 2006). Most grape studies have focused on regulation of phenolic compound accumulation during berry ripening and only a few studies have investigated the influence of environmental factors (Downey et al. 2004). A recent study investigated grape color variation related to expression of flavonoid 3'-hydroxylase and flavonoid 3'5'-hydroxylase genes (Castellarin et al. 2006).

Accumulation of anthocyanins. Anthocyanins begin accumulating in the grape skins starting at véraison and continuing through ripening until approximately 24 Brix is reached. In grape berries, all of the genes involved in accumulation of

phenolic compounds were detected in flower and grape skins up to 4 weeks post flowering except for UFGT (Boss et al. 1996). At 10 weeks post flowering, expression of CHS, CHI, F3H, DFR, LDOX, and UFGT increased, coinciding with the onset of anthocyanin synthesis (**Figure 1.7**) (Boss et al. 1996b). The early expression of the genes in the pathway with the exception of UFGT specific to anthocyanins, are thought to be involved in the biosynthesis of other phenolic compound s such as flavones, flavonols, and proanthocyanidins. In most studies, it has been found that the proportion of 3' and 3' 5' substituted anthocyanins does not change over the course of ripening (Boss et al., 1996a). This suggests that the rate of flux down either branch of the biosynthetic pathway is nearly constant through ripening (Boss et al., 1996a).

As anthocyanins begin to accumulate, expression of all the genes involved in anthocyanin synthesis increased suggesting there is coordinated regulation of all these genes at this time in the grape skin (Boss et al., 1996a). The pattern of expression seen prior to véraison in the grape flower and skin suggests that UFGT is under different regulation controls. This regulation occurring at the last step of anthocyanin biosynthesis differs from the regulation found in other species such as petunia, snapdragon, and maize where control is generally found to be earlier in the pathway (Boss et al., 1996a).

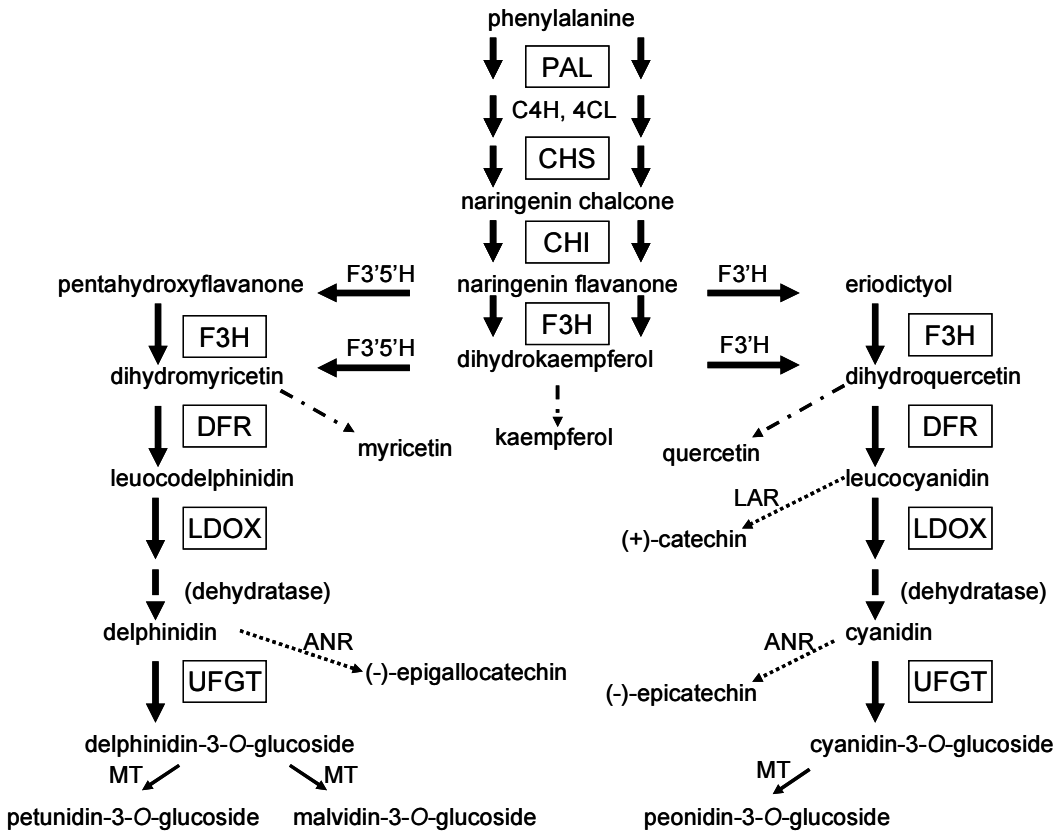


Figure 1.7. Simplified phenolic biosynthetic pathway showing products from flavonoid 3'-hydroxylase (F3'H) and flavonoid 3',5'-hydroxylase (F3'5'H) activity, flavonols = dashed arrows, flavan-3-ols = dotted arrows, and anthocyanins = solid arrows, PAL, phenylalanine ammonia lyase; CHS, chalcone synthase; CHI, chalcone isomerase; DFR, dihydroflavonol-4-reductase; LDOX, leucoanthocyanidin dioxygenase; UFGT, UDP glucose: flavonoid-3-*O*-glucosyltransferase; LAR, leucoanthocyanidin reductase; ANR, anthocyanin reductase; MT, methyltransferase

Accumulation of proanthocyanidins in grape skins and seeds. Grapes can have up to 4 seeds per berry but generally have fewer as often times one or more will abort. Seeds obtain full size before or near véraison and then fresh seed mass declines as the seed loses water and the seed coat turns brown and hardens. The number of seeds per berry is more important in total seed proanthocyanidin per berry

than the concentration per seed (Harbertson et al. 2002). The general pattern of grape seed 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 (Downey et al. 2003b; Kennedy et al. 2000). EC and C account for the major proportion of monomers with differences reported between varieties. In Pinot noir the flavan-3-ol monomers were about 70% C and 30% EC (Pastor del Rio & Kennedy 2006) while in Shiraz they were ~ 70% EC and 30% C (Kennedy et al. 2000b) and in Cabernet sauvignon the ratio was found to be about 50:50 (Kennedy et al. 2000a).

Seed proanthocyanidin extension subunits were highest at véraison (Kennedy et al. 2000a) or several weeks post véraison (Downey et al. 2003b; Pastor del Rio & Kennedy 2006) and then declined leading to harvest. Seed proanthocyanidin extension subunits have been found to be ~ 70% EC in several grape varieties (Kennedy et al. 2000a; Kennedy et al. 2000b; Pastor del Rio & Kennedy 2006). Seed proanthocyanidin terminal subunits were reported to be about the same proportions of EC and C in Cabernet Sauvignon and Shiraz and over 50% C in Pinot noir (Pastor del Rio & Kennedy 2006). Seed mean degree of polymerization (mDP) was found to be in the range of 5-9 (Pastor del Rio & Kennedy 2006; Kennedy et al. 2000b).

Grape skins have very low concentrations of flavan-3-ol monomers primarily consisting of C (Kennedy et al. 2001; Pastor del Rio & Kennedy 2006). Previous studies have shown that skin proanthocyanidin concentration peaks near véraison and then declines with increasing maturity (Downey et al. 2003b; Kennedy et al. 2002; Pastor del Rio & Kennedy 2006). Skin extension subunits in Pinot noir were

found to be ~ 63% EC and ~ 34% EGC with only a small percent of ECG (Pastor del Rio & Kennedy 2006). The average molecular weight of skin proanthocyanidin in Shiraz grapes was found to increase with berry development (Kennedy et al. 2001) while Downey et al. (2003b) reported that skin mDP increased during the early phase of berry development then decreased after véraison. Skin mDP in Pinot noir was reported to be from 27-42 at harvest (Pastor del Rio & Kennedy 2006).

Grape proanthocyanidins are thought to branch from the phenolic pathway at the point of leucocyanidin or leucodelphindin. From leucocyanidin, the dihydroxylated flavan-3-ols (+)-catechin and its epimer (-)-epicatechin are synthesized through the activity of leucoanthocyanidin reductase (LAR). In grape skins, (-)-epigallocatechin is synthesized through the activity of LAR from the intermediate leucodelphindin (Dixon et al. 2005). Bogs et al. (2005) 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. This makes sense as seeds do not produce EGC from the F3'5'H branch of the pathway. The formation of proanthocyanidins from flavan-3-ols is poorly understood but is thought that extension subunits arise by condensation of an electrophile derived from leucoanthocyanidin with the nucleophilic 8 or 6 position of the starter unit (Dixon et al. 2005).

Accumulation of flavonols. Flavonols synthase (FLS) has been found to have high levels of expression in grape berries between flowering and fruitset and then again during ripening in Shiraz (Downey et al 2003a). Flavonol biosynthesis

appears to be ongoing during berry development; however two main periods of synthesis were noted; one around flowering and the second occurring after véraison (Downey et al 2003b). The second post-véraison increase occurred after the main period of anthocyanin biosynthesis and the authors suggested this might have to do with copigmentation with anthocyanins or in preventing photobleaching of anthocyanins (Yamasaki et al. 1996; Downey et al. 2003a).

INFLUENCES ON ACCUMULATION OF PHENOLIC COMPOUNDS

Under conditions of low water and nutrient availability, plants can reduce growth and shift carbon into producing more secondary plant metabolites (Chaves & Escudero). Ultraviolet radiation and water stress have been shown in numerous studies to be the most relevant factors in the induction of phenolic biosynthesis (Chaves & Escudero Bohm, 1988; Chalker-Scott, 1999; Winkel-Shirley, 2002). Although the accumulation of phenolic compounds is an integral part of berry ripening, a shift into higher biosynthesis of secondary metabolites can occur in vineyards based on high UV exposure or soil and site differences such as soil depth, available nutrients and water holding capacity.

Variations in vine growth/vigor can be due to management practices or to environmental characteristics of the site and can influence berry ripening and phenolic accumulation in the fruit (**Figure 1.8)** (Jackson and Lombard, 1993).

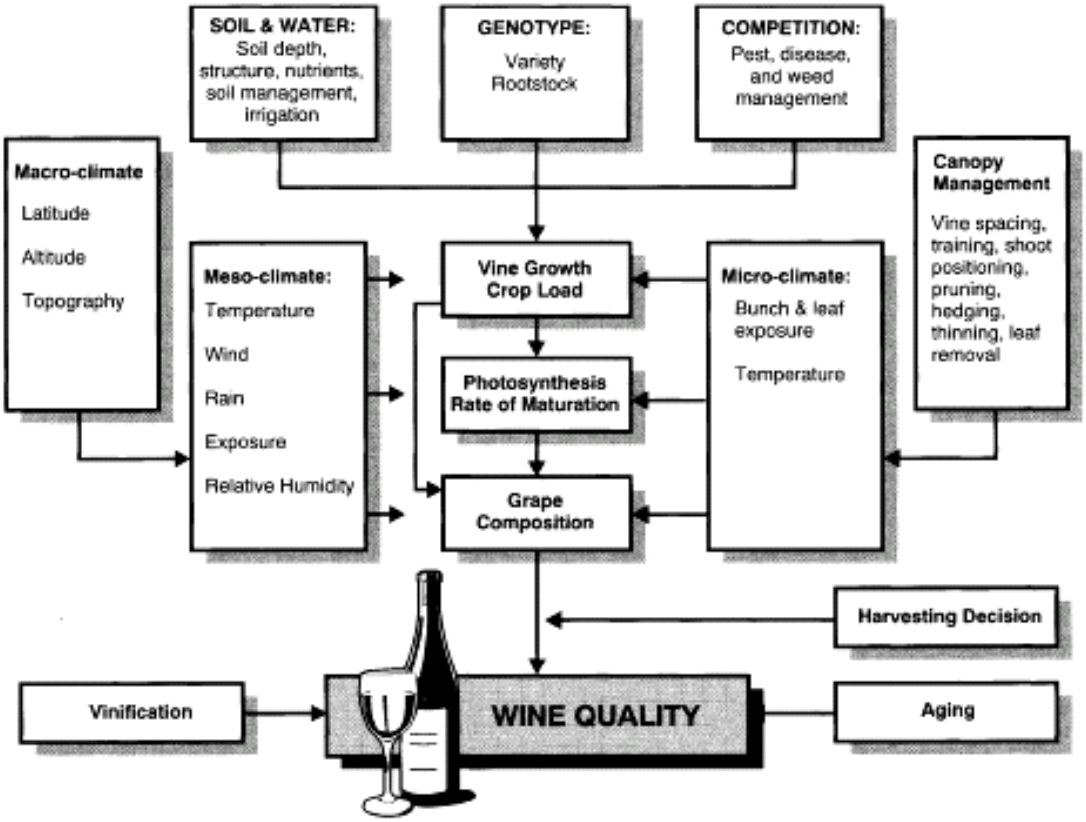


Figure 1.8. Diagram showing relationships among vineyard site environment and management practices on fruit composition and wine quality (Jackson and Lombard 1993).

Precision viticulture (PV) is one approach being investigated for studying spatial variations in vineyards. PV has been defined by Lamb (2000), as monitoring and managing spatial variation in productivity–related variables (yield and quality) within single vineyards. According to Hall et al. (2002), spatial variations in topography, climatic conditions, physical and chemical characteristics of the soil and pests and diseases, can cause spatial variations in yield and quality within a vineyard. In Australia, the use of remotely sensed images and ground measurements to create

geospatial vineyard maps have found variation in yield, soil characteristics (Lamb, 2000) and more recently in berry color and phenolics (Holzapfel et al. 1999, 2000; Bramley and Proffitt, 1999, 2000; Proffitt et al. 2000; Lamb, 2000; Bramley et al. 2000; Bramley 2001; Lamb et al. 2004). There is particular interest in investigating the influence of site environment factors on the accumulation of phenolic compounds due to their importance in wine.

Plants have evolved to be well adapted to minimize damage from excess sunlight exposure. UV-B causes damage indirectly through the production of free radicals, such as superoxide, singlet oxygen, and hydroxyl radicals. Several possible mechanisms have been proposed for how plants respond to UV stress. One is through the increased biosynthesis of compounds that provide protection through UV screening (Li et al., 1993; Bieza and Lois, 2001). The second is through increased production of phenolics that function as free radical scavengers (Nagata et al., 2003).

Sunlight exposure is thought to be one of the main factors influencing phenolic accumulation and composition in grapes (Smart et al. 1988; Jackson and Lombard, 1993). Sunlight exposure in the fruiting zone can be influenced by vine vigor or management practices. Various viticultural practices have been found to influence anthocyanin accumulation and composition including nitrogen supply (Keller & Hrazdina 1998; Hilbert et al. 2003), vine canopy management (Reynolds et al. 2005), water deficit (Ojeda et al., 2002), soil amendments (Yokotsuka, 1999) and others. A difficulty in assessing many of these results is that there are confounding factors where practices modify vine growth and canopy structure

causing changes in light exposure or temperature in the fruiting zone.

A number of exposure studies show a range of results in terms of anthocyanin accumulation (Smart et al. 1988; Morrison & Noble 1990; Gao & Cahoon 1994; Price et al. 1995; Dokoozlian & Kliewer 1996; Bergqvist et al. 2001; Spayd et al. 2002; Downey et al. 2004). Light was found to have its greatest impact on anthocyanin and phenolic accumulation during the initial stages of growth rather than during the fruit ripening period (post- véraison) (Dokoozlian and Kliewer 1996). In addition, high bloom time N particularly with low light irradiance was reported to interfere with phenolic biosynthesis leading to a lower total amount at maturity (Keller & Hrazdina 1998). Consequently, the variable results may be due to the large number of possible interactions.

In several studies, higher accumulation of anthocyanins was found in grapes with a cool day and night temperatures compared to high day or night temperatures (Buttrose et al. 1971; Mori et al. 2005). In the hot San Joaquin Valley, anthocyanins in two red varieties, increased linearly with increasing sunlight exposure on the north side of vines while anthocyanins declined on the south side due to high temperatures (Bergqvist et al. 2001). In an experiment designed to separate the effects of light and temperature, Spayd et al. (2002) found that sunlight increased anthocyanin concentration while high berry temperatures reduced anthocyanin concentration in west exposed fruit. Sunlight exposed berries have been reported to have increased temperatures from 3-13°C (Dokoozlian & Kliewer 1996; Spayd et al. 2002; Reynolds et al. 1986; Kliewer & Lider 1968) compared to nonexposed fruit due to

incident radiation. A net loss of anthocyanins in Merlot was associated with the number of hours over 35°C the fruit experienced (Spayd et al 2002).

The effect of sunlight exposure on proanthocyanidin accumulation and composition has been less studied. In a number of sun exposure studies either proanthocyanidins were not investigated or they were included in general measurements that do not discriminate between phenolic classes. However, recent results with winegrapes suggest that berry skins are more responsive to light exposure than seeds in accumulation of proanthocyanidins (Downey et al, 2004).

Flavonols are highly responsive to light exposure and appear to function as UV protectants (Winkel-Shirley, 2001; Flint et al. 1985; Smith and Markham, 1998; Price et al., 1995). Several light exposure studies in wine grapes have shown that flavonol concentrations increase in response to UV exposure (Price et al., 1995, Spayd et al, 2002). High total flavonol levels appear to be related to thick skinned varieties and good sun exposure (Morag et al. 1998; Price et al. 1995; Spayd et al. 2002).

As mentioned above, water stress can also cause a shift into higher biosynthesis of phenolic compounds. In grapes, anthocyanin accumulation was found to be most rapid during the first two weeks after véraison at the onset of anthocyanin biosynthesis and that accumulation of anthocyanins was more sensitive to water deficit before véraison than post-véraison (Matthews & Anderson 1988). Fruit that failed to mature and color properly has been found due to severe water deficit (Hardie & Considine 1981). However, in a number of irrigation studies, reduced

water applications or increased water stress was found to have a direct effect of increased concentration of anthocyanin biosynthesis or an indirect effect of a reduction in fruit size (Ojeda et al. 2002; Kennedy et al. 2002; Matthews & Anderson 1988; Hardie et al. 1981; Ginestar et al. 1998; Salón et al. 2005).

The influence of water status on proanthocyanidin accumulation in fruit has been investigated in winegrapes. In general, the studies have shown minimal influence from vine water status on seed or total proanthocyanidins (Kennedy et al. 2000a; Roby et al. 2004a). However, in one study, vines grown under water deficits had a greater dry weight of skin in addition to a higher concentration of skin proanthocyanidins (Kennedy et al. 2002). The higher concentration of skin proanthocyanidins may have been directly related to vine water status or indirectly to variations in light exposure in the fruiting zone. Thicker skins may have some benefit in a water deficit situation while an increase in skin proanthocyanidin concentration may play a role in UV protection (Solvochenko & Schmitz-Eiberger 2003). In addition, a plant's response will depend on the degree of exposure to stress and can be additive in response to both water deficit and UV irradiance (Chaves & Escudero 1999). Consequently, many questions remain in how UV light induces biosynthesis of specific phenolic compounds and what roles these phenolics play in UV protection (Winkel-Shirley, 2002).

INFLUENCES ON COMPOSITION OF PHENOLIC COMPOUNDS

There is also evidence that phenolic composition can change in response to environmental factors. Plants have been shown to shift anthocyanin composition toward higher levels of B-ring hydroxylation in response to UV light (Ryan et al., 2002; Downey et al. 2004, Jaakola et al. 2004). Ryan et al. (2002) found Wild-type Arabidopsis L. leaves produced primarily kaempferol glycosides under low UVB light while higher UVB resulted in double the concentration and an increase in the ratio of quercetin:kaempferol. In the tt7 mutant, only kaempferol was accumulated at a higher level; however, the mutant had less tolerance of UVB radiation suggesting that kaempferol is a less effective photopotectant than quercetin.

Compositional changes in proanthocyanidins have been less studied, however; recent results with grape skins suggest that light exposure can result in a compositional shift toward increased trihydroxylation (EGC subunits) (Downey et al. 2004). In bilberry (*Vaccinium myrtillus* L.) sun-exposed leaves had higher prodelphinidins (Jaakola et. al, 2004).

Although a number of authors have concluded that anthocyanin composition is primarily determined by genetic factors (Roggero et al. 1986; Brossaud et al. 1999; Mazza & Miniata 1993; Boss et al. 1996A), the proportional composition specific for a variety may be modified by environmental influences. Previous studies have found vintage effects related to environmental conditions (Downey et al 2004; Ryan & Revilla 2003). Lower levels of the anthocyanins (Dp and Pt derivatives), were found in a warmer year compared to a cooler year (Ryan & Revilla 2003). In Merlot, in

response to cool seasons, a higher concentration of Cy and Pn were reported in one study (Yokotsuka et al. 1999) and a higher percent of Dp and Cy and lower Pn and Mv were found in another study (Spayd et al. 2002).

The percent of Mv has been reported to increase with ripening (Ryan & Revilla 2003) and with high rates of nitrogen at bloom and low light intensity at véraison (Keller & Hrazdina 1998). Mv accumulation was found to be less sensitive to the environmental influences of nitrogen and light compared to the other anthocyanins (Keller and Hrazdina 1998; Hilbert et al. 2003).In assessing the results by Spayd et al. (2002), cooling sun exposed fruit decreased the percent of Mv and Pn (one out of two years) derivatives while heating shaded fruit increased Mv and decreased Dp. This suggests the proportion of Mv may be closely associated with temperature. According to Roggero et al. (1986), the levels of Cy and Dp peak first, three to four weeks after véraison and then decrease rapidly whereas Pn and Mv continue to be formed. Keller and Hrazdina (1998) reported that the relative proportions of individual anthocyanins were most equal with low bloom time nitrogen and high light intensity.

Highly sun-exposed fruit was associated with a lower content of Mv and higher Dp and Pt (Tomasi et al 2003). In grape skins, the use of UV barriers that site UVB light but did not change temperature had an approximate 3% reduction in the percent composition of delphinidin derivatives and an increase of about 6% in malvidin-based compounds (Spayd et al., 2002). Downey et al. (2004) found that shading clusters in Shiraz increased the proportion of Pn while exposure increased

the proportion of Dp and Pt. It appears that Dp and Pt are the most responsive to light exposure in a number of different grape varieties. Others have found a higher proportion of Pn in response to water stress (Bao do & Cormier 1991) and jasmonic acid (Curtin et al. 2003). This shows the complexity of anthocyanin biosynthesis as they can have a similar response to different environmental factors.

EXTRACTION OF PHENOLIC COMPOUNDS INTO WINE

In addition to the amount of phenolic compounds in the berry, the extractability of the various classes of compounds influences the wine profile. Ease of extraction of specific compounds is related to both solubility and localization of the compound within the berry. Winemaking practices such as skin contact time, fermentation temperature, and the use of macerating enzymes also influence the extraction of phenolic compounds as reviewed by (Sacchi et al. 2005).

In the past, it was difficult to study the percent extraction of seed versus skin proanthocyanidin in wines due to the inability to separate them by analytical methods. Studies were done to determine proanthocyanidin amount in wine by comparing wines made with or without pomace contact (Kantz & Singleton 1991), addition of seeds (Kovac et al. 1995), or isolating seeds and stems after fermentation (Sun et al. 1999). Recently, a method based on the presence of EGC proanthocyanidin extension subunits in skins but not seeds, has been developed to analyze the percent extraction of seed and skin proanthocyanidins in wine (Peyrot de Gachons & Kennedy 2003)

Extraction of proanthocyanidins from seeds is generally quite low although the seed contain a higher amount of flavan-3-ols and proanthocyanidins than found in the skins. Two studies suggested that about half of the extractable flavan-3-ol monomers and proanthocyanidins in grape seeds were transferred into wine (Singleton & Draperi 1964; Sun et al. 1999). The number of seeds per berry is more important in extraction than the amount of proanthocyanidin per seed due to the increased surface area for extraction. Although skins contain a lower amount of proanthocyanidins, they are more readily extractable and are an important contribution to wine phenolics (Meyer & Hernandez 1970). Skin proanthocyanidins were found to account for ~ 75-90% of total proanthocyanidins in wine in the first 4 days of fermentation as they are extracted earlier than seed proanthocyanidins (Peyrot des Gachons & Kennedy 2003). By the 8th day of fermentation, the wine contained closer to 50% each of seed and skin proanthocyanidin (Peyrot des Gachons & Kennedy 2003). Skin proanthocyanidins also have a higher mDP than seeds proanthocyanidins so they are a major source of polymeric proanthocyanidins in wine (Sun et al. 1999). This is important in terms of wine perception as skin proanthocyanidins are generally thought to provide an improved mouthfeel compared to seed derived proanthocyanidins while flavan-3-ol monomers are reported to have a negative attribute of increasing the bitterness of wine (Cheynier et al. 1998).

Berry size was thought to play a role in extraction due to the relationship between seed, skin and pulp (Coombe et al. 1987; Matthews and Anderson 1988)

although recent studies found that berry size alone did not have a major impact on extraction (Roby et al. 2004B; Walker et al. 2005). Small berries were found to have a similar skin to fruit ratio and similar juice yield compared to large berries and they did not find any indication of a higher anthocyanin content or improved color density in wine from assessing a range of berry sizes except for the very smallest berry category tested (0.3-0.55 g.) (Walker et al. 2005).

Higher anthocyanins or color density in wines was found from vines with greater sun exposure in the fruiting zone (Price et al. 1995; Mazza et al. 1999), from riper fruit (Sims and Bates 1994; Perez-Magarino & Gonzalez-San Jose 2004; Canals et al. 2005), and from deficit irrigation (Bravdo et al. 1985; Sipiora et al. 1998; Salon et al. 2005). However, in many of these studies it is hard to determine if the differences found in the wine were strictly related to berry size, anthocyanin content or if some other variable such as ease of extraction was also playing a role.

Anthocyanins are more water soluble then proanthocyanidins so the ease which anthocyanins can move from the vacuole and from the hypodermal cell into the wine is important (Sacchi et al. 2005). A recent study investigated the ease of anthocyanin extraction based on the different cell wall components and found that pectin and cellulose content were important (Ortega-Regules et al. 2006). Anthocyanin concentration reaches a maximum early in fermentation followed by a decrease (Nagel and Wulf 1979; Watson et al. 1995; Gao et al. 1997). Once anthocyanins have been extracted into the wine matrix, they rapidly form copigmentation complexes (Boulton 2001; Brouillard et al. 1994) and begin

undergoing numerous reactions. It has been suggested that having high levels of total phenolics and specifically copigments such as flavonols may help keep anthocyanins in solution through the phenomenon of copigmentation (Boulton, 2001; Schwarz et al. 2005; Lorenzo et al. 2005). Copigmentation has also been described as the first step toward the formation of more stable pigments (Liao et al. 1992; Brouillard; Boulton 2001). Several families of new pigmented compounds have been identified and described (Salas et al. 2005).

FORMATION OF PIGMENTED POLYMERS

Numerous studies have shown a strong relationship between pigmented polymers and color density (Somers 1971; Mazza 1995; Gao et al 1997). This is due to incorporation of monomeric anthocyanins into several classes of pigmented polymers. Peng et al. (2002) reported that pigmented polymers accounted for 50% of the color after two years of aging and Lee et al. (2004) found that pigmented polymers accounted for close to 70% of color after one year. However, much of the formation is thought to occur rapidly and early during fermentation (Morel-Salmi et al. 2006; Harbertson et al. 2002). The fermentation process has been described as a decrease in free anthocyanins with a concomitant increase in pigmented polymers (Somers 1971; Gao et al, 1997; Parley et al. 2001).

One mechanism is described as being a cycloaddition between anthocyanins and vinyl derivatives results in a class of pigments known as pyranoanthocyanins (Bakker & Timberlake 1997; Fulcrand et al. 1998). The formation of these

compounds involves a two electron oxidation to reform the anthocyanin moiety (Lee et al. 2004). Vitisin A, one of the first compounds of this class identified, is formed from the union of malvidin-3-glucoside with pyruvate (**Figure 1.9**) (Bakker and Timberlake 1997; Fulcrand et al. 1998). Pyruvic acid is a product of yeast glycolysis during fermentation. Pyruvic acid reaches maximum concentration when half of the sugars have been fermented so Vitisin A is mainly formed in wine in the period between 20% and 85% glucose utilization (Asenstorfer et al. 2003). The anthocyanin can react with a number of compounds that have a polarizable double-bond to form this class of compounds. At wine pH, they are orange-red pigments and have an absorbance maximum at 500nm (Lee et al. 2004). These compounds are resistant to oxidation and to bisulfite bleaching. Although these compounds have longevity in wine they play a limited role in red wine color due to their concentration.

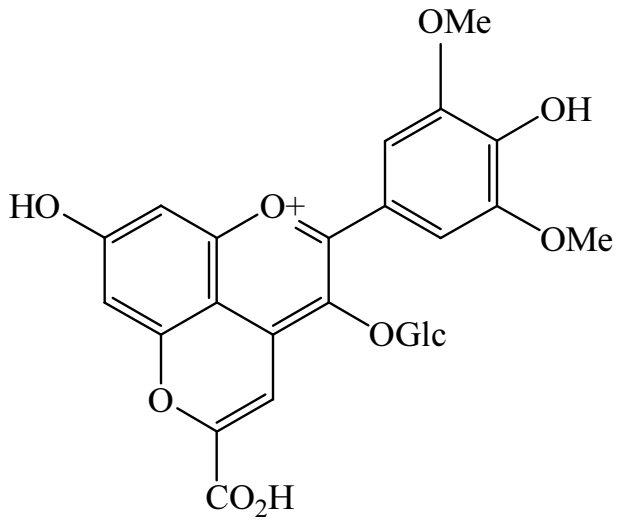


Figure 1.9. Structure of pigment formed by addition of pyruvic acid to malvidin 3-*O*-glucoside

The combination of an anthocyanin and a proanthocyanidin can occur through several mechanisms. One is through the formation of an ethyl-bridge via the enolic form of acetaldehyde (**Figure 1.10**) (Timberlake and Bridle, 1976; Saucier et al. 1997; Bishop and Nagel 1984). Acetaldehyde can be produced through yeast metabolism, ethanol oxidation or decarboxylation of pyruvic acid during fermentation (Liu 2000). These ethyl-linked pigments are formed early in fermentation and rapidly consume much of the monomeric anthocyanins. They have maximum absorbance at around 544 nm at wine pH and have a purple color. Compared to monomeric anthocyanins, they have increased resistance to hydration and bisulfite bleaching due to steric hindrance to nucleophilic attack at position 4 of the anthocyanin. These compounds are thought to be degraded relatively easily possible becoming reactive intermediates for other reactions.

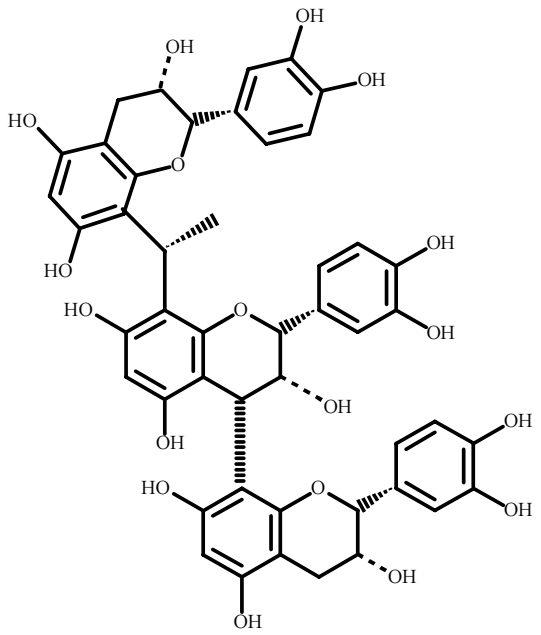


Figure 1.10. Structure showing an ethyl bridge.

Over time, the predominant pigment in aged red wine is from the direct reaction between anthocyanins and proanthocyanidins to form pigmented polymers (Remy et al. 2000). These mechanisms lead to tannin-anthocyanin (T - A+) and anthocyanin-tannin (A+ - T) adducts (**Figure 1.11**) (Salas et al. 2003). Both reactions are expected to be pH dependent and the T – A+ adducts are susceptible to acid-catalyzed cleavage and hydration (Salas et al. 2005; Salas et al. 2003). The existence of these compounds in wines has been recently confirmed by mass spectrometry (Hayasaka and Kennedy 2003). The structural diversity of pigmented polymers is expected to be large due to all the possible combinations of proanthocyanidins and anthocyanins.

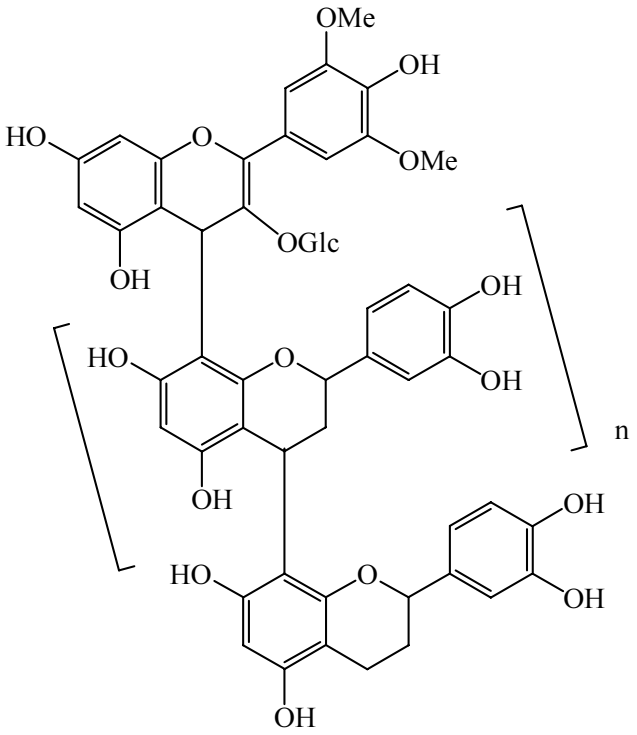


Figure 1.11. A-T pigmented polymer formed through direct condensation of malvidin-3-*O*-glucoside and flavan-3-ol subunits.

Although there has been recent evidence of pigmented polymers formed in the grape through direct condensation of anthocyanins (Vidal et al. 2004) this may be an artifact of sample processing. Pigmented polymer formation relates to the vineyard and fruit composition as the initial concentration of anthocyanins, proanthocyanidins and other phenolic compounds and compositional differences are likely to play a role in the rate of formation of these new compounds in wine (Fulcrand et al. 2004). The level of a specific anthocyanin-derived pigment was found to be related to the initial concentration of native anthocyanin precursors (Gómez-Cordovés 2004).

WINE ASTRINGENCY AND BITTERNESS

Astringency is an important sensory attribute of wine. Astringency and bitterness in wine is provided primarily by flavan-3-ols and proanthocyanidins phenolic compounds originating from the fruit (Noble 1994). Flavan-3-ols and proanthocyanidins are also important in wine because of their role in the formation of pigmented polymers, long-term color stability (Somers, 1971) and human health benefits (Santos-Buelga & Scalbert 2000; Dixon et al. 2004). There is interest in understanding the relationships between the chemical analyses of fruit and wine and the perception of astringency. Astringency has been difficult to study because of its long persistence and carry over effects in sensory studies (Lee & Lawless 1991; Valentová et al. 2002).

Astringency is a tactile sensation that can be described sensorially as mouth drying and puckering. Tannins are chemically defined as having molecular weights between 500 and 3000 and having the ability to precipitate proteins. In the case of wine, tannins precipitate salivary proteins. While monomeric flavan-3-ols are primarily bitter, as molecular weight increases with polymerization; astringency becomes predominate over bitterness (Noble 1994; Peleg et al. 1999). Consequently, large polymeric tannins from skins and seeds are the major contributors to wine astringency.

There are differences in grape skin and seed tannin composition and these are thought to have different astringent qualities in wine. Studies have shown there are differences in sensory properties related to the identity of the monomeric unit, the specific linkages, degree of galloylation and formation of derivatives (Peleg et al. 1999; Vidal et al. 2003; Lesschaeve & Noble 2005). Although it had been suggested that skin tannin played an important role in wine tannin (Meyer & Hernandez 1970), at this time studies have not been done to characterize the differences in skin and seed astringency. The impact of pigmented polymers on wine astringency has not been determined.

Other taste factors modify the intensity of astringency through enhancement or suppression. Increasing the ethanol content increased the intensity of bitterness but had no effect on astringency (Fischer et al. 1994). The addition of acid increased the astringency of wines (Kallithraka et al. 1997) while lowering the pH of wines increased the sourness but had no effect on bitterness (Fischer & Noble 1994).

Increasing sweetness in wine with sucrose decreased astringency (Ishikawa & Noble 1995). Increasing viscosity in wine has also been found to reduce astringency (Smith et al. 1996). Consequently, the mouth feel of wine can be modified by a number of compounds and interactions.

RESEARCH JUSTIFICATION AND OBJECTIVES

In Oregon, Pinot noir fruit sells for between $1500 to $3000/ton with an average price of $2100 in 2005 (OR AG. Statistics Service, 2005). Increasingly, winemakers have contracts for specific vineyard sites and pay by the ton or by the acre. Some premium quality fruit sells for $6,000 an acre or more with the average crop of approximately two tons. Unlike many other crops, a high proportion of the fruit is thinned off in order to achieve adequate ripening and high quality fruit. In addition, more wines are being sold as “vineyard designate” wines where the label lists a specific vineyard as the source of fruit.

Our understanding of how variations within vineyard site influence the development of phenolic compounds in fruit is not well documented. Research on the influences of vineyard sites on wine composition is particularly important in Oregon for several reasons: 1) there is an emphasis on producing premium wine, 2) many vineyards are non-irrigated or receive minimal irrigation, 3) most vineyards are planted on slopes consisting of more than one soil type, 4) sites of fruit based on a specific geographical location are deemed to be of superior quality, and 5) Oregon

is near the climatological limit in terms of being able to adequately ripen fruit for wine production and therefore site management is a critical concern.

In designing this project, we were interested in determining whether vineyard designated price differences were based on definable chemical differences in fruit and wine phenolic chemistry. This involved determining the extent of differences in phenolic accumulation and differences in composition due to spatial variations in vineyard sites. An additional objective was to assess the relative importance of environmental factors that played a role in differences in phenolic compounds in the fruit. The other major part of the research was to investigate the relationships between fruit and wine phenolic chemistry.

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

Jessica M. Cortell, Michael Halbleib, Andrew V. Gallagher,

Timothy L. Righetti, James A. Kennedy

Journal of Agricultural Food Chemistry
PO Box 3337,
Columbus, OH, 43210, USA
Volume 53, Number 14, pages 5798-5808

ABSTRACT

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 vineyard sites to produce research wines to investigate the vine-fruit-wine continuum.There was not a 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 while 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, while 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 phenolic compounds specifically important to red wine quality due to their astringent properties (Gawel 1998) and their role in long-term color stability (Somers 1971). There is also increasing interest in the potential role that proanthocyanidins have in human health (Santos-Buelga & Scalbert 2000). Grape-based proanthocyanidins contain the flavan-3-ol subunits (+)-catechin (C), (-)-epicatechin (EC), (-)-epicatechin-3-*O*-gallate (ECG) and (-)-epigallocatechin (EGC) (Prieur et al. 1994; Souquet et al. 1996; Romeyer, F.M.; Macheix & Sapis 1986; Czochanska, Foo & Lawrence 1979) (**Figure 2.1**). Skin proanthocyanidins differ from those found in seeds in that skins contain prodelphinidins (EGC), have a higher degree of polymerization and a lower proportion of galloylated subunits (Cheynier et al. 1998).

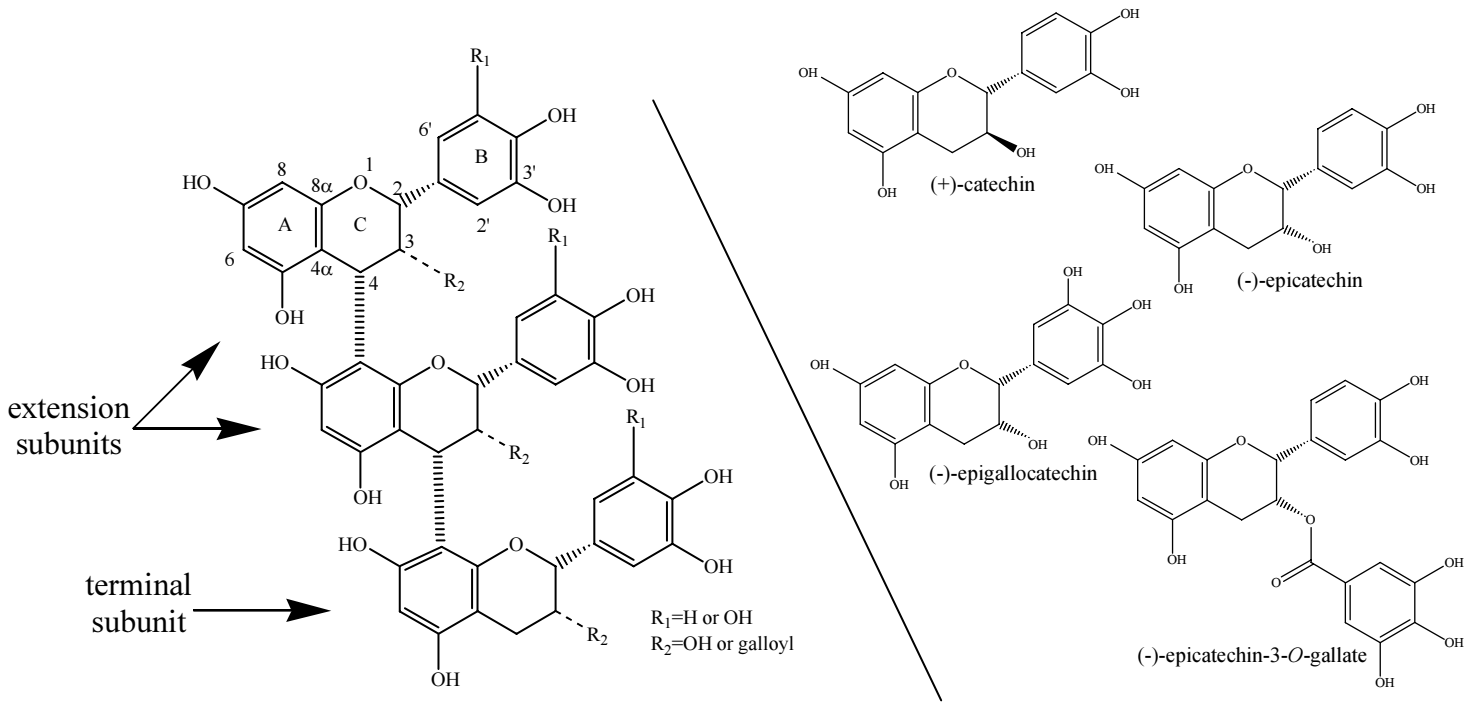


Figure 2.1. Generalized proanthocyanidin structure and grape-based proanthocyanidin subunits.

Given the complexity of plant growth, it can be difficult to separate the specific factors that cause changes in fruit composition. While relationships between environmental factors and grape composition have been investigated (Jackson & Lombard, 1993; Hardie & Considine 1976; Dokoozlian & Kliewer 1996; Bravdo et al. 1985; Gladstones 1992; Roby & Matthews 2003; Smart 1997), examples are limited (Spayd et al. 2002; Downey et al. 2004) in which the essential components that might affect fruit composition have been individually manipulated (i.e.: light, heat, water relations, nutrient content). Specific studies focused on grape seed and skin proanthocyanidin indicate that proanthocyanidins can significantly change in the developing berry (Ricardo da Silva et al. 1991; Kennedy, Matthews & Waterhouse 2000a; Kennedy et al. 2000b; Downey, Harvey & Robinson 2003), 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. While 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 (Hall et al. 2002; Bramley & Hamilton 2004).

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 the global positioning system

(GPS), remote sensing and geographical information systems (GIS) to link novel and traditional on-site measurements (physical, chemical and biological) to specific locations within a vineyard. Out of 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 (Hall et al. 2002; Bramley & Hamilton 2004). Previous research found a relationship between canopy structure and sunlight exposure and subsequent fruit phenolics (Mabrouk & Sinoquet 1998). 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 (Lamb et al. 2004). 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 in order 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 specific vineyard sites, A and B (**Figure 2.2**), known to produce wines with distinctly different price points (US $38.00/bottle versus US $75.00/bottle, respectively). These vineyard sites were in close proximity to each other, 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.

MATERIAL 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, USA. Vines were planted at a spacing of 1 m (within row) X 2.8 m (between rows) with approximately 5113 vines per hectare (**Figure 2.3a**). The training system was a vertical shoot position with each vine pruned to 10-12 buds. Two vineyard sites (A and B) were selected for study based upon historic evidence for phenolic variation, and were 1.28 and 0.21 hectares, respectively. These sites 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 approximately 25 soil cores per hectare. 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 based on 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 (-0.033Mpa 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 mm of water.

Vine Measurements. Data vines were established on a grid pattern in each site (consisting of every 15th vine in every other row, approximately 220 vines per hectare). The location was recorded by both vine and row coordinate and with a global positioning system (GPS) which had a measurement accuracy of ± 1 meter. The goal was to collect vine growth data between budbreak and véraison in order to divide the sites 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 meter, Minolta, USA) (one 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 precent 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 based upon 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 extrapolate a surface derived from the collected point feature field data. This technique, common to Precision Agriculture, has been previously applied in vineyards (Hall et al. 2002; Bramley & Hamilton 2004). With respect to the krieging, no trend removal was applied, a spherical search radius was used and the neighborhood was set to include all the data points.

Fruit Sampling and Extraction. Fruit samples were collected from the same grid spacing as was used for soil sampling. A second sample was collected across each vigor zone (3 replicates/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 (Kennedy et al. 2000a).

Winemaking. Triplicate wines were produced from each vigor zone. For each replicate, 35 kg fruit was destemmed with a Velo DPC 40 crusher/stemmer operated without the crusher, underwent a 2.5 day pre-fermentation cold maceration (10 °C) and then inoculated with Lalvin RC 212 yeast according to the manufacturer's guidelines. On day two of fermentation, wines were transferred to a water bath maintained at 32°C. Wines were punched down 2 times per day and pressed 6 days after inoculation (bladder-type press, Wilmes, Germany), to a maximum pressure of 2 bars.

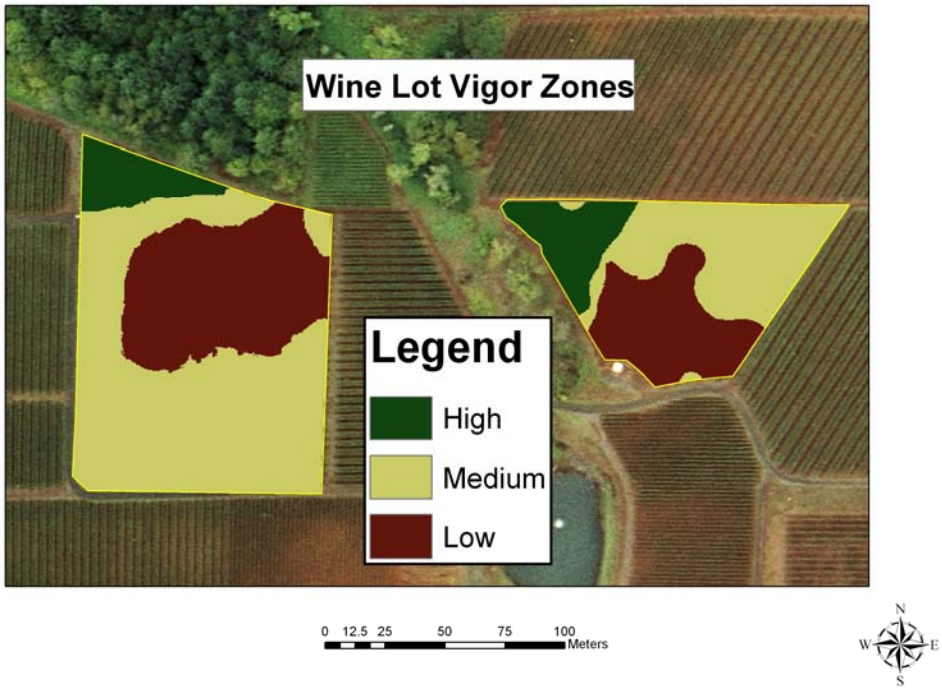


Figure 2.2. High resolution image with delineation of wine production vigor zones.

Wines were transferred into 5-gallon 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, 35 ppm SO_2 was added followed by 4 weeks cold stabilization and then bottled. The same time/temperature profile was maintained during all fermentations in order to reflect vineyard derived differences.

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). 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. An Agilent, Model 1100 HPLC (Palo Alto, CA) consisting of a vacuum degasser, autosampler, quaternary pump, diode array detector, 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 as previously described (Lamuela-Raventos &Waterhouse 1994). Aqueous extracts and wines were filtered using Teflon filters (0.45µm, 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 (Kennedy & Jones 2001) under modified HPLC conditions (Kennedy and Taylor, 2003). Phloroglucinolysis provided information on subunit composition, conversion yield and mean degree of polymerization (mDP). To prepare seed and skin extracts for analysis, 3 mL aqueous extract was freeze dried and then dissolved in 5 mL (seed) or 2 mL (skin) 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 water, and then applied to a C18-SPE column (1 g Alltech) after activation with 10 mL methanol followed by 15 mL water. After sample application, the column was washed with 15 mL water and eluted with 10 mL methanol.The methanolic solution was divided into two 5 mL samples. One sample was prepared for phloroglucinolysis and the other for GPC. For phloroglucinolysis, the methanolic sample was evaporated under reduced pressure and 40 °C, reconstituted into 1 mL 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 (Peyrot des Gachons & Kennedy 2003). The percent skin proanthocyanidin extracted from the fruit into the wine was calculated based on the ratio of EGC/EC in the fruit and wine for each vigor zone/rep combination.

Gel Permeation Chromatography. Gel Permeation Chromatography (GPC) was used to analyze intact proanthocyanidins. 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 previously described (Kennedy & Taylor 2003). Samples were prepared as described above; however, after freeze drying they were dissolved in mobile phase. Malvidin-3-glucoside was obtained from Polyphenols Labs (Sandness, Norway) and used as a standard for GPC analysis at 520 nm, while (+)-catechin was used as the quantitative standard at 280 nm.

SO_2 Bleaching of Wines. Wines were subjected to bleaching with SO_2 using a previously described method (Somers & Evans 1977).

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 $\alpha = 0.05$ or less. For vine growth, data vines within vigor zones were treated as independent samples. Tukey's adjusted p-values were 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 PAB (photosynthetically active biomass), based upon a relative index (**Figure 2.3b**) of average shoot length, trunk circumference and leaf chlorophyll content were used to delineate high, medium, and low vigor zones within each vineyard site so that research wines could be produced (**Figure 2.2, Table 2.1**). 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 (Hall et al. 2002). 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 (Ponder, 1998; Romero et al. 2004).

Table 2.1. Vine growth and yield between sites and vigor zones[a]

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

Average shoot length and leaf chlorophyll content (SPAD units) decreased with decreasing vigor in both sites. Trunk cross sectional area was similar between high and medium vigor zones in both sites while trunks in the low vigor zone were significantly smaller. The vigor index was significant in separating different levels of vigor in both vineyard sites (**Figure 2.3b, Table 2.1).** 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 2.1**). When each site 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, leaf chlorophyll content) and fruit yield and composition such as total phenolics and color (Mabrouk & Sinoquet 1998; Hall et al. 2002; Bramley and Hamilton 2004). Yield variations of up to 10 fold in the vineyard have been

associated with fruit composition differences (Bramley & Hamilton 2004). In our study, there was a 40% reduction in yield (A-low vs B-low, **Table 2.1**); however, vigor variation did not vary linearly with yield. The medium vigor zones had the highest yield while vigor extremes were lower yielding. B-low had a significant yield reduction and based upon 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 fruit set.

Table 2.2. Average berry weight, soluble solids, and seeds per berry between vine vigor zones[a].

Site	Zone	Average berry weight (g)	Soluble solids (°Brix)	Seeds per berry
A	high	0.99[a]	23.5[d]	1.31[c]
	med	0.91[ab]	24.3[a]	1.37[bc]
	low	0.87[bc]	24.1[b]	1.56[a]
B	high	0.82[bc]	23.7[c]	1.45[abc]
	med	0.87[bc]	24.0[b]	1.50[ab]
	low	0.78[c]	24.4[a]	1.59[a]
p-value[a]		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 $p \geq 0.05$.

In general, berry weight increased with vigor (**Table 2.2**). However, the only significant difference in berry weight was in comparing A-high with B-low where B-low was 0.21 gm/berry lower (Tukey adj. p=0.0053, (CI; 0.063, 0.35)). Based upon surface area to volume ratio, berry weight is generally thought to influence wine phenolic concentration (Coombe et al. 1987), although phenolic concentration has been shown to vary independently of berry size (Roby et al. 2004).

Figure 2.3. High resolution image of vineyard site A (west) and site B (east) highlighted (a) and vine vigor index variation (b).

Analysis of Seed. In general, there were a greater number of seeds per berry in low vigor zones compared to high vigor zones (**Table 2.2**). Dry seed mass per berry showed a similar trend (data not shown).

Although there was an overall reduction in total flavan-3-ol monomers per seed with a reduction in vigor (**Table 2.3**), there was no significant difference when calculated on a per berry basis. This was due to the higher number of seeds per berry in the low vigor zones (**Table 2.2**). 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 2.3**). It has been observed that during fruit ripening the amount of flavan-3-ol monomer declines, and the proportion of C declines (Kennedy et al. 2000a; Kennedy et al. 2000b; Downey et al. 2003). Given 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-med (**Table 2.3**); 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 (Kennedy et al. 2000b; Roby et al. 2004) and light exclusion (Downey et al. 2004). 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 (Harbertson et al. 2002). 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 while EC and ECG decreased. Overall, the results of this study indicate that the amount of grape seed proanthocyanidins was independent of vine vigor while 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 (Kennedy et al. 2002; Monagas et al. 2003), these components were not quantified.

By phloroglucinolysis, per berry (**Figure 2.4a, Table 2.4**) and per berry weight proanthocyanidin amount increased substantially in skins with decreasing vine vigor. B-low had an increase of approximately 42% in total extension subunits compared to A-high ($p=0.0014$, $n=3$).

Table 2.3. Seed flavan-3-ol monomer and proanthocyanidin concentration and percent composition analysis by phloroglucinolysis.

Flavan-3-ol Monomers

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

Procyanidin

		Concentration[b]			Extension[c]			Terminal[c]		
Site	Zone	Extension nmol/seed	Terminal nmol/seed	Total nmol/seed	C %	EC %	ECG %	C %	EC %	ECG %
A	high	6205.4[a]	1733.5[a]	7938.9[a]	12.0[c]	76.2[a]	11.7[a]	53.2[ab]	33.9[a]	12.9[b]
	med	6268.5[a]	1516.7[a]	7785.1[a]	13.8[bc]	75.2[ab]	11.0[ab]	43.9[b]	39.0[a]	17.1[a]
	low	6027.9[ab]	1637.8[a]	7665.6[ab]	15.2[ab]	74.3[ab]	10.5[bc]	54.9[ab]	31.8[a]	13.3[ab]
B	high	5200.7[b]	1288.1[a]	6488.9[b]	15.5[ab]	74.4[ab]	10.1[bc]	54.6[ab]	32.0[a]	13.4[ab]
	med	5940.2[ab]	1712.9[a]	7653.1[ab]	16.5[a]	73.4[b]	10.1[c]	58.5[a]	30.2[a]	11.4[b]
	low	5601.7[ab]	1480.6[a]	7082.3[ab]	16.5[a]	73.8[b]	9.7[c]	57.9[ab]	29.5[a]	12.6[b]
p-value[a]		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.

In comparing A-high with B-low, there was approximately a 19% 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 2.5)** a significant increase was also found in total proanthocyanidin amount (mg/berry) in both sites with decreasing vine vigor, consistent with results observed by phloroglucinolysis (**Table 2.4**). The greatest increase was between A-high and B-low where there was an approximate 69 % increase in total proanthocyanidin (mg/berry) (**Table 2.5**). Small differences in total proanthocyanidin amount at harvest have been observed with respect to light exposure (Downey et al. 2004) and vine water status (*37*).

Skin extension subunits consisted of C, EC, ECG and EGC, in agreement with others (Souquet et al. 1996; Downey et al. 2003; Harbertson et al. 2002). EC and EGC were the primary extension subunits, also in agreement with other studies (Souquet et al. 1996; Downey et al. 2003; Kennedy et al. 2002; Kennedy et al. 2001). 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 2.4**). A similar pattern was observed in the surface map of percent EGC (**Figure 2.4b**). In comparing the extremes in vine vigor zones, EGC increased by 6.4 % (Tukey adj. p=0.0023, (CI; 2.50, 10.73)) in B-low compared to A-high. Calculated on a nmol per berry basis instead of percent, this was an approximate two-fold increase (from B-low to A-high) in EGC containing a

trihydroxlated B-ring. In previous research, a shift was found toward a decrease in trihydroxylation compared to dihydroxylation of the B-ring with cluster shading (Downey et al. 2004). This suggests the substitution pattern on the B-ring may be influenced by differences in fruit sun exposure. In addition to environmental factors (Downey et al. 2004; Kennedy et al. 2001), fruit maturity appears to have an influence on EGC proportion (Downey et al. 2003; Kennedy et al. 2002). In this study, it could not be determined if the differences observed in proanthocyanidin proportion were due to maturity, the environment or to a combination of these effects.

By phloroglucinolysis, the mDP for A-high was lower than B-low by 10.54 (Tukey adj. p=0.002, (CI; 4.19, 16.88)). The difference between the extremes was more obvious than the intermediate levels of vigor. In other work, the molecular weight of skin proanthocyanidin has been found to increase with maturity (Souquet et al. 1996; Kennedy et al. 2001; Kennedy et al. 2002). Skin mDP has been observed to increase during the early phase of berry development but then decreases after véraison (Downey et al. 2003). Downey et al. (2004) 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 vines is related to differences in ripening. This explanation seems less likely given the minimal differences in soluble solids (**Table 2.2**).

Table 2.4. Skin proanthocyanidin concentration and percent composition analysis by phloroglucinolysis.

Site	Zone	Extension nmol/berry[b]	Terminal nmol/berry[c]	mDP	Total nmol/berry[d]	C[e] %	EC %	EGC %	ECG %
A	high	2002.9[c]	74.4[c]	27.95[c]	2077.3[c]	2.2[a]	71.9[a]	24.4[c]	1.6[a]
	med	2453.6[cb]	69.4[c]	36.23[ab]	2523.0[cb]	2.2[a]	68.9[b]	27.2[b]	1.7[a]
	low	3439.0[a]	107.3[a]	33.04[b]	3546.3[a]	2.2[a]	66.8[bc]	29.2[ab]	1.8[a]
B	high	2892.4[ab]	83.0[bc]	35.71[ab]	2975.4[ab]	1.7[b]	66.6[bc]	30.0[a]	1.7[a]
	med	3331.5[a]	95.9[ab]	35.78[ab]	3427.3[a]	2.1[a]	65.7[c]	30.7[a]	1.6[a]
	low	3459.5[a]	91.8[b]	38.71[a]	3551.3[a]	2.3[a]	65.1[c]	30.8[a]	1.8[a]
p-value[a]		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.

By GPC, the molecular mass at 50% elution increased with a reduction in vigor (**Table 2.5**). 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 (Kennedy et al. 2002).

GPC was used to determine pigmented polymer content in grape skins (**Figure 2.4c, Table 2.5**). In this experiment, there was an approximate 75% increase in per berry pigmented polymer content from A-high to B-low (p=0.001, n=3). While the presence of pigmented polymer in the grape has been observed previously (Kennedy et al. 2001; Kennedy et al. 2002), its origin is not clear. Recent evidence suggests that pigmented polymers may include oligomeric anthocyanins (Vidal et al. 2004). However, the presence of pigmented compounds could also be an artefact of sample preparation and extraction.

Table 2.5. Skin proanthocyanidin analysis by gel permeation chromatography.

Site	Zone	Molecular mass 50% (g/mol)	Tannin (mg/berry)	Pigmented polymer (mg/berry)
A	high	9915^{d}	1.15^{d}	0.32^{d}
	med	10680^{c}	1.34^{cd}	0.44^{bc}
	low	11183^{bc}	1.79^{ab}	0.48^{abc}
B	high	12224^{a}	1.52^{bc}	0.39^{cd}
	med	11258^{b}	1.69^{ab}	0.49^{ab}
	low	11517^{b}	1.94^{a}	0.56^{a}
p-value[a]		<.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 $p \geq 0.05$.

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 2.4a-c**). An apparent relationship was observed between vine vigor (**Figure 2.2b**) and the concentration of skin proanthocyanidin (mg/berry, **Figure 2.4a**), percent skin EGC extension subunits (**Figure 2.4b**) and pigmented polymers (mg/berry, **Figure 2.4c**). The findings in this study of minimal differences in seed proanthocyanidin while there were substantial variations in skin proanthocyanidin in response to vine vigor agrees with previous findings on a differential response between seed and skin proanthocyanidins (Downey et al. 2004).

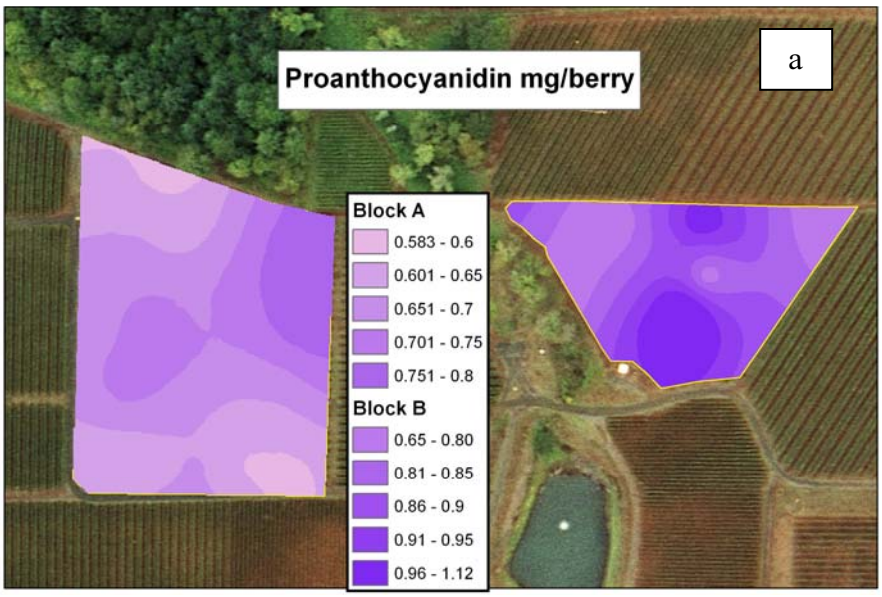
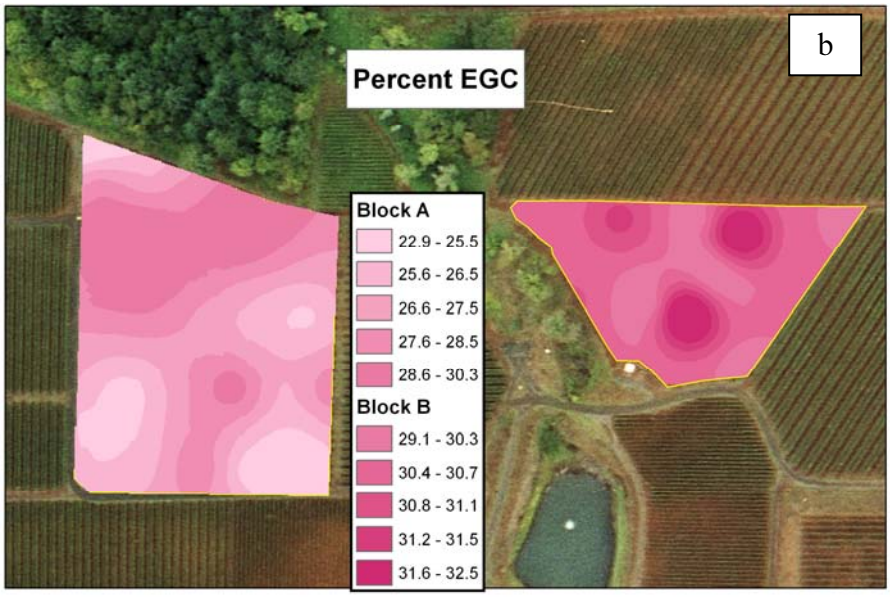
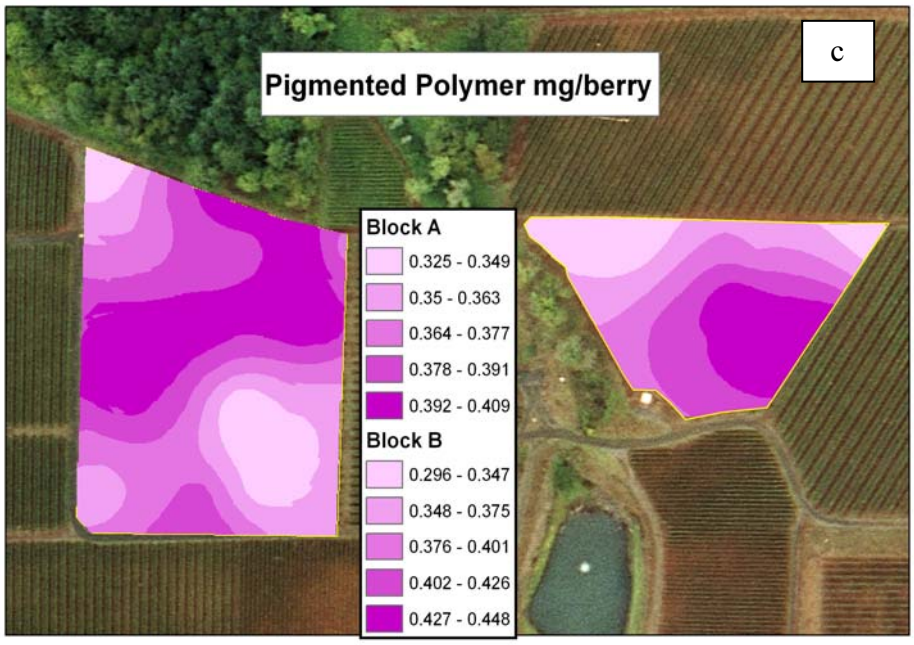


Figure 2.4. Grape skin proanthocyanidin chemistry including concentration of grape skin proanthocyanidins (a) and % (-)-epigallocatechin extension subunits (b) determined by phloroglucinolysis.





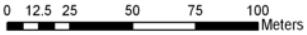


Figure 2.4 cont. Grape skin proanthocyanidin chemistry including incorporation of 520 nm absorbing material or pigmented polymer (c) determined by GPC.

In this particular study that used light exclusion boxes in cv. Shiraz (Downey et al. 2004), 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 2.5**). In this study, Total (skin + 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 high vigor regions (**Figure 2.5**). Total proanthocyanidin amount in the fruit increased approximately 60 % in comparing A-high 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).

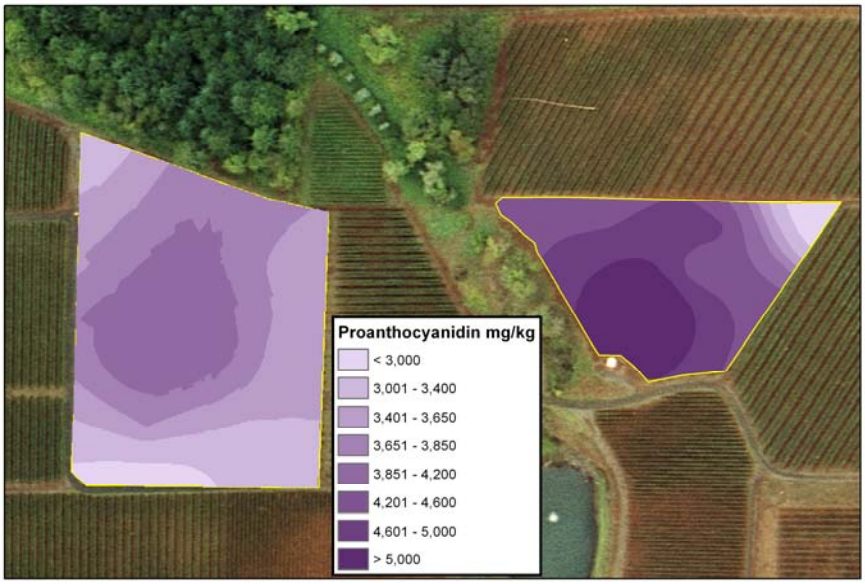


Figure 2.5. Surface map of total (seed + skin) proanthocyanidin (mg/kg) in fruit at harvest.

An increase in flavan-3-ol monomers in wines was observed with an increase in grapevine vigor (**Table 2.6**). In comparing A-high to B-low, A-high had a 0.06 mM (Tukey adj. p=0 0.0001, (CI; 0.03, 0.09)) increase in monomer concentration compared to the B-low vigor zone. The proportion of C and EC was similar to the relationship found in seeds (**Table 2.3**) although there was an approximate 10-20% increase in catechin compared to epicatechin in the wine (**Table 2.6**). This increase in the proportion of C in wine relative to seed has been observed in other studies (Sun et al. 1999; De Freitas et al. 2000). 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) to 20% (A-high) of the total flavan-3-ol fraction (**Table 2.6**). This is similar to other research where the polymeric fraction in wines represented 75-81% of total flavan-3-ols in seeds and 94-98% in skins (Monagas et al. 2003). The presence of low molecular weight flavanols may be important in terms of increasing the perception of bitterness in wine (Cheynier et al. 1998).

Overall, there appeared to be a relationship between the total proanthocyanidin (skin + seed) concentration in the fruit expressed by weight (**Figure 2.5**), and the proanthocyanidin concentration in the wines (**Table 2.8**). This is of interest because winemakers in general would like to develop a means to predict 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 2.6**). 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. In terms of compositional differences, there was a higher proportion and concentration of galloylated flavan-3-ols in wines made from lower vigor vines (**Table 2.6**). There was a little over a three-fold increase in galloylated derivatives between the A-high and B-low wine on a mM 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 (Pérez-Magarino & González 2004).

A direct relationship between the distribution of seed and skin proanthocyanidins in fruit and those in wine does not exist (Peyrot des Gachons & Kennedy 2003). Given the apparent differences in sensory properties between seed and skin proanthocyanidins in wine (Cheynier et al. 1998), it is of interest to better understand the relationship between fruit proanthocyanidin distribution in fruit and differential extraction into wine. Based upon seed and skin subunit analysis (**Table 2.7**), seed proanthocyanidin extraction into wine remained relatively constant with vigor. The proportion and amount of EGC in wine increased indicating that skin proanthocyanidin extraction increased dramatically with a decrease in grapevine vigor (**Table 2.7**, **Figure 2.6**). 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 (Monagas et al. 2003). In wine there was a trend toward an increase in mDP in site B with decreasing vigor but not in site A (**Table 2.8**). However, molecular mass at 50 % elution determined by GPC showed an increase in wine proanthocyanidin molecular size with decreasing vigor.

There was a strong relationship between the vigor index, proanthocyanidin production in the grape and resulting proanthocyanidin concentration in the wines (**Figure 2.7**).

Table 2.6. Wine flavan-3-ol monomer and proanthocyanidin concentration and composition by phloroglucinolysis.

Flavan-3-ol monomers

Site	Zone	Monomer mM	C %	EC %
A	high	0.18[a]	77.3[c]	22.7[a]
	med	0.17[ab]	75.7[c]	24.3[a]
	low	0.16[ab]	77.6[c]	22.4[a]
B	high	0.13[c]	84.0[b]	16.1[b]
	med	0.12[c]	86.6[a]	13.4[c]
	low	0.12[c]	88.0[a]	12.0[c]
p-value[a]		<.0001	<.0001	<.0001

Proanthocyanidins

		Concentration			Extension				Terminal	
		Extension	Terminal	Total	C	EC	ECG	EGC	C	EC
Site	Zone	mM	mM	mM	%	%	%	%	%	%
A	high	0.64[d]	0.08[c]	0.72[d]	5.0[ab]	78.2[a]	4.8[a]	12.0[d]	73.0[a]	27.0[a]
	med	0.95[c]	0.09[bc]	1.05[c]	3.9[b]	76.1[b]	3.1[b]	16.9[c]	86.9[a]	13.1[a]
	low	1.24[b]	0.15[a]	1.39[b]	4.1[ab]	74.4[bc]	2.1[c]	19.4[b]	78.2[a]	21.8[a]
B	high	1.20[b]	0.13[ab]	1.33[b]	5.3[a]	73.6[cd]	0.9[d]	20.3[b]	74.1[a]	25.9[a]
	med	1.20[b]	0.11[abc]	1.31[b]	4.6[ab]	72.1[ed]	0.7[d]	22.7[a]	66.3[a]	33.7[a]
	low	1.46[a]	0.13[ab]	1.59[a]	4.2[ab]	71.3[e]	0.7[d]	23.8[a]	67.4[a]	32.6[a]
p-value[a]		<.0001	0.0358	<.0001	0.2006	0.0001	<.0001	<.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 2.7. Extraction of skin and seed proanthocyanidin into wine as determined by phloroglucinolysis.

Site	Zone	Total Proanthocyanidin (mg/L)	Skin extracted (%)	Skin proanthocyanidin (mg/L)	Seed proanthocyanidin (mg/L)
A	high	268.6[d]	52.8[d]	142.0[c]	127.04[ab]
	med	361.9[c]	64.3[c]	232.9[b]	129.0[ab]
	low	457.6[ab]	68.0[bc]	311.1[a]	146.5[a]
B	high	432.8[b]	70.0[ab]	319.5[a]	113.3[b]
	med	423.5[b]	75.3[ab]	307.4[a]	116.1[ab]
	low	504.3[a]	77.9[a]	387.9[a]	116.3[b]
p-value[a]		<.0001	<.0001	<.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 2.8. Wine tannin analysis by gel permeation chromatography and sulfite bleaching.

Site	Zone	Tannin (mg/L)	MW 50 % elution (gm/mol)	Pigmented polymer (mg/L)	Sulfite resistant pigment
A	high	1040[e]	1146.5[c]	632[e]	0.94[f]
	med	1340[d]	1235.5[c]	844[d]	1.28[e]
	low	1586[c]	1506.2[b]	1090[b]	2.04[d]
B	high	1611[c]	1478.3[b]	989[c]	1.59[c]
	med	1792[b]	1751.3[a]	1223[b]	2.56[b]
	low	2051[a]	1778.4[a]	1459[a]	3.30[a]
p-value[a]		<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 $p \geq 0.05$.

There was almost a two-fold increase in total tannin in wines made from the B-low vigor zone compared to wines made from the to A-high vigor zone (**Table 2.8**). Although there was a strong relationship overall (**Figure 2.7c**), the relationship appears to be driven by the skin proanthocyanidins (**Figure 2.7b**) as opposed to the seed procyanidins (**Figure 2.7a).** In total, approximately 11% of total proanthocyanidins were extracted from the grape across all vigor zones (r^2=0.87). When only skin proanthocyanidins were considered, extraction increased to 30% with a stronger correlation (r^2=0.93). Overall, these data suggest that wine proanthocyanidin composition is driven by the amount of proanthocyanidin material present in the fruit (assuming constant winemaking), and diffusion of skin proanthocyanidins into wine did not appear to vary with vigor.

The pigmented polymer concentration in wine was determined by GPC (**Table 2.8**). As described previously, there was an approximate 70.5 % increase in pigmented polymers in the grape skins with decreasing vigor (A-high to B-low, **Table 2.5**). In wine, this difference was greater (than found in skins) with a two-fold increase in comparing A-high to B-low (**Table 2.8**). Overall, these quantities seemed to be quite high compared with previous work (Peng et al. 2002), and upon comparing the results with those by reversed-phase HPLC, it was realized that the response of the standard to the GPC conditions was different than 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.

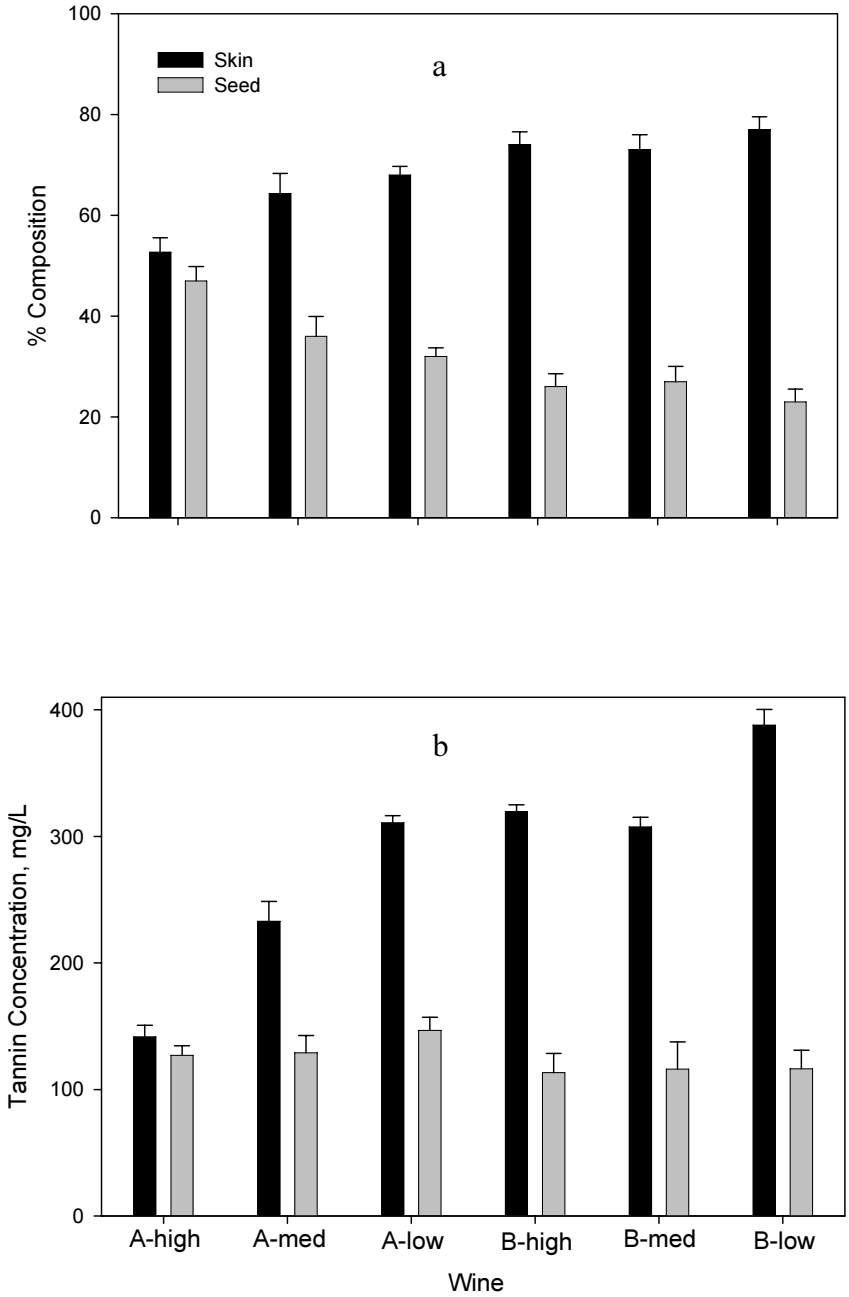


Figure 2.6. Fractional composition (a) and concentration (b) of skin and seed proanthocyanidins in red wines made from grapes sourced from different vigor zones, and with error bars indicating ±SEM (n=3).

Nevertheless, the trends across vigor zones were similar when GPC results were compared with sulfite resistant pigments (**Table 2.8**). Furthermore, the strong relationship between pigmented polymer by GPC and sulfite resistant pigment (r^2=0.97) suggests that while questions remain with regard to the nature and source of pigmented polymers in grapes (artefact 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.

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 ramifications related to wine quality.

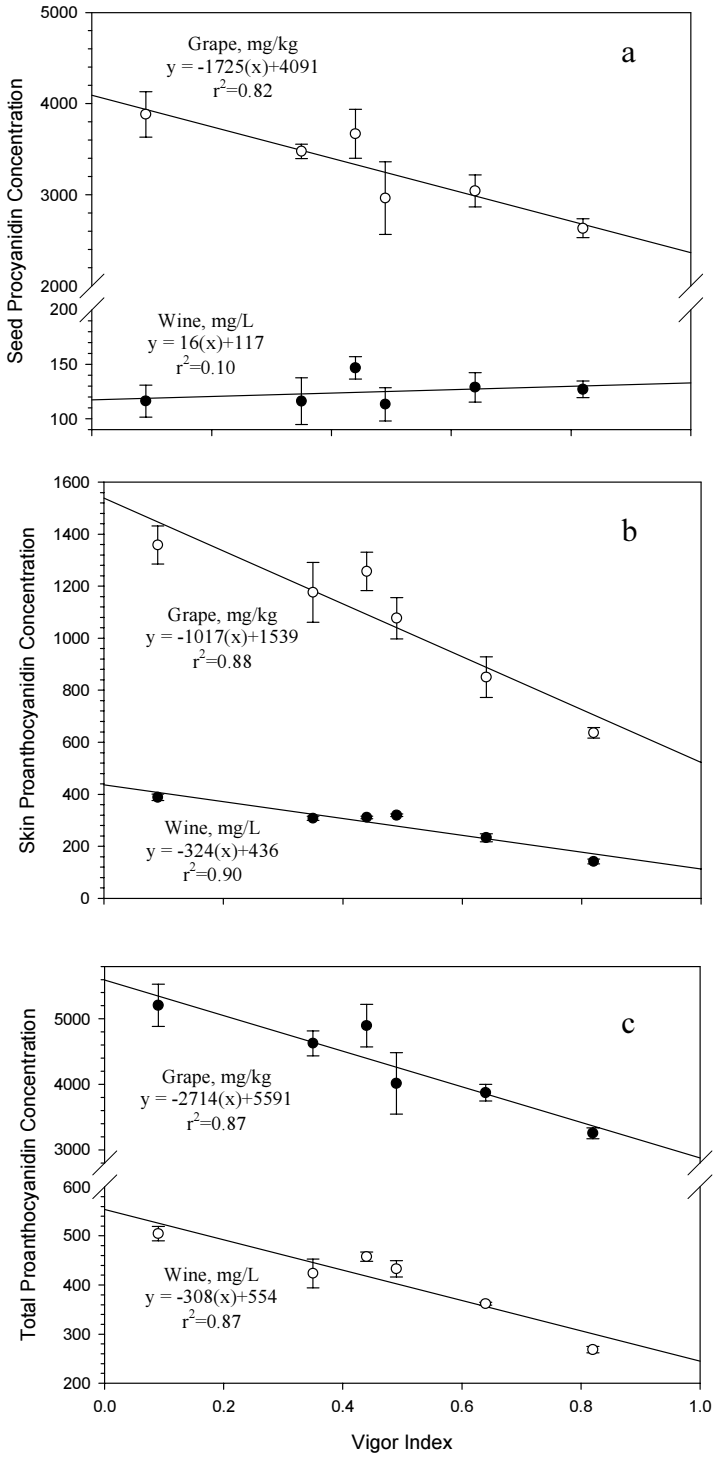


Figure 2.7 Concentration of proanthocyanidins in seed (a), skin (b) and skin + seed (c) in grapes at harvest and in the corresponding wine, and with error bars indicating ±SEM (n=3).

For example, skin proanthocyanidins and pigmented polymers in wines are considered to have an affect on proanthocyanidin perception (Cheynier et al. 1998; Glories & Saucier 2000). 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.

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 ramifications related to wine quality. For example, skin proanthocyanidins and pigmented polymers in wines are considered to have an affect on proanthocyanidin perception (Cheynier et al. 1998; Glories & Saucier 2000). 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 sites that were considered by the winemaker to produce wine of differing quality. 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: 1) reduce the time needed to divide sites by vine vigor and wine composition, 2) develop rapid vineyard fruit sampling assessment techniques and 3) utilize these results to modify vineyard practices to produce fruit to specification.

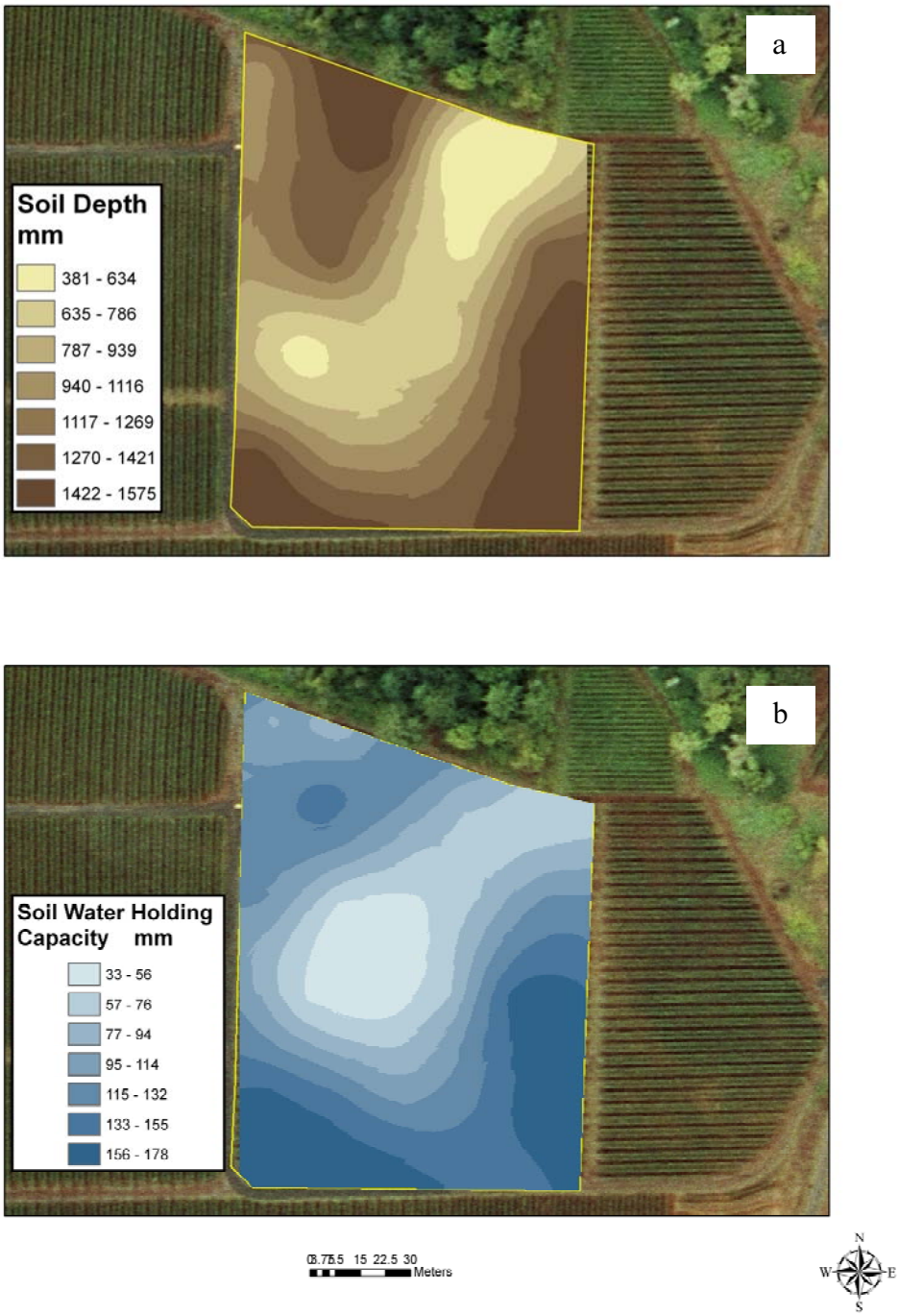


Figure 2.8. Surface map of soil depth (a) and corresponding water holding capacity (b) for site A.

To reduce analysis time, the use of high resolution images is a preferred choice that is being investigated by several researchers (Hall et al. 2002; Bramley & Hamilton 2004; Mabrouk & Sinoquet 1998).

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 (**Figures 2.8a** and **b**); 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 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 towards understanding the differences in plant response to environment in terms of fruit ripening biochemistry.

ABBREVIATIONS USED

(+)-catechin (C), (-)-epicatechin (EC), (-)-epicatechin-3-*O*-gallate (ECG) and (-)-epigallocatechin (EGC), Precision agriculture (PA), gel permeation chromatography (GPC), mean degree of polymerization (mDP), available water holding capacity

(AWHC), National Soil Survey Laboratory (NSSL), 95% Confidence Interval (CI).

ACKNOWLEDGMENTS

We thank Archery Summit Winery for the use of their vineyards and fruit for wine production as well as Leigh Bartholomew and Anna Matzinger specifically for their support. In addition, we would like to thank Jason Bell, Fiorella Cerpa, Jose Luis Pastor del Rio and Chris Zielinski for their help. Funding for this project was provided by the USDA Northwest Center for Small Fruit Research and the American Society for Enology and Viticulture.

LITERATURE CITED

Bramley, R.G.V.; Hamilton, R.P. Understanding variability in winegrape production systems 1. Within vineyard variation in yield over several vintages. *Aust. J. Grape Wine Res.* **2004,** 1, 32-45.

Bravdo, B.; Hepner, Y.; Loinger, C; Cohen, S; Tabacman, H. Effect of irrigation and crop level on growth, yield and wine quality of cv. Cabernet Sauvignon. *Am. J. Enol. Vitic.* **1985,** 36, 132-139.

Gawel, R. Red wine astringency: a review. *Aust. J. Grape Wine Res.* **1998,** 4, 74-95.

Cheynier, v.; Fulcrand, H.; Brossaud, F.; Asselin, C.; Moutounet, M. Phenolic composition as related to red wine flavor. In *Chemistry of Wine Flavor*. A.L. Waterhouse and S.E. Ebeler Eds. Oxford University Publishing, New York, NY. **1998.** pp. 125-141.

Coombe, B.G.; Bovio, M.; Schneider, A. Solute accumulation by grape pericarp cells V. Relationship to berry size and the effects of defoliation. *Journal of Experimental Biology.* **1987**. 38, 1789-1798.

Czochanska, Z.; Foo, L.Y.; Lawrence, J. P. Compositional changes in lower molecular weight flavans during grape maturation. *Phytochemistry.* **1979,** 18, 1819-1822.

De Frietas, V.A.P.; Glories, Y.; Monique, A. Developmental changes of procyanidins in grapes of red *Vitis vinifera* varieties and their composition in respective wines. *Am. J. Enol. Vitic.* **2000**, *51*, 397-403.

Dokoozlian, N.K.; Kliewer, W.M. Influence of light on grape berry growth and composition varies during fruit development. *J. Amer. Soc. Hort. Sci.* **1996,** 121, 869-874.

Downey, M.O.; Harvey, J.S.; Robinson, S.P. Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. *Aust. J. Grape Wine Res.* **2003,** 9, 15-27.

Downey, M.O.; Harvey, J.S.; Robinson, S.P. The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. *Aust. J. Grape Wine Res.* **2004,** 1, 55-73.

Gladstones, J.S. *Viticulture and Environment* Winetitles, Adelaide, Soth Australia, 1992.

Glories, Y.; Saucier, C. Tannin evolution from grape to wine. Effects on wine taste. *Proc. Amer. Soc. Vit. Enol. 50th Anniversary Annual* Meeting, Seattle, Washington. **2000,** 353-355.

Hall, A.; Lamb, D.W.; Holzapfel, B.; and Louis, J. Optical remote sensing applications for viticulture – a review. *Aust. J. Grape Wine Res.* **2002,** 8, 36-47.

Harbertson, J.F.; Kennedy, J. A.; Adams, D.O. Tannin in skins and seeds of Cabernet Sauvignon, Syrah, and Pinot noir berries during ripening. *Am. J. Enol. Vitic.* **2002,** 53, 54-59.

Hardie, W.J.; Considine, J. A. Response of grapes to water deficit stress in particular stages of development. *Am. J. Enol. Vitic.* **1976,** 27, 55-61.

Jackson, D.I.; Lombard, P.B. Environmental and management practices affecting grape composition and wine quality – A review. *Am. J. Enol. Vitic.* **1993,** 44, 409-430.

Kennedy, J.A.; Matthews, M.A.; Waterhouse, A.L. Changes in grape seed polyphenols during fruit ripening. *Phytochemistry.* **2000a.** 55, 77-85.

Kennedy, J.A.; Troup, G.J.; Pilbrow, J.R.; Hutton, D.R.; Hewitt, D.; Hunter, C.R.; Ristic, R.; Iland, P.G.; Jones, G.P. Development of seed polyphenolics in berries from *Vitis vinifera* L. cv Shiraz. *Aust. J. Grape Wine Res.* **2000b,** 6, 244-254.

Kennedy, J.A.; Jones, G.P. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J. Agric. Food Chem.* **2001,** 49, 1740-1746.

Kennedy, J.A.; Hayasaka, Y; Vidal, S; Waters, E.J.; Jones, G.P. Composition of grape skin proanthocyanidins at different stages of berry development. *J. Agric. Food Chem.* **2001,** 49, 5348-5355.

Kennedy, J.A.; Matthews, M.A.; Waterhouse, A.L. Effect of maturity and vine water status on grape skin and wine flavonoids. *Am. J. Enol. Vitic.* **2002,** 53, 268-274.

Kennedy, J.A.; Taylor, A.W. Analysis of proanthocyanidins by high-performance gel permeation chromatography. *J. Chromatogr. A.* **2003,** 995, 99-107.

Lamb, D.W.; Weedon, M.M.; Bramley, R.G.V. Using remote sensing to predict grape phenolics and colour at harvest in a Cabernet Sauvignon vineyard: Timing observations against vine phenology and optimizing image resolution. *Aust. J. Grape Wine Res.* **2004,** 10, 46-54.

Lamuela-Raventos, R.M.; Waterhouse, A.L. A direct HPLC separation of wine phenolics. *Am. J. Enol. Vitic.* **1994,** 45, 1-5.

Mabrouk, H; Sinoquet, H. Indices of light microclimate and canopy structure of grapevines determined by 3D digitizing and image analysis, and their relationship to grape quality. *Aust. J. Grape Wine Res.* **1998,** 4, 2-13.

Monagas, M.; Gómez-Cordovés, C.; Bartolomé, B.; Laureano, O.; Ricardo Da Silva, J. Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from Vitis vinifera L. Cv. Graciano, Tempranillo, and Cabernet Sauvignon. *J. Agric. Food Chem.* **2003,** 51, 6475-6481.

Peng, Z.; Iland, P.G.; Oberholster, A.; Sefton, M.A.; Waters, E.J. Analysis of pigmented polymers by reverse phase HPLC. *Aust. J. Grape Wine Res.* **2002,** 8, 70-75.

Peyrot des Gachons, C.; Kennedy, J.A. Direct Method for Determining seed and skin proanthocyanidin extraction in red wine. *J. Agric. Food Chem.* **2003,** 51, 5877-5881.

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

Prieur, C; Rigaud, J.; Cheynier, V; Moutounet, M. Oligomeric and polymeric proanthocyanidins from grape seeds. *Phytochemistry.* **1994,** 36, 781-784.

Ponder, F. Jr.; Fertilizer combinations benefit diameter growth of plantation black walnut. *J. of Plant Nutrition.* **1998.** 21, 1329-1337.

Ricardo da Silva, J. M.; Rigaud, J.; Cheynier, V.; Cheminat, A.; Moutounet, M. Procyanidin dimers and trimers from grape seeds. *Phytochemistry.* **1991,** 4, 1259-1264.

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

Roby, G.; Matthews, M.A. Relative proportions of seed, skin and flesh, in ripe berries from Cabernet Sauvignon grapevines grown in a vineyard either well irrigated or under water deficit. *Aust. J. Grape Wine Res.* **2003,** 10, 74-82.

Romero, P.; Botia, P.; Francisco, G. Effects of regulated deficit irrigation under subsurface drip irrigation conditions on vegetative development and yield of mature almond trees. *Plant and Soil.* **2004.** 260, 169-181.

Romeyer, F.M.; Macheix, J.J.; Sapis, J.C. Changes and importance of oligomeric proanthocyanidins during maturation of grape seeds. *Phytochemistry.* **1986,** 25, 219-221.

Santos-Buelga, C.; Scalbert, A. Review: Proanthocyanidins and tannin-like compounds – nature, occurrence, dietary intake and effects on nutritional health. *J. Sci. Food Agric.* **2000.** 80, 1094-1117.
Somers, T.C. The polymeric nature of wine pigments. *Phytochemistry* **1971.** 10, 2175-2186.

Smart, R. E. Influence of light on composition and quality of grapes. *Acta Horticulture.* **1997,** 206, 37-47.

Somers, T.C.; Evans, M.E. Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO_2, “chemical age”. *J. Sci. Food Agric.* **1977**, 28, 279-286.

Souquet, J.M.; Cheynier, V.; Brossaud, F.; Moutounet, M. Polymeric proanthocyanidins from grape skins. *Phytochemistry.* **1996,** 43, 509-512.

Spayd, S.E.; Tarara, J.M.; Mee, D.L.; Ferguson, J.C. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *Am. J. of Enol. and Vitic.* **2002.** 53, 171-182.

Sun, B.S.; Pinto, T.; Leandro, M.C.; Ricardo-Da-Silva, J.M.; Spranger, M.I. Transfer of catechins and proanthocyanidins from the solid parts of the grape cluster into wine. *Am. J. Enol. Vitic.* **1999**, *50*, 179-184.

Vidal, S.; Meudec, E.; Cheynier, V.; Skouroumounis, G.; Hayasaka, Y. Mass spectrometric evidence for the existence of oligomeric anthocyanins in grape skins. *J. Agric. Food Chem.* **2004,** 52, 7144-7151.

Influence of Vine Vigor on Grape (*Vitis vinifera* L. Cv. Pinot noir) Anthocyanins: Part I. Anthocyanin Concentration and Composition in Fruit.

Jessica M. Cortell, Michael Halbleib, Andrew V. Gallagher,

Timothy L. Righetti, James A. Kennedy*

Journal of Agricultural Food Chemistry
PO Box 3337,
Columbus, OH, 43210, USA
To be submitted October15, 2006

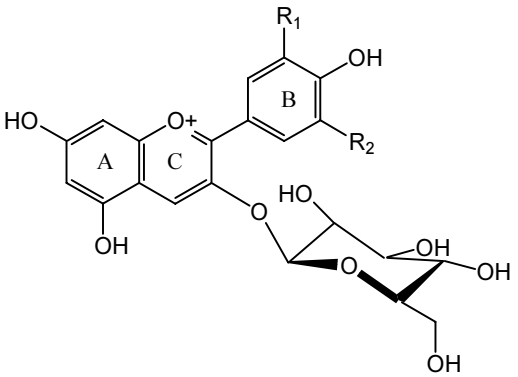
ABSTRACT

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

KEYWORDS: temperature; light exposure

INTRODUCTION

Anthocyanins are a class of pigmented phenolic compounds responsible for the red color of grapes and wine. In fruit, they play a role in attracting seed dispersal organisms and possibly in UV-light protection (Chalker-Scott 1999). Anthocyanins have also been reported to have human health benefits (Wang et al. 1997). The hydroxylation pattern on the B-ring results in five anthocyanin glucosides which include delphinidin-3-*O*-glucoside (Dp) cyanidin-3-*O*-glucoside (Cy), petunidin-3-*O*-glucoside (Pt), peonidin-3-*O*-glucoside (Pn) and malvidin-3-*O*-glucoside (Mv) (**Figure 3.1**). In addition to producing 3-monoglucosides, *V. vinifera* varieties usually also produce 3-acetylglucoside and 3-*p*-coumarylglucoside derivatives of the aglycons. Malvidin derivatives are generally the major forms present. Pinot noir, a cool climate variety, only produces non-acylated forms (Fong et al. 1971). Consequently, it differs from other varieties in terms of its anthocyanin profile and in how this impacts color density and color stability of wines.



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 3.1. Structures of anthocyanin-3-*O*-glucoside monomers based on substitution pattern commonly found in *V. vinifera*.

Grape berry growth follows a double sigmoid curve separated by a lag phase (Coombe 1976). Ripening begins after the lag phase at a time termed véraison by viticulturalists. Anthocyanin accumulation (coloring) begins at véraison and continues through ripening. Although the biosynthesis of all anthocyanins is stimulated at véraison, they have been reported to accumulate at different rates during fruit ripening (Roggero et al. 1986; Keller & Hrazdina 1998).

Many environmental factors and viticultural practices influence the accumulation and composition of anthocyanins in the fruit. High vine vigor resulting from excessive soil moisture and high levels of available nitrogen can modify the vine microclimate (Smart 1985) and can influence the accumulation of anthocyanins (Smart 1985; Jackson & Lombard 1993). Low vine vigor vineyards are characterized by greater light exposure in the fruiting zone (Smart 1985; Smart et al. 1988; Jackson & Lombard 1993). Spatial variation in vineyard topography, climatic conditions, physical and chemical characteristics of the soil and pests and diseases have been associated with spatial variation in vine vigor, yield and fruit soluble solids (Hall et al. 2002; Bramley and Hamilton 2004). A relationship between grapevine canopy size and vine vigor measured as NDVI (normalized difference vegetation index) and grape color has been reported (Lamb et al. 2004).

Various viticultural practices have been found to influence anthocyanin accumulation and composition including nitrogen supply (Keller & Hrazdina 1998; Hilbert et al. 2003), vine canopy management (Mazza 1995; Reynolds et al 1996; Reynolds et al. 2005), water deficit (Ojeda et al. 2002), soil amendments (Yokotsuka

et al. 1999) and many other examples. A difficulty in assessing many of these results is that the practices modify vine growth and canopy structure causing changes in light exposure and temperature in the fruiting zone.

Sunlight exposure is thought to be one of the main factors influencing anthocyanin accumulation and composition in grapes (Smart et al. 1988; Jackson & Lombard 1993). Light was found to have its greatest impact on anthocyanin accumulation during the initial stages of growth rather than during the fruit ripening period (post- véraison) (Dokoozlian & Kliewer 1996). It has also been reported that anthocyanin accumulation increased linearly with increasing sunlight exposure while high berry temperatures reduced anthocyanin concentration in highly exposed fruit (Bergqvist et al. 2001; Spayd et al. 2002). In several studies, higher accumulation of anthocyanins were found in grapes with cool day and night temperatures compared to high day or night temperatures (Buttrose et al. 1971; Mori et al. 2005). A number of exposure studies show a range of results in terms of anthocyanins accumulation (Keller & Hrazdina 1998; Smart et al. 1988; Dokoozlian & Kliewer; 1996; Bergqvist et al. 2001; Spayd et al. 2002; Morrison & Noble 1990; Gao & Cahoon 1994; Price et al. 1995; Downey et al. 2004). The variable results may be due to temperature differences or other factors. In addition, a plant's response will depend on the degree of exposure to stress and can be additive in response to both water deficit and UV irradiance (Chaves & Escudero 1999).

Environmental influences can also modify anthocyanin composition in grape skins. Several studies have shown that plants shift anthocyanin composition toward

higher levels of B-ring hydroxylation in response to UV light (Spayd et al. 2002; Downey et al. 2004; Ryan et al. 2002; Jaakola et al. 2004. In Pinot noir, high night temperatures compared to low night temperatures reduced the proportion of Dp, Cy, and Pt (Mori et al. 2005).

In our assessment of vineyard spatial variations and the impact of vine vigor on phenolic accumulation, we found limited differences in seed proanthocyanidins while substantial differences were found in skin proanthocyanidin accumulation and composition (Cortell et al. 2005). There was an interest in determining whether differences in composition could be detected in grapes from two specific vineyard sites known to produce wines with distinctly different price points (US $38.00/bottle versus US $75.00/bottle). In this paper, we are addressing the influence of vine vigor on the concentration and composition of anthocyanins in fruit. The objective was to investigate the importance of vineyard spatial variation on anthocyanin accumulation and composition in the variety Pinot noir. In Part II of this paper, we address relationships between fruit and wine anthocyanin composition and wine color.

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, USA. Vine spacing was 1 m (within row) X 2.8 m (between rows) with ~ 5113 vines per hectare. The training system was a vertical shoot position with each vine pruned to 10-12 buds. The vineyard

experimental design and delineation of vigor zones was previously described (Cortell et al. 2005).

Surface Maps. Surface maps were made using ESRI software (Redlands, CA) with the ordinary kriging utility. Surface maps were made as previously described (Cortell et al. 2005).

Fruit Sampling and Extraction. Fruit samples were collected from a grid pattern across each site for the surface maps and for the model wine extraction. A sample was also collected across each vigor zone (3 replicates/zone) to reflect the fruit used for wine production. Harvest dates (September 21 in 2003 and September 9 and 10th in 2004) were determined by the cooperating winery. Fruit samples were frozen and stored at -35°C until processed. Frozen berries were removed from the rachis and samples of 150 berries were randomly collected, weighed and then processed as previously described (Kennedy et al. 2000).

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

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

Reversed-Phase HPLC of Anthocyanins. Anthocyanin content and composition in grape skins was measured by reversed-phase HPLC (Lamuela-Raventos & Waterhouse 1994). Aqueous extracts were filtered using Teflon filters (0.45µm, Acrodisc CR13) before injection. Eluting anthocyanins were identified and quantified with a malvidin-3-*O*-glucoside standard.

Statistical Analyses. Statistical data analysis was performed using analysis of variance (ANOVA) and the least significant difference (LSD) test to determine statistically different values at a significance level of $\alpha \leq 0.05$. Since vigor zones (high, medium and low) were relative levels within each site and because both year and site were significantly different for most variables, a separate ANOVA was run to compare vigor zones for each site and year combination. For vineyard site and year comparisons, weighted averages were calculated and analyzed to take into account the contribution of the vigor zones to the total area within each vineyard site. All statistical analyses were performed using SAS version 8.2.

Table 3.1. Temperature data (°C) from 2004 showing variation by site, month and time period.

Site	May 12 am -6 am	May 10 am- 1 pm	May 1 pm – 4 pm	May 4 pm – 7 pm
A	9.82	18.63	20.05	18.75
B	9.68	19.07	20.54	19.69
SEM	0.33	0.33	0.33	0.33
p-value	0.6908	0.1835	0.1347	0.0047

Site	June 12 am -6 am	June 10 am- 1 pm	June 1 pm – 4 pm	June 4 pm – 7 pm
A	12.76	22.61	25.04	23.46
B	12.75	23.94	25.85	25.58
SEM	0.33	0.33	0.33	0.33
p-value	0.9571	<.0001	0.0147	<.0001

Site	July 12 am -6 am	July 10 am- 1 pm	July 1 pm – 4 pm	July 4 pm – 7 pm
A	14.23	26.24	30.44	27.70
B	14.69	28.30	31.15	31.17
SEM	0.33	0.33	0.38	0.33
p-value	0.1689	<.0001	0.0620	<.0001

Site	August 12 am -6 am	August 10 am- 1 pm	August 1 pm – 4 pm	August 4 pm – 7 pm
A	16.07	25.80	29.39	27.62
B	15.66	27.58	30.35	29.67
SEM	0.41	0.33	0.38	0.33
p-value	0.2153	<.0001	0.0116	<.0001

Site	September 12 am -6 am	September 10 am- 1 pm	September 1 pm – 4 pm	September 4 pm – 7 pm
A	6.69	22.46	24.81	22.75
B	6.45	23.67	25.52	24.15
SEM	0.33	0.34	0.38	0.38
p-value	0.4674	0.0005	0.0602	0.0003

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

Table 3.2. Temperature data (°C) from 2004 showing variation by site, vigor zone, month and time period.

Site	Zone Time	May 12 am – 6 am	May 10 am- 1 pm	May 1 pm – 4 pm	May 4 pm – 7 pm
A	High	9.98[a]	18.10[b]	19.46[b]	18.45[e]
A	Medium	9.56[b]	18.81[ab]	20.39[a]	19.03[c]
A	low	9.77[ab]	19.16[a]	20.42[a]	18.79[d]
B	High	9.73[bc]	18.78[ab]	20.28[a]	19.38[b]
B	Medium	9.56[c]	19.33[a]	20.62[a]	19.76[b]
B	low	9.77[b]	19.10[a]	20.73[a]	19.92[a]
SEM		0.05	0.28	0.15	0.11
p-value		0.0051	0.1220	0.0026	<.0001

Site	Zone Time	June 12 am -6 am	June 10 am- 1 pm	June 1 pm – 4 pm	June 4 pm – 7 pm
A	High	12.69[ab]	22.44[d]	24.71[d]	23.02[d]
A	Medium	12.86[a]	22.65[cd]	25.39[bc]	23.94[c]
A	low	12.71[ab]	22.79[bcd]	25.03[cd]	23.39[cd]
B	High	12.76[ab]	23.59[abc]	25.55[abc]	25.12[b]
B	Medium	12.59[b]	23.77[ab]	25.82[ab]	25.56[ab]
B	low	12.89[a]	24.45[a]	26.18[a]	26.05[a]
SEM		0.08	0.33	0.20	0.21
p-value		0.1363	0.0129	0.0060	<.0001

Site	Zone Time	July 12 am -6 am	July 10 am- 1 pm	July 1 pm – 4 pm	July 4 pm – 7 pm
A	High	13.41[d]	24.07[c]	missing	26.03[e]
A	Medium	14.82[ab]	27.56[b]	30.75[ab]	29.3[c]
A	low	14.59[bc]	27.52[b]	29.98[b]	27.78[d]
B	High	14.64[bc]	28.41[ab]	30.82[ab]	30.47[b]
B	Medium	14.51[c]	28.79[a]	31.19[a]	30.87[b]
B	low	14.92[a]	27.70[ab]	31.44[a]	32.18[a]
SEM		0.08	0.35	0.28	0.27
p-value		<.0001	<.0001	0.0883	<.0001

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

Table 3.2 (continued). Temperature data (°C) from 2004 showing variation by site, vigor Zone, month and time period.

Site	Zone Time	August 12 am -6 am	August 10 am- 1 pm	August 1 pm – 4 pm	August 4 pm – 7 pm
A	High	16.3[a]	26.1[cd]	missing	27.64[d]
A	Medium	16.09[ab]	25.38[d]	29.61[b]	28.2[cd]
A	low	15.68[c]	25.98[cd]	29.06[b]	26.72[e]
B	High	15.71[c]	27.69[ab]	29.84[ab]	28.66[c]
B	Medium	15.49[c]	28.3[a]	30.51[a]	29.51[b]
B	low	15.78[bc]	26.76[bc]	30.7 [a]	30.84[a]
SEM		0.27	0.27	0.27	0.27
p-value		0.0003	0.0033	0.0228	<.0001

Site	Zone Time	September 12 am -6 am	September 10 am- 1 pm	September 1 pm – 4 pm	September 4 pm – 7 pm
A	High	6.99[a]	22.88[ab]	missing	missing
A	Medium	6.68[ab]	22.00[b]	24.98[ab]	23.43[c]
A	low	6.48[b]	22.56[b]	24.55[a]	21.75[d]
B	High	6.54[b]	23.48[ab]	25.07[ab]	23.19[c]
B	Medium	6.38[b]	24.48[a]	25.79[a]	24.12[b]
B	low	6.42[b]	23.06[ab]	25.7[a]	25.16[a]
SEM		0.25	0.45	0.30	0.14
p-value		<.0001	0.0250	0.1361	<.0001

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

RESULTS AND DISCUSSION

Vine vigor and temperature differences. Figure 3.2 shows seasonal monthly daily maximum temperatures for 2003 and 2004. Although temperatures were similar during bloom for both years, it was very warm from March through May in 2004 compared to 2003. The remainder of the ripening period was similar

but cooled off more rapidly in 2004 with early September rains.

Site A and B are oriented differently in the vineyard so this resulted in differences in light interception and temperature. Site B was 2-3°C higher in June through August from 4-7 pm and 1-2°C higher from 10 am-1 pm. in August compared to site A (**Table 3.1**). Site B is also lower in vigor than site A. Sunlight exposure and temperature differences have been previously reported in the vine microclimate between high and low vigor vines (Jackson & Lombard 1993).

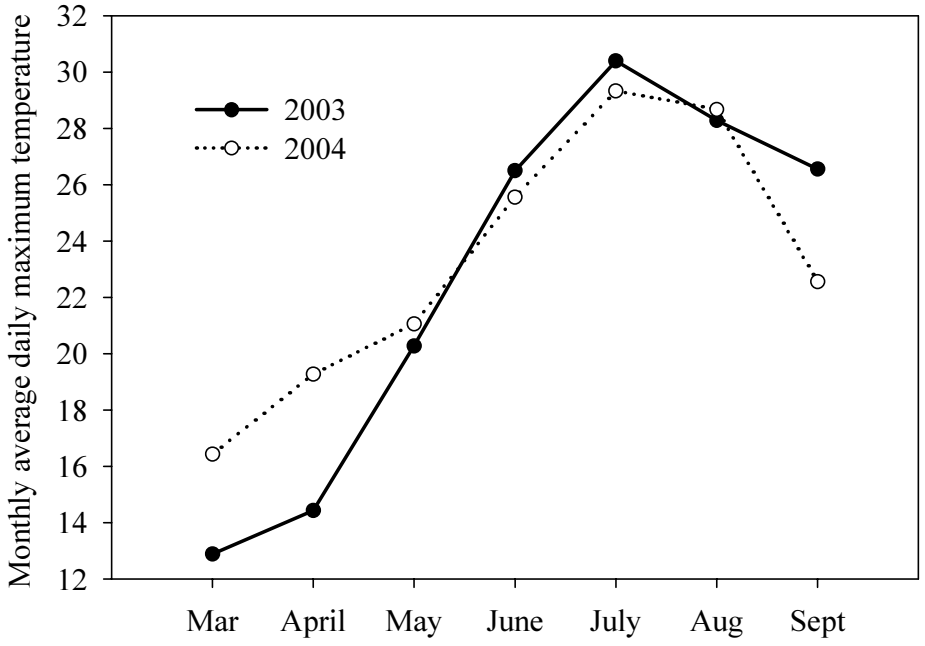


Figure 3.2. Monthly average of daily maximum temperatures in °C for 2003 and 2004 from March through September.

Vine vigor differences across the vineyard sites were previously described (Cortell et al. 2005). An increase in vine vigor from greater water and nitrogen availability modified the vine microclimate due to a larger canopy with increased leaf size. For this specific vineyard, the relationship between leaf chlorophyll and leaf size is shown in **Figure 3.3** where there was a positive correlation (r^2=0.84) between increasing leaf greenness (chlorophyll) and leaf surface area for fully expanded mature leaves. The shadier canopy in the high vigor zones was observed to reduce light exposure in the fruiting zone with the indirect effect of also causing cooler temperatures. As seen in **Table 3.2**, there were differences in temperature between the vigor zones in both sites with the greatest temperature differences occurring in the 4-7 pm. time period where vigor zones with reduced vine vigor were generally warmer. These differences in temperature could effect anthocyanin accumulation positively or negatively.

Fruit composition. The accumulation of soluble solids (°Brix) was higher in 2003 compared to 2004 as rains forced an earlier harvest in the second year (**Table 3.3**). Soluble solids were higher in site A in both years compared to site B (**Table 3.3**). Soluble solids were about 1 °Brix lower in the high vigor zone than the medium and low vigor zones in site A in 2003 (**Table 3.4**). In site B, soluble solids increased with decreasing vine vigor in 2003. There was a similar pattern for site A in 2004 with the high vigor zone having lower soluble solids than the medium or low vigor zones (**Table 3.5**) while in site B the medium vigor zone was higher in soluble solids

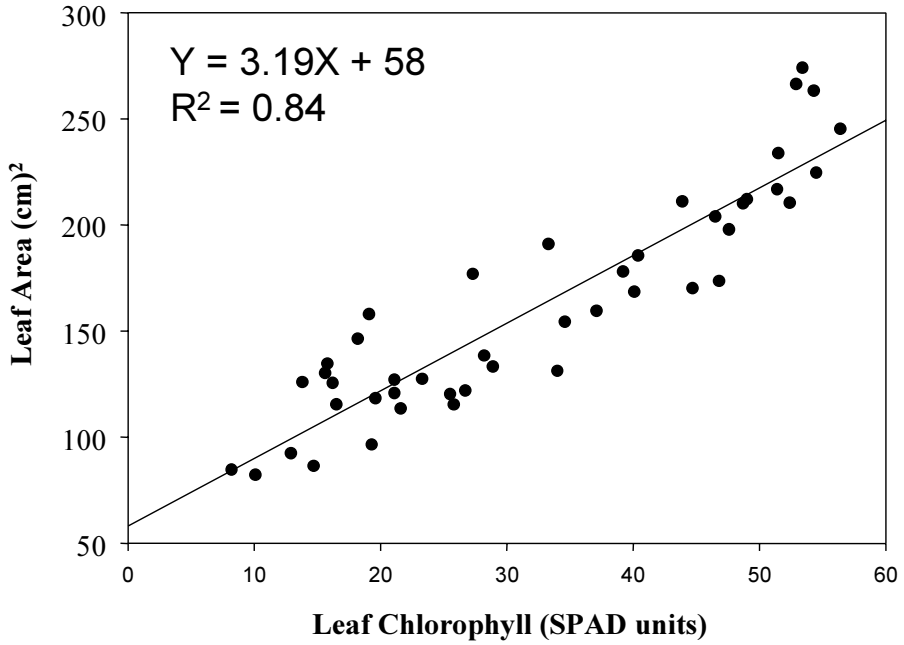


Figure 3.3. Correlation between Pinot noir leaf chlorophyll (SPAD units) and leaf area (cm^2) representing a range of leaf sizes from low, medium and high vigor zones.

than the high or low vigor zone. Reduced sugar accumulation has been reported due to fruit shading (Kliewer & Lider 1967; Gao & Cahoon 1994; Price et al. 1995; Reynolds et al. 1996) and in high vigor canopies (Jackson & Lombard 1993). However, other studies specifically on fruit shading did not find differences in soluble solids accumulation (Crippen & Morrison 1986; Haselgrove et al. 2000; Morrison & Noble 1990; Spayd et al. 2002; Downey et al. 2004).

In comparing vintages, 2003 was lower in titratable acidity (TA) and had a higher pH than in 2004 (**Table 3.3**). For TA and pH, there was also a site and a site by year interaction (**Table 3.3**). Site A had a higher average pH than site B in 2003

and was similar in 2004. For TA, site B was slightly higher than in site A in 2003 while site A was substantially higher than site B in 2004. Site A may have had a higher TA than site B in 2004 as it contains higher vigor vines. In both years and both sites, a reduction in TA was associated with a reduction in vine vigor except for the medium and low vigor zones in site A were similar in 2003 (**Table 3.4 and 3.5**). Higher TA was reported in canopies with excessive soil moisture and less than 60% cluster exposure (Jackson & Lombard 1993). In 2003, pH was higher in site A than B most likely due to higher available water and greater cation uptake (**Table 3.3**) (Jackson & Lombard 1993). In 2004, the sites were similar for pH.

Average berry weight was lower in 2004 than 2003 (**Table 3.3)**. The average berry weight was higher in site A in 2003 and higher in site B in 2004 (**Table 3.3)**. The lower average berry weight in 2004 compared to 2003 may have been due to cool rainy weather during bloom and fruitset that reduced ovule fertility and number of seeds per berry (data not included). Temperature has been reported to reduce ovule fertility and number of seeds per berry (Ewart & Kliewer 1977). The site by year interaction was also probably related to fruitset problems in 2004 where site A had more small shot berries while site B had compensation for poor fruitset in fewer but larger berries.

In comparing the vigor zones in site A, the high vigor zone had a higher average berry weight than the medium amd low vigor zones in 2003 (**Table 3.4**). There were no differences between vigor zones in site B in 2003. There was a site by vigor interaction in 2003 for average berry weight.

Table 3.3. Weighted mean (± SEM) of average berry weight (g), dry average skin weight (mg), soluble solids (°Brix), titratable acidity (g/L) and pH in vineyard sites in 2003 and 2004.

Site	Year	Berry weight (gm)	Dry skin weight (mg)	Soluble solids °Brix	Titratable acidity (g/L)	pH
A	2003	0.91	32.0	24.2	4.8	3.49
B	2003	0.84	27.9	24.0	5.1	3.27
A	2004	0.63	17.5	23.3	6.5	3.23
B	2004	0.73	17.6	22.9	5.2	3.23
SEM		0.02	1.0	0.1	0.1	0.01
Site p-value[a]		0.5541	0.0614	0.0483	0.0012	<0.0001
Year p-value[a]		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Site*Year p-value[a]		0.0004	0.0481	0.9980	<.00001	<0.0001

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

Table 3.4. Mean (± SEM) of average berry weight (gm), dry average skin weight (mg), soluble solids (°Brix), titratable acidity (g/L) and pH in vigor zones in 2003.

Site	Year	Vigor Zone	Berry weight (g)	Dry skin weight (mg)	Soluble solids °Brix	Titratable acidity (g/L)	pH
A	2003	High	0.99[a]	28.0[b]	23.5[b]	5.7[a]	3.50[a]
A	2003	Medium	0.91[b]	32.8[a]	24.3[a]	4.7[b]	3.50[a]
A	2003	Low	0.87[b]	31.3[ab]	24.1[a]	4.6[b]	3.47[a]
SEM			0.02	1.2	0.05	0.1	0.02
Vigor p-value[a]			0.0490	0.1135	0.0010	0.0035	0.4444
B	2003	High	0.82[a]	29.6[a]	23.7[c]	5.7[a]	3.20[b]
B	2003	Medium	0.87[a]	27.7[a]	24.0[b]	4.9[b]	3.30[a]
B	2003	Low	0.78[a]	26.5[a]	24.4[a]	4.7[c]	3.30[a]
SEM			0.03	2.0	0.05	0.05	0.02
Vigor p-value[a]			0.2856	0.4758	0.0010	0.0002	<0.0001
Site*vigor			0.0079	0.1478	<0.0001	<0.0001	<0.0001

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

Table 3.5. Mean (± SEM) of average berry weight (gm), dry average skin weight (mg), soluble solids (°Brix), titratable acidity (g/L) and pH in vigor zones in 2004.

Site	Year	Vigor Zone	Berry weight (g)	Dry skin weight (mg)	Soluble solids °Brix	Titratable acidity (g/L)	pH
A	2004	High	0.63[a]	16.0[a]	21.7[b]	7.4[a]	3.19[b]
A	2004	Medium	0.60[a]	17.1[a]	23.6[a]	6.5[b]	3.24[a]
A	2004	Low	0.72[a]	20.1[a]	23.4[a]	5.9[c]	3.21[ab]
SEM			0.05	1.6	0.1	0.1	0.009
Vigor p-value[a]			0.3792	0.1925	0.0004	0.0001	0.0509
B	2004	High	0.69[a]	17.2[a]	22.5[b]	5.8[a]	3.19[c]
B	2004	Medium	0.72[a]	18.1[a]	23.3[a]	5.2[b]	3.23[b]
B	2004	Low	0.78[a]	17.2[a]	22.5[b]	4.8[c]	3.26[a]
SEM			0.03	1.8	0.07	0.03	0.003
Vigor p-value[a]			0.1635	0.9167	0.0014	<0.0001	0.0002
Site*vigor			0.1144	0.5274	<0.0001	<0.0001	<0.0001

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

In 2004, there were no differences between vigor zones in either site (**Table 3.5**). Larger berries were previously reported due to fruit shading (Reynold et al 1986; Crippen & Morrison 1986). As mentioned above, the anticipated berry size related to vigor did not occur in 2004 due to poor fruitset that affected the vigor zones differently.

For average dry skin weight (mg), there was a vintage effect with the higher berry weight in 2003 also resulting in a higher dry skin weight (**Table 3.3**). There was a site by year interaction with a higher average dry skin weight in site A than in site B in 2003 and no differences in 2004 (**Table 3.3**). Skin weight appeared to be more strongly related to berry size then vigor differences. Differences in average dry skin weight were found in site A in 2003 where the medium vigor zone was higher than the high vigor zone while no differences were found in site B (**Table 3.4**). No differences were found in average dry skin weight between vigor zones in either site in 2004 (**Table 3.5**). Research on deficit irrigation has shown an increase in skin weight (Kennedy et al 2002; Roby et al. 2004) and a higher skin to pulp ratio (Ojeda et al. 2002).

Anthocyanin accumulation in fruit. Spatial variation across the vineyard sites for total anthocyanin accumulation in mg/berry in 2004 is shown in **Figure 3.4**. There was a response of greater anthocyanin accumulation in the low vigor zones of both vineyard sites (**Figure 3.4**). In 2004, there was an increase in the total amount per berry and for all individual anthocyanins in site A with a reduction in vine vigor (**Table 3.6**). For site B, there were no significant vine vigor differences for total, Pn

and Mv amount per berry while Dp, Cy and Pt increased with a reduction in vigor. This berry sample was taken at 5 random locations (5 replicates) within each vigor zone. The fruit was also used for the model extraction discussed in Part II of this paper.

In addition, fruit samples (3 replicates) were collected that were representative of the fruit used to make wines. For this fruit sample, there was higher total anthocyanin accumulation (mg/berry) in the fruit in 2003 than in 2004 (**Table 3.7**). In comparing the sites, there were no differences between sites or a site by year interaction (**Table 3.7**).

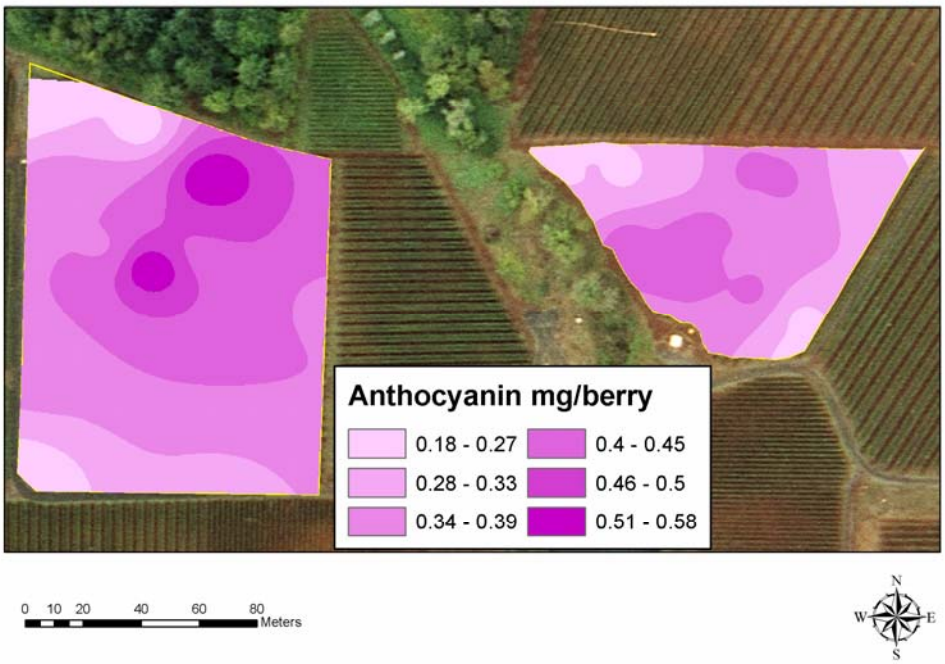


Figure 3.4. Surface map of spatial variation in total anthocyanin accumulation in mg per berry in 2004.

Table 3.6. Mean and SEM of total and individual anthocyanins in mg/berry for vine vigor zones in 2004. Fruit samples were used for the spatial surface map and model extractions (Part II).

Site	Vigor Zone	Total (mg/berry)	Delphinidin (mg/berry)	Cyanidin (mg/berry)	Petunidin (mg/berry)	Peonidin (mg/berry)	Malvidin (mg/berry)
A	High	0.20[b]	0.007[b]	0.003[b]	0.011[b]	0.043[c]	0.13[b]
A	Medium	0.31[b]	0.013[b]	0.005[b]	0.020[b]	0.066[b]	0.21[b]
A	Low	0.48[a]	0.024[a]	0.009[a]	0.036[a]	0.10[a]	0.31[a]
SEM		0.04	0.002	0.0007	0.003	0.006	0.02
p-value[a]		0.0067	0.0094	0.0060	0.079	0.0036	0.0096
B	High	0.29[a]	0.009[b]	0.003[b]	0.015[b]	0.055[a]	0.21[a]
B	Medium	0.34[a]	0.013[ab]	0.005[ab]	0.020[ab]	0.064[a]	0.24[a]
B	Low	0.38[a]	0.015[a]	0.005[a]	0.023[a]	0.068[a]	0.26[a]
SEM		0.03	0.002	0.0005	0.002	0.006	0.02
p-value[a]		0.2027	0.0400	0.0758	0.0704	0.3294	0.2430
Site * vigor		0.0015	0.0002	<0.0001	0.0003	0.0004	0.0015

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

Vintage variation was reported in some studies (Spayd et al. 2002; Brossaud et al. 1999) while others have reported minimal influence of the season (Mazza et al. 1999). In the present study, it seems more probably that the low accumulation of anthocyanins in 2004 was in response to rapid vine growth due to warm spring weather and high available nitrogen leading to a depletion of carbon reserves during fruit ripening which restricted secondary metabolism in favor of primary metabolism (Keller & Hrazdina 1998). High bloom time N particularly with low light irradiance was reported to interfere with phenolic biosynthesis leading to a lower total amount at maturity (Keller & Hrazdina 1998).

In 2003, the medium and low vigor zones in site A were higher in anthocyanin concentration (mg/berry) compared to the high vigor zone (**Table 3.9)**. In 2004, there were no differences between zones in site A (**Table 3.10)**. In site B, there were no differences in total anthocyanin concentration (mg/berry) in either year (**Table 3.9 and 3.10**). In 2003, there was a trend toward higher Dp and Pt with a reduction in vine vigor and no differences in Cy, Pn or Mv in site A (**Table 3.9).** In 2003 in site B, there was a trend for higher Cy with reduced vine vigor and no differences in the Dp, Pt, Pn or Mv (**Table 3.9)**. In 2004, there were no vine vigor differences in total or individual anthocyanins in site A or B (**Table 3.10)**. However, as seen above in **Table 3.6**, a different sampling strategy with greater replication was able to detect differences. This suggests that in 2004 three replicates of 150 berries was not an adequate sample size possibly due to high fruit variability.

The observed reduction in anthocyanin concentration in the high vigor zones was likely due to a larger canopy with increased shading within the fruiting zone in

addition to higher available water and nutrients. Vines in the high vigor zones had reduced exposure and cooler temperatures (**Table 3.2**). In general cool day and night temperature from 15-20°C have been found to increase anthocyanin biosynthesis compare to high day or night temperatures of 30-35°C (Kliewer & Torres 1972; Spayd et al. 2002). On the other hand, sunlight exposure has a positive linear effect on anthocyanin biosynthesis although high berry temperatures have been shown to reduce anthocyanin accumulation (Haselgrove et al 2000; Bergqvist et al. 2001; Spayd et al. 2002). Therefore, the reduced sun exposure would be anticipated to lower anthocyanin accumulation while cooler temperatures in the fruiting zone might increase anthocyanin biosynthesis in high vigor vines.

Anthocyanin accumulation was found to be most rapid during the first two weeks after véraison at the onset of anthocyanin biosynthesis and accumulation of anthocyanins was more sensitive to water deficit before véraison than post-véraison (Matthews & Anderson 1988). Consequently, pre-véraison water deficit could have also affected the vines in the lowest vigor zone (B-low), as high stress may have reduced anthocyanin accumulation. Vines in the B-low vigor zone had higher sun exposure but also experienced more temperatures above 30-35°C as noted in July and August (**Table 3.2**). Sunlight exposed berries have been reported to have increased temperatures from 3-13°C (Kliewer & Lider 1968; Reynold et al. 1986; Dokoozlian & Kliewer 1996; Spayd et al. 2002) compared to nonexposed fruit due to incident radiation. A net loss of anthocyanins in Merlot was associated with the number of hours over 35°C the fruit experienced.

Table 3.7. .Mean and SEM of total anthocyanin content in mg/berry and mg/kg and in mg/berry for delphinidin, cyanidin, petunidin, peonidin, and malvidin anthocyanin glucosides in 2003 and 2004.

Site	Year	Total (mg/berry)	Total (mg/kg)	Delphinidin (mg/berry)	Cyanidin (mg/berry)	Petunidin (mg/berry)	Peonidin (mg/berry)	Malvidin (mg/berry)
A	2003	0.81	896	0.045	0.021	0.061	0.21	0.48
B	2003	0.87	1043	0.049	0.020	0.065	0.19	0.55
A	2004	0.25	411	0.011	0.004	0.017	0.05	0.17
B	2004	0.30	413	0.012	0.004	0.018	0.05	0.21
SEM		0.03	51	0.003	0.0009	0.004	0.008	0.02
Site	p-value[a]	0.8871	0.1484	0.3908	0.9909	0.5171	0.2752	0.0110
Year	p-value[a]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Site*Year	p-value[a]	0.1525	0.1610	0.6364	0.6852	0.8305	0.1205	0.4267

[a]ANOVA to compare data (*P* indicated), n=9. Anthocyanin content was quantified in malvidin equivalents.

Table 3.8. Mean and SEM of percent composition for delphinidin, cyanidin, petunidin, peonidin, malvidin, dioxygenated and trioxygenatedanthocyanin glucosides for site A and B in 2003 and 2004.

Site	Year	Delphinidin (percent)	Cyanidin (percent)	Petunidin (percent)	Peonidin (percent)	Malvidin (percent)	3'4'-OH (percent)	3'4'5'-OH (percent)
A	2003	5.5	2.6	7.4	25.9	58.6	28.5	71.5
B	2003	5.7	2.3	7.5	21.7	62.9	24.0	76.0
A	2004	4.2	1.5	6.3	19.3	68.71	20.8	79.2
B	2004	4.10	1.4	6.0	17.6	70.9	19.0	81.0
SEM		0.3	0.1	0.3	0.5	0.6	0.5	0.5
Site	p-value[a]	0.6118	0.0662	0.6723	<0.0001	0.0003	<0.0001	<0.0001
Year	p-value[a]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Site*Year	p-value[a]	0.8356	0.3599	0.6092	0.0255	0.1841	0.0192	0.0192

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

The fruit in the B-low vigor zone had inconsistent ripening, particularly in 2004 where clusters had a combination of purple, pink and green berries. This phenomenon has been reported in fruit that failed to mature due to water deficit (Hardie & Considine 1976).

In a number of irrigation studies, reduced water applications or increased water stress was found to have a direct effect of increased concentration due to higher anthocyanin biosynthesis or an indirect effect from a reduction in fruit size (Ojeda et al. 2002; Kennedy et al. 2002; Matthews & Anderson 1988; Hardie et al. 1981; Ginestar et al. 1998; Salón et al. 2005). This positive response in anthocyanin accumulation to water deficit may help explain why the B-medium vigor zone had similar levels of anthocyanins compared to the B-low zone. The medium zones may have benefited from moderate water stress, good light exposure and moderate canopy temperatures compared to the more extreme conditions found in the B-low vigor zone. Consequently, it is likely a combination of factors including water status, sun exposure and canopy temperature moderated anthocyanin accumulation in the vigor zones. This may explain why a number of exposure studies in *Vitis vinifera* grapes have shown a range of results (Smart et al. 1988; Dokoozlian & Kliewer 1996; Bergqvist et al. 2001; Spayd et al. 2002; Gao & Cahoon 1994; Price et al. 1995; Downey et al. 2004).

When the same data was expressed on a (mg/kg) basis to reflect differences in fruit weight, 2003 was substantially higher than 2004 (**Table 3.7**). There was not a consistent site effect or site by year response (**Table 3.7**). In 2003, site B was higher

in anthocyanin accumulation when expressed as (mg/kg) while in 2004 the sites were similar. In 2003 for the vigor zones, there was greater anthocyanin accumulation (mg/kg) in the medium and low vigor zones compared to the high vigor zone in site A and no differences between vigor zones in site B (**Table 3.9**). In 2004, there were no differences in anthocyanin accumulation (mg/kg) among zones in either site (**Table 3.10**).

In assessing the anthocyanin available to be extracted into wine, both the concentration per berry and the berry size needs to be taken into account. Determining concentration as mg/kg gives the potential amount that could be extracted into wine. Berry size can affect the skin to pulp ratio as well as the skin surface area. In part II of this paper, we investigate the influence of vine vigor on the relationships between concentration, berry size, extraction in a model system and extraction into wine. In addition, the impact of anthocyanin concentration and composition on the formation of pigmented polymers and red wine color is discussed.

Anthocyanin composition in fruit. There were also differences in the anthocyanin proportional composition between years (**Table 3.8**). In 2003, the proportion of Dp, Cy, Pt, and Pn were higher and only the proportion of Mv was lower in comparison to 2004. Although a number of authors have concluded that anthocyanin composition is primarily determined by genetic factors (Roggero et al. 1986; Mazza & Miniata 1993; Boss et al. 1996a; Brossaud et al. 1999),

Table 3.9. Mean and SEM of total anthocyanin content (mg/berry and mg/kg), delphinidin, cyanidin, petunidin, peonidin, and malvidin glucoside content in mg/berry for vine vigor zones in 2003.

Site	Year	Vigor Zone	Total (mg/berry)	Total (mg/kg)	Delphinidin (mg/berry)	Cyanidin (mg/berry)	Petunidin (mg/berry)	Peonidin (mg/berry)	Malvidin (mg/berry)
A	2003	High	0.61[b]	619.5[b]	0.03[b]	0.02[a]	0.04[b]	0.15[a]	0.38[a]
A	2003	Medium	0.83[a]	913.7[a]	0.05[ab]	0.02[a]	0.06[ab]	0.22[a]	0.48[a]
A	2003	Low	0.87[a]	994.5[a]	0.06[a]	0.03[a]	0.07[a]	0.22[a]	0.50[a]
SEM			0.08	79.9	0.007	0.002	0.008	0.02	0.04
p-value[a]			0.1394	0.0610	0.0929	0.2449	0.1174	0.1527	0.1755
B	2003	High	0.86[a]	1056.2[a]	0.04[a]	0.02[a]	0.06[a]	0.19[a]	0.55[a]
B	2003	Medium	0.87[a]	996.4[a]	0.05[ab]	0.02[a]	0.06[a]	0.19[a]	0.55[a]
B	2003	Low	0.87[a]	1116.4[a]	0.05[b]	0.02[a]	0.07[a]	0.18[a]	0.54[a]
SEM			0.04	81.0	0.004	0.001	0.005	0.01	0.03
p-value[a]			0.9756	0.3977	0.0889	0.1523	0.3082	0.4894	0.9494

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

Table 3.10. Mean and SEM of total anthocyanin content (mg/berry and mg/kg), delphinidin, cyanidin, petunidin, peonidin, and malvidin glucoside content in mg/berry for vine vigor zones in 2004

Site	Year	Vigor Zone	Total (mg/berry)	Total (mg/kg)	Delphinidin (mg/berry)	Cyanidin (mg/berry)	Petunidin (mg/berry)	Peonidin (mg/berry)	Malvidin (mg/berry)
A	2004	High	0.23[a]	362.1[a]	0.009[a]	0.004[a]	0.01[a]	0.05[a]	0.15[a]
A	2004	Medium	0.22[a]	384.6[a]	0.009[a]	0.003[a]	0.01[a]	0.04[a]	0.15[a]
A	2004	Low	0.39[a]	544.4[a]	0.02[a]	0.007[a]	0.03[a]	0.08[a]	0.25[a]
SEM			0.05	86.8	0.004	0.001	0.006	0.01	0.03
p-value[a]			0.1187	0.3648	0.1148	0.1135	0.1367	0.1097	0.1295
B	2004	High	0.26[a]	381.2[a]	0.009[a]	0.004[a]	0.015[a]	0.05[a]	0.19[a]
B	2004	Medium	0.31[a]	439.5[a]	0.013[a]	0.005[a]	0.020[a]	0.06[a]	0.22[a]
B	2004	Low	0.31[a]	396.6[a]	0.014[a]	0.005[a]	0.020[a]	0.06[a]	0.21[a]
SEM			0.05	82.9	0003	0.001	0.004	0.01	0.03
p-value[a]			0.7087	0.8701	0.3025	0.6267	0.4990	0.7534	0.7468

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

Table 3.11. Mean and SEM of percent composition for delphinidin, cyanidin, petunidin, peonidin, malvidin, dioxygenated, and trioxygenated anthocyanin glucosides for vine vigor zones in 2003.

Site	Year	Vigor Zone	Delphinidin (percent)	Cyanidin (percent)	Petunidin (percent)	Peonidin (percent)	Malvidin (percent)	3'4'-OX (percent)	3'4'5'-OX (percent)
A	2003	High	4.3[b]	3.1[a]	6.4[b]	23.9[a]	62.2[a]	27.0[a]	73.0[a]
A	2003	Medium	5.5[ab]	2.4[b]	7.4[ab]	26.5[a]	58.3[a]	28.8[a]	71.2[a]
A	2003	Low	6.4[a]	2.9[a]	8.0[a]	25.0[a]	57.7[a]	27.9[a]	72.1[a]
SEM			0.5	0.1	0.4	1.3	1.3	1.2	1.2
p-value[a]			0.0953	0.0009	0.0809	0.2983	0.1305	0.4777	0.4777
B	2003	High	5.1[b]	2.2[b]	7.0[a]	22.2[bcd]	63.5[a]	24.4[a]	75.6[a]
B	2003	Medium	5.7[ab]	2.3[b]	7.4[a]	21.8[cd]	62.8[a]	24.1[a]	75.9[a]
B	2003	Low	6.3[a]	2.5[a]	7.9[a]	20.9[d]	62.4[a]	23.4[a]	76.6[a]
SEM			0.3	0.04	0.3	0.5	0.8	0.6	0.6
p-value[a]			0.0459	0.0095	0.1499	0.3275	0.6472	0.5235	0.5235

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

Table 3.12. Mean and SEM of percent composition for delphinidin, cyanidin, petunidin, peonidin, malvidin, dioxygenated, and trioxygenated anthocyanin glucosides for vine vigor zones in 2004.

Site	Year	Vigor Zone	Delphinidin (percent)	Cyanidin (percent)	Petunidin (percent)	Peonidin (percent)	Malvidin (percent)	3'4'-OX (percent)	3'4'5'-OX (percent)
A	2004	High	4.1[b]	1.6[ab]	6.0[a]	20.2[a]	68.1[ab]	21.8[a]	78.2[a]
A	2004	Medium	3.8[b]	1.4[b]	6.0[a]	18.9[a]	69.9[a]	20.3[a]	79.7[a]
A	2004	Low	5.6[a]	1.9[a]	7.6[a]	20.4[a]	64.6[b]	22.3[a]	77.7[a]
SEM			0.4	0.1	0.5	0.6	1.5	0.7	0.7
p-value[a]			0.0832	0.0608	0.1697	0.1114	0.0813	0.0802	0.0802
B	2004	High	3.6[a]	1.4[a]	5.6[a]	17.6[a]	71.8[a]	19.0[a]	81.0[a]
B	2004	Medium	4.1[a]	1.4[a]	6.1[a]	17.4[a]	71.2[a]	18.7[a]	81.3[a]
B	2004	Low	4.6[a]	1.5[a]	6.5[a]	18.0[a]	69.4[a]	19.5[a]	80.5[a]
SEM			0.3	0.1	0.3	0.9	1.4	1.0	1.0
p-value[a]			0.0650	0.4069	0.0678	0.9002	0.4657	0.8647	0.8647
Site* vigor			0.0110	0.0131	0.0415	0.0487	0.0119	0.0401	0.0401

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

the proportional composition specific for a variety may be modified by environmental influences. Previous studies have found vintage effects related to environmental conditions (Ryan & Revilla 2003; Downey et al. 2004). Lower levels of the anthocyanins (Dp and Pt), were found in a warmer year compared to a cooler year (Ryan & Revilla 2003). In Merlot, in response to cool seasons, a higher concentration of Cy and Pn were reported in one study (Yokotsuka et al. 1999) and a higher percent of Dp and Cy and lower Pn and Mv (Spayd et al. 2002) were found in another study. The percent of Mv has been reported to increase with ripening (Ryan & Revilla 2003). According to Roggero et al. (1986), the levels of Cy and Dp peak first, three to four weeks after véraison and then decrease rapidly whereas Pn and Mv continue to be formed. Consequently, the proportion may change over time and may also be confounded with total anthocyanin accumulation.

These results of a higher percent Mv with a warmer year or greater maturity do not explain the results in the present study. In 2003, the fruit was harvested riper (based on soluble solids) and the weather was warmer near the time of harvest (**Table 3.3**; **Figure 2**). However, 2004 had a higher proportion of Mv than 2003. One possible explanation is that the composition was influenced during early berry development by atypical spring weather in 2004. March and April were unusually warm (**Figure 2**) resulting in rapid vegetative growth and high levels of nitrogen in the plant; however, during bloom and fruitset the weather suddenly turned cool and rainy resulting in poor fruitset and inflorescence necrosis. The percentage of Mv was previously found to be predominant with high rates of nitrogen at bloom and low

light intensity at véraison (Keller and Hrazdina 1998). The high N and low light at bloom may explain the high levels of Mv in 2004 compared to 2003. It was also reported that the relative proportions of individual anthocyanins were most equal with low bloom time nitrogen and high light intensity which is more characteristic of the 2003 anthocyanin profile (Keller and Hrazdina 1998). Another possibility is that the differences in proportion are simple related to differences in total accumulation as accumulation of individual anthocyanins is comfounded with total anthocyanin accumulation.

There were differences in the proportion of anthocyanins between sites (**Table 3.8**). The most notable and consistent pattern between the sites was in site A having proportionally higher Pn and lower Mv than site B. The higher Pn and lower Mv found in site A might be explained by the higher degree of shade in this site as it has overall higher vigor than site B. Shading has been found to increase the proportion of Pn (Downey et al 2004). In a light exclusion experiment done in the low vigor zone of site A, shading resulted in an increase of around two times the proportion of Pn compared to exposed clusters (Cortell & Kennedy 2006). Additionally, the lower vigor site (B) had greater sun exposure and higher temperatures during the day which might explain the higher proportion of Mv. In assessing the results by Spayd et al. (2002), cooling sun exposed fruit decreased the percent of Mv and Pn (one out of two years) derivatives while heating shaded fruit increased the proportion of Mv and Pn in the fruit. This shows that the percent of Mv may be closely associated with higher temperatures as observed in site B (**Table 3.2**).

Although accumulation increased for all anthocyanins in fruit with a reduction in vine vigor (**Table 3.6; Figure 5**), vine vigor resulted in proportional variations in anthocyanin composition (**Table 3.11 and 3.12**). In 2003, there was a trend for a higher proportion of Dp and Pt in site A with a reduction in vine vigor (**Table 3.11)**. In site B, there was a trend for higher Dp and Cy with a reduction in vine vigor in 2003. In 2004, there was a trend for higher Dp and Cy with a reduction in vine vigor in site A and no differecnces between vigor zones in site B (**Table 3.12**). Although, shading was found to increase the proportion of Pn (Cortell & Kennedy 2006) and site A had a higher proportion of Pn then site B, there were no differences found in the vigor zones in 2003 or 2004. There was a site by vigor interaction in both years for the proportion of Pn. Mv was similar to Pn in that there were no differences between vigor zones within a site for either year but there was a site by vigor interaction for both years.

The higher proportion of Dp and Pt found in the low vigor fruit in site A for 2003 agrees with previous results where highly sun-exposed fruit was associated with higher Dp and Pt (Tomasi et al. 2003). Our results also agree with findings with Shiraz where shading resulted in a decrease in the relative proportions of Dp and Pt and differs from this study where they found a decrease in Mv and higher Pn and Cy (Downey et al. 2004).

In the previously mentioned Pinot noir shading experiment, shading resulted in decreases in the proportions of Dp, Cy, Pt and Mv with only an increase in the proportion of Pn (Cortell & Kennedy 2006). Hence, it seems likely that increased

sunlight exposure in the low vigor canopy played a role in the higher proportion of Dp and Pt. It does not explain the response of Pn or Mv as clearly. Mv accumulation was found to be less sensitive to the environmental influences of nitrogen and light compared to the other anthocyanins (Keller & Hrazdina 1998). It is possible the higher Mv in the low vigor zones was related to greater methylation occurring in fruit exposed to higher temperatures as seen in **Table 3.2**.

The percent of dioxygenated and trioxygenated anthocyanins was calculated. Both vintage and site varied and there was also a site by year interaction (**Table 3.8**). In 2003, the proportion of dioxygenated anthocyanins was approximately 6% higher than in 2004. Site B was consistently lower than site A in the proportion of dioxygenated in both years. In 2003, there was not an apparent pattern to the proportion of dioxygenated anthocyanins across vigor zones in either site; howver there was a site by vigor interaction (**Table 3.11**). In 2004, there was a trend for a higher proportion of dioxygenated anthocyanins with a reduction in vine vigor in site A and while no differences were found in site B (**Table 3.12**).

Light exposure was reported to shift the biosynthesis toward a higher proportion of trioxygenated anthocyanins compared to dioxygenated through the upregulation of 3’5’flavonoid hydroxylase (Ryan et al. 2002; Downey et al. 2004; Jaakola et al. 2004). In this study, this response was most noticeable between the vineyard sites, where site B had a lower proportion of dioxygenated anthocyanins (Cy and Pn) or in particular a lower proportion of Pn and higher Mv (**Table 3.8**). As Mv has not been found to be particularly sensitive to light or nitrogen (Keller &

Hrazdina 1998; Hilbert et al 2003); this may be more of a temperature effect. The proportional increase in Dp and Pt in the low vigor zones may be more specifically related to light exposure as these have been previously reported to be light responsive (Keller & Hrazdina 1998; Downey et al. 2004) and were found to be lower in a shading experiment carried out in the B-low vigor zone (Cortell & Kennedy 2006). With regard to Pn, we have seen increases in the proportion of Pn in response to shading in Pinot noir (Cortell & Kennedy 2006); however, others have found a higher proportion of Pn in response to water stress (Bao Do & Cormier 1991) and jasmonic acid (Curtin et al. 2003). This shows the complexity of anthocyanin biosynthesis as they can have a similar response to different environmental factors. In addition, as previously mentioned the apparent response could be a direct or indirect effect.

Conclusions. As seen in this study, a number of environmental influences can cause complex relationships in the vineyard related to anthocyanin accumulation in the fruit. This can be due to interactions between available water and nutrients, light interception in the fruiting zone, canopy temperatures and seasonal weather patterns from bloom until ripeness. Environmental factors may result in differences related indirectly to berry size or directly to the accumulation of anthocyanins. Compositional differences can also be a response to environmental conditions but may be simple confounded with total anthocyanin accumulation. We found differences between vintages, vineyard sites and vigor zones in both the amount of anthocyanin accumulation and composition. In 2004, we found smaller berries while

there was a higher concentration of anthocyanins in 2003. Although anthocyanin accumulation does not begin until véraison, light has been reported to have a strong effect on berry development during the early stages of berry development (Keller & Hrazdina 1998; Dokoozlian & Kliewer 1996). As many of the same genes involved in anthocyanin accumulation are expressed early in development, likely due to biosynthesis of other phenolics such as flavonols and proanthocyanidins (Boss et al 1996b), it is possible that anthocyanin biosynthesis and composition could be modified in early berry development. This may help explain way we saw such large vintage compositional effects.

In relation to wine, accumulation of anthocyanins in the fruit is only part of the story as differences in cell structure and winemaking techniques also play a role in anthocyanin extraction. The ease with which anthocyanins can diffuse out of vacuolar membranes and the cell itself is likely the limiting factor in anthocyanin extraction rather than solubility (Sacchi et al. 2005). This is where berry size and the skin to pulp ratio in addition to fruit ripeness (Sims & Bates 1994; Pérez-Magarino & González 2004; Canals et al. 2005) could influence extraction. It has been reported that small berries have a higher ratio of skin surface to volume (Matthews & Anderson 1988) and this could result in a greater concentration of anthocyanins in wine.

Once anthocyanins have been released into the wine matrix, they rapidly begin undergoing reactions that can form pigmented polymers. Both anthocyanins and pigmented polymers contribute to wine color; however, as wine begins to age the

pigmented polymers play an increasingly important role in wine color (Ribéreau-Gayon 1974; Somers 1971; Nagel & Wulf 1979). In Part II of this paper, we investigate some aspects of the relationship between fruit anthocyanin composition as modified by vine vigor and evolution of anthocyanins and formation of pigmented polymers during winemaking.

ABBREVIATIONS USED

TA, titratable acidity (mg/L); Dp, delphinidin-3-*O*-glucoside; Cy, cyanidin-3-*O*-glucoside; Pt, petunidin-3-*O*-glucoside; Pn, peonidin-3-*O*-glucoside; CI, 95% confidence interval.

ACKNOWLEDGMENTS

We thank Archery Summit Winery for the use of their vineyards and fruit for wine production as well as Leigh Bartholomew and Anna Matzinger specifically for their support. In addition, we would like to thank students: Jason Bell, Fiorella Cerpa, Jose Luis Pastor del Rio and Chris Zielinski for their help. Funding for this project was provided by the USDA Northwest Center for Small Fruit Research and the American Society for Enology and Viticulture.

LITERATURE CITED

Bao Do, C.; Cormier, F. Accumulation of peonidin-3-glucoside enhanced by osmotic stress in grape (*Vitis vinifera* L.) cell suspension. *Plant Cell, Tissue and Organ Culture.* **1991,** 24, 49-54.

Bergqvist, J.; Dokoozlian, N.; Ebisuda, N. Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the central San Joaquin Valley of California. *Am. J. Enol. Vitic.* **2001,** 52, 1-7.

Boss, P. K.; Davies, C.; Robinson, S. P. Analysis of the expression of anthocyanin pathway genes in developing Vitis vinifera L. cv Shiraz grape berries and the implications for pathway regulation. *Plant Physiol.* **1996a,** 111, 1059-1066.

Boss, P. K.; Davies, C.; Robinson, S. P. Expression of anthocyanin biosynthesis pathway genes in red and white grapes. *Plant Mol. Biol.* **1996b,** 32, 565-569.

Bramley, R.G.V.; Hamilton, R.P. Understanding variability in winegrape production systems 1. Within vineyard variation in yield over several vintages. *Aust. J. Grape Wine Res.* **2004,** 1, 32-45.

Brossaud, F.; Cheynier, V.; Asselin, C.; Moutounet, M. flavonoid compositional differences of grapes among site test plantings of Cabernet franc. *Am. J. Enol. Vitic.* **1999,** 50, 277-284.

Buttrose, M. S.; Hale, C. R.; Kliewer, W. M. Effect of temperature on the composition of Cabernet Sauvignon berries. *Am. J. Enol. Vitic.* **1971,** 22, 71-75.

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

Chalker-Scott, L. Environmental significance of anthocyanins in plant stress responses. *Photochem. Photobiol.* **1999,** 70, 1-9.

Chaves, N.; Escudero, J. C. Variation of flavonoid synthesis induced by ecological factors. In *Principles and Practices of Plant Ecology, Allelochemical Interactions.* (Eds Inderjit, K.M.M. Dakshini & C.L. Foy) CRC Press, Boca Raton, Florida, USA. **1999;** pp 267-285.

Coombe, B. G. The development of fleshy fruits. *Ann. Rev. Plant Physiol.* **1976,** 27, 507-528.

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

Cortell, J. M.; Kennedy, J. A. Effect of shading on accumulation of flavonoid compounds in *Vitis vinifera* L. Pinot noir fruit and extraction in a model system. *J. Agric. Food Chem.* **2006,** in press.

Crippen, D. D.; Morrison, J. C. The effects of sun exposure on the compositional development of Cabernet Sauvignon berries. *Am. J. Enol. Vitic.* **1986,** 38, 235-242.

Curtin, C.; Zhang, W.; Franco, C. Manipulating anthocyanin composition in *Vitis vinifera* suspension cultures by elicitation with jasmonic acid and light irradiation. *Biotechnology Letters,* **2003,** 25, 1131-1135.

Dokoozlian, N. K; Kliewer W. M. Influence of light on grape berry growth and composition varies during fruit development. *J. Am. Soc. Hort. Sci.* **1996,** 121, 869-874.

Downey, M. O.; Harvey, J. S.; Robinson, S. P. The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. *Aust. J. Grape Wine Res.* **2004,** 1, 55-73.

Ewart, A.; Kliewer, W. M. Effects of controlled day and night temperatures and nitrogen on fruit-set, ovule fertility, and fruit composition of several grape cultivars. *Am. J. Enol. Vitic.* **1977,** 28, 88-95.

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

Gao, Y.; Cahoon, G. A. Cluster shading effects on fruit quality, fruit skin color, and anthocyanin content and composition in Reliance (*Vitis* hybrid). *Vitis* **1994,** 33, 205-209.

Ginestar, C.; Eastham, J.; Gray, S.; Iland, P. Use of sap-flow sensors to schedule vineyard irrigation. II. Effects of post-véraison water deficits on composition of Shiraz grapes. *Am. J. Enol. Vitic.* **1998,** 49, 421-428.

Hall, A.; Lamb, D.W.; Holzapfel, B.; and Louis, J. Optical remote sensing applications for viticulture – a review. *Aust. J. Grape Wine Res.* **2002,** 8, 36-47.

Hardie, W. J.; Considine, J. A. Response of grapes to water-deficit stress in particular stages of development. *Am. J. Enol. Vitic.* **1976,** 27, 55-61.

Hardie, W. J.; Johnson, J. O.; Weaver, R. J. The influence of vine water regime on ethephon enhanced ripening of zinfandel. *Am. J. Enol. Vitic.* **1981**, 32, 115-121.

Haselgrove, L.; Botting, D.; van Heeswijck, R.; HØj, P. B.; Dry, P.R.; Ford, C.; Iland, P. G. Canopy microclimate and berry composition: The effect of bunch exposure on the phenolic composition of *Vitis vinifera* L. cv Shiraz grape berries. *Aust. J. Grape Wine Res.* **2000,** 6, 141-149.

Hilbert, G.; Soyer, J. P.; Molot, C.; Giraudon, J.; Milin, G. S.; Gaudillere, J. P. Effects of nitrogen supply on must quality and anthocyanin accumulation in berries of cv. Merlot. *Vitis* **2003,** 42, 69-76.

Jaakola, L.; Määttä-Riihinen, K.; Kärenlampi, S.; Hohtola, A. Activation of flavonoid synthesis by solar radiation in bilberry (*Vaccinium myrtyillus* L.) leaves. *Planta* **2004,** 218, 721-728.

Jackson, D. I.; Lombard, P. B. Environmental and management practices affecting grape composition and wine quality – A review. *Am. J. Enol. Vitic.* **1993,** 44, 409-430.

Keller, M. K.; Hrazdina, G. Interaction of nitrogen availability during bloom and light intensity during véraison. II. Effects on anthocyanin and phenolic development during grape ripening. *Am. J. Enol. Vitic.* **1998,** 49, 341-349.

Kennedy, J.A.; Matthews, M.A.; Waterhouse, A.L. Changes in grape seed polyphenols during fruit ripening. *Phytochemistry.* **2000.** 55, 77-85.

Kennedy, J. A.; Matthews, M. A.; Waterhouse, A. L. Effect of maturity and vine water status on grape skin and wine flavonoids. *Am. J. Enol. Vitic.* **2002,** 53, 268-274.

Kliewer, W. M.; Lider, L. A.; Schultz, H. B. Influence of artificial shading of vineyards on the concentration of sugar and organic acids in grapes. *Am. J. Enol. Vitic.* **1967,** 18, 78-86.

Kliewer, W. M.; Lider, L. A. Influence of cluster exposure to the sun on the composition of 'Thompson seedless' fruit. *Am. J. Enol. Vitic.* **1968,** 19, 175-184.

Kliewer, W. M.; Torres, R. E. Effect of controlled day and night temperatures on grape coloration. *Am. J. Enol. Vitic.* **1972,** 23, 71-103.

Lamb, D.W.; Weedon, M.M.; Bramley, R.G.V. Using remote sensing to predict grape phenolics and colour at harvest in a Cabernet Sauvignon vineyard: Timing observations against vine phenology and optimizing image resolution. *Aust. J. Grape Wine Res.* **2004,** 10, 46-54.

Lamuela-Raventos, R. M.; Waterhouse, A. L. A direct HPLC separation of wine phenolics. *Am. J. Enol. Vitic.* **1994,** 45, 1-5.

Matthews, M. A.; Anderson, M. M. Fruit ripening in *Vitis vinifera* L.: Response to seasonal water deficits. *Am. J. Enol. Vitic.* **1988,** 39, 313-320.

Mazza, G.; Miniata, E. Grapes. In *Anthocyanins in Fruits, Vegetables, and Grain;* G., Miniata, E., Eds.; CRC Press: Boca Raton, 1993; pp 149-199.

Mazza, G. Anthocyanins in grapes and grape products. *Crit. Rev. Food Sci. Nutr.* **1995,** 35, 341-371.

Mazza, G.; Fukumoto, L.; Delaquis, P.; Girard, B.; Ewert, B. Anthocyanins, phenolics, and color of Cabernet franc, Merlot, and Pinot noir wines from British Columbia. *J. Agric. Food Chem.* **1999,** 47, 4009-4017.

Mori, K.; Saito, H.; Goto-Yamamoto, N.; Kitayama, M.; Kobayashi, S.; Sugaya, S.; Gemma, H.; Hashizume, K. Effects of abscisic acid treatment and night temperature on anthocyanin composition in Pinot noir grapes. *Vitis,* **2005,** 4, 161-165.

Morrison, J. C.; Noble, A. C. The effects of leaf and cluster shading on the composition of Cabernet Sauvignon grapes and on fruit and wine sensory properties. *Am. J. Enol. Vitic.* **1990,** 41, 193-200.

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

Ojeda, H.; Andary, C.; Kraeva, E.; Carbonneau, A.; Deloire, A. Influence of pre- and post-véraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. *Am. J. Enol. Vitic.* **2002,** 53, 261-267.

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

Price, S. F.; Breen, P. J.; Valladao, M.; Watson, B. T. Cluster exposure and quercetin in Pinot noir grapes and wine. *Am. J. Enol. Vitic.* **1995,** 46, 187-194.

Reynolds, A. G.; Pool, R. M.; Mattick, L. R. Influence of cluster exposure on fruit composition and wine quality of Seyval blanc grapes. *Vitis* **1986,** 25, 85-95.

Reynolds, A. G.; Yerle, S.; Watson, B.; Price, S. F.; Wardle, D. A. Fruit environment and crop level effects on Pinot noir. III. Composition and descriptive analysis of Oregon and British Columbia wines. *Am. J. Enol. Vitic.* **1996,** 47, 329-339.

Reynolds, A. G.; Molek, T.; De Savigny, C. Timing of shoot thinning in *Vitis vinifera*: Impacts on yield and fruit composition Variables. *Am. J. Enol. Vitic.* **2005,** 56, 343-356.

Ribéreau-Gayon, P. The chemistry of red wine color. In *Chemistry of Winemaking*. (Eds Webb, A. D.) American Chemical Society, Washington, D.C. 1974; pp.81.

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

Roggero, J. P.; Coen, S.; Ragonnet, R. High performance liquid chromatography survey on changes in pigment content in ripening grapes of Syrah. An approach to anthocyanin metabolism. *Am. J. Enol. Vitic.* **1986,** 37, 77-83.

Ryan, J. M.; Revilla, E. Anthocyanin composition of Cabernet Sauvignon and Tempranillo grapes at different stages of ripening. *J. Agric. Food Chem.* **2003,** 51, 3372-3378.

Ryan, K. G.; Swinny, E. E.; Markham, K. R.; Winefeld, C. Flavonoid gene expression and UV photoprotection in transgenic and mutant Petunia leaves. *Phytochemistry* 59, **2002,** 23-32.

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

Salón , J. L.; Chirivella, C.; Castel, J. R. Response of cv. Bobal to timing of deficit irrigation in Requena, Spain: Water relations, yield, and wine quality. *Am. J. Enol. Vitic.* **2005**, 56, 1-8.

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

Smart, R. E. Principles of grapevine canopy microclimate manipulation with implications for yield and quality. A review. *Am. J. Enol. Vitic.* **1985,** 36, 230-239.

Smart, R. E.; Smith, S. M.; Winchester, R. V. Light quality and quantity effects on fruit ripening for Cabernet sauvignon. *Am. J. Enol. Vitic.* **1988,** 39, 250-258.

Somers, T. C. The polymeric nature of wine pigments. *Phytochemistry.* **1971,** 10, 2175-2186.

Spayd, S. E.; Tarara, J. M.; Mee, D. L.; Ferguson, J.C. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *Am. J. Enol. Vitic.* **2002,** 53, 171-182.

Tomasi, D.; Pitacco, A.; Pascarella, G. Bunch and berry temperature and anthocyanin synthesis and profile in Cabernet sauvignon. *Rivista di Viticoltura e di Enologia,* **2003,** 56, 3-15.

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

Yokotsuka, K.; Nagao, A.; Nakazawa, K.; Sato, M. Changes in anthocyanins in berry skins of Merlot and Cabernet sauvignon grapes grown in two soils modified with limestone or oyster shell versus a native soil over two years. *Am. J. Enol. Vitic.* **1999,** 50, 1-12.

Influence of Vine Vigor on Grape (*Vitis vinifera* L. Cv. Pinot noir) Anthocyanins: Part II. Anthocyanins and Pigmented Polymers in Wine.

Jessica M. Cortell, Michael Halbleib, Andrew V. Gallagher,

Timothy L. Righetti, James A. Kennedy*

Journal of Agricultural Food Chemistry
PO Box 3337,
Columbus, OH, 43210, USA
To be submitted October 15, 2006

ABSTRACT

The relationships between grapevine (*Vitis vinifera*) vigor variation and resulting wine anthocyanin concentration and composition and pigmented polymer formation were investigated. The study was conducted in a commercial vineyard consisting of the same clone, rootstock, age, and vineyard management practices. Vine vigor parameters were used to designate vigor zones within two vineyard sites to produce research wines (2003 and 2004) and conduct a model extraction experiment (2004 only) to investigate the vine-fruit-wine continuum. Wines and model extracts were analyzed by HPLC and spectrophotometry. For the model extraction, there were no differences between sites for pomace weight while juice volume was higher in site A. This was not related to a larger berry size. Site A had a higher anthocyanin concentration (mg/L) in the model extracts than site B particularly for the medium and low vigor zone. For anthocyanin composition, In the model extraction, site B had a greater proportion of malvidin and less of the other four anthocyanin glucosides compared to site A. In the wines, there was a vintage effect with the 2003 wines having a higher anthocyanin concentration (mg/L) than in 2004. This appears to have been primarily due to a greater accumulation of anthocyanins in the fruit. In general, the medium vigor zone wines had higher anthocyanin concentrations than either the high or low vigor zones wines. There was also vintage variation related to anthocyanin composition with the 2003 wines having a higher proportion of delphinidin and petunidin glucosides and lower malvidin-3-*O*-glucoside compared to 2004. In both years, there were higher proportions of delphinidin and petunidin

glucosides in wines made from low vigor zone fruit. Wines made from low vigor zones showed a greater propensity to form vitisin A as well as pigmented polymers. Low vigor zone wines had ~ a two-fold increase in pigmented polymer concentration (mg/L) over high vigor zones. There was a strong positive relationship between pigmented polymer concentration, sulfite bleaching resistant pigments, proanthocyanidin concentration and color density in wines. Overall, differences found in the wines magnified variation in the fruit.

KEYWORDS: pigmented polymers; proanthocyanidins; color density; hue; berry size

INTRODUCTION

The color of red wine is an important sensory attribute that originates from anthocyanins in the fruit. Anthocyanins also have human health benefits (Wang et al. 1997; Mũnoz-Espada et al. 2004). Grape varieties may have a complex profile of up to 20 different anthocyanins (Wulf & Nagel 1976) or a relatively simple profile as in Pinot noir, a cool climate variety which only produces the five non-acylated forms (Fong et al. 1971) Consequently, the specific anthocyanin profile in Pinot noir will impact color density and color stability of the wine.

In addition to the anthocyanin amount in the berry, the extractability of anthocyanins from skins influences the wine profile. Berry size was reported to play a role in extraction (Matthews & Anderson 1988) although recent studies found that berry size alone did not have a major impact on extraction (Roby et al. 2004; Walker

et al. 2005). Fruit ripeness was also reported to improve extraction of anthocyanins and phenolic compounds (Sims & Bates 1994; Pérez-Magarino & González-San José 2004; Canals et al. 2005).

Winemaking practices such as skin contact time, fermentation temperature, and the use of macerating enzymes influence the extraction of anthocyanins as reviewed by Sacchi et al. (2005). Anthocyanin concentration reaches a maximum early in fermentation followed by a decrease (Nagel & Wulf 1979; Watson et al. 1995; Gao et al. 1997). Once anthocyanins have been extracted into the wine matrix, they rapidly form copigmentation complexes (Brouillard et al. 1994; Boulton 2001) and begin undergoing numerous reactions. Several families of new pigmented compounds have been identified and described (Salas et al. 2005).

One reaction mechanism is described as a cycloaddition between anthocyanins and vinyl derivatives to form pigments known as pyranoanthocyanins (Bakker & Timberlake 1997; Fulcrand et al. 1998). Vitisin A was one of the first compounds identified in this family (Bakker & Timberlake 1997; Fulcrand et al. 1998). At wine pH, they are orange-red pigments, have an absorbance maximum at 500nm, and are resistant to oxidation and bisulfite bleaching (Lee et al. 2004). Although these compounds have longevity in wine, they play a limited role in red wine color due to their concentration.

Another family of pigmented compounds is formed through the combination of an ethyl-bridge via the enolic form of acetaldehyde (Timberlake & Bridle 1976; Bishop & Nagel 1984; Saucier et al. 1997). These pigments are formed early in

fermentation and rapidly consume much of the monomeric anthocyanins. They have maximum absorbance at around 544 nm at wine pH and have a purple color. Compared to monomeric anthocyanins, they have increased resistance to hydration and bisulfite bleaching. These compounds are thought to be degraded relatively easily possibly becoming reactive intermediates for other reactions.

The predominant pigments in aged red wine are thought to come from the direct reaction between anthocyanins and proanthocyanidins (Remy et al. 2000). Pigmented polymers account for 50 to 70% of the color in a one year old wine (Riberéau-Gayon et al. 1970; Somers 1971; Nagel & Wulf 1979). Two mechanisms lead to tannin-anthocyanin (T - A+) and anthocyanin-tannin (A+ - T) adducts (Salas et al. 2003). Both reactions are expected to be pH dependent and the T – A+ adducts are susceptible to acid-catalyzed cleavage and hydration (Salas et al. 2005; Salas et al. 2003).

While the existence of these compounds in wines has been recently confirmed (Hayasaka & Kennedy 2003) the structural diversity of pigmented polymers is expected to be large due to all the possible combinations of proanthocyanidins and anthocyanins. Vineyard related fruit differences are thought to play a role in pigmented polymer formation based on the initial concentration and composition of anthocyanins and proanthocyanidins (Fulcrand et al. 2004). The level of a specific anthocyanin-derived pigment was found to be related to the initial concentration of native anthocyanin precursors (Gómez-Cordovés 2004).

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

MATERIALS AND METHODS

Vineyard. The study was conducted in 2003 and 2004 in a 7-year-old commercial *Vitis vinifera* L., cv. Pinot noir vineyard (clone Dijon 777 grafted onto *Riparia gloire* rootstock) located in the Willamette Valley in Oregon, USA. Vine spacing was 1 m (within row) X 2.8 m (between rows) with approximately 5113 vines per hectare. The training was a vertical shoot positioned system with each vine pruned to 10-12 buds. The experimental design and delineation of vigor zones was previously described (Cortell et al. 2005).

Fruit Sampling and Extraction. A fruit sample was collected across each vigor zone (3 replicates/zone) to reflect the fruit used for wine production. Harvest date was determined by the cooperating winery. Fruit samples were harvested and processed as described in Part I of this paper ((Cortell et al. in press).

Model extraction. Approximately 15 clusters were used for each rep of the model extraction for a total of 5 reps collected from random data vines locations within each vigor zone. Fruit was collected off of the data vines and 3-4 adjacent

vines. The berries were carefully removed from the rachis in order to avoid losing juice. Berries were mixed then a 300 g sample was taken for each replicate. The number of berries in the 300 gram 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. 300 ml of the ethanol solution was added to the 300g berry sample resulting in a ~ 20% v/v ethanol solution. Samples were sparged with nitrogen and then placed on a shaker table for 48 hours at 38 °C. After 48 hours, the musts were pressed using a buchner funnel (69 cm^2 surface area) with an applied vacuum of 1.6 bars. The pressed pomace was weighed and frozen. The must volume was determined before and after pressing. After pressing, musts were frozen at -10°C until analyzed.

Winemaking. Triplicate wines were produced from each vigor zone in 2003 and 2004. The winemaking protocol for the 2003 wines was previously described in (Cortell et al. 2005). The 2004 wines were made with the same protocol as the 2003 wines except for smaller replicates consisting of 22 kg were used. In 2004, samples were taken at the end of cold soak and every day during fermentation until pressing. Samples were taken before and after pressing in the finished wines. The samples were frozen at -10°C until analyzed. This differed from 2003 where wines were not analyzed until after malolactic fermentation at 3-4 months old.

Chemicals. All solvents were HPLC grade. Chemicals were as described in (Cortell et al. 2006). Additional chemicals used included potassium metabisulfite and potassium hydroxide purchased from J.T. Baker (Phillipsburg, NJ). N,N-dimethylforamide (DMF) was purchased from Burdick and Jackson (Muskegon, MI). Hydrochloric acid was purchased from E.M. Science (Gibbstown, NJ) and lithium chloride was purchased from Mallinckrodt (Phillipsburg, NJ).

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

Reversed-Phase HPLC. Anthocyanin content and composition in grape skins and wine were measured by reversed-phase HPLC (Lamuela-Raventos & Waterhouse 1994). Aqueous extracts and wines were filtered using Teflon filters (0.45µm, Acrodisc CR13) before injection. Eluting anthocyanins were identified and quantified with a malvidin-3-*O*-glucoside standard.

Gel Permeation Chromatography. Gel Permeation Chromatography (GPC) was used to analyze monomeric 520nm absorbing material and pigmented polymers. By using GPC, information on the size distribution as well as pigment content could be obtained. The GPC method used has been described previously (Kennedy & Taylor 2003). Samples were prepared as previously described (Cortell et al. 2005); however, after freeze drying the samples were dissolved in the GPC mobile phase. Malvidin-3-*O*-glucoside was used as a standard for GPC analysis at 520 nm.

SO_2 Bleaching of Wines. Wine color density was calculated as absorbance at 420nm + 520nm. Hue was determined as 420nm/520nm absorbance. Percent red pigment in wine was determined as 520nm/ 520nm + HCl *100 absorbance. Wines were subjected to bleaching with SO_2 using a previously described method (Somers & Evans 1977).

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

RESULTS AND DISCUSSION

Model extraction anthocyanin concentration. The influence of vine vigor on fruit composition in 2003 and 2004 was described in (Cortell et al. in press). In 2004, a model extraction was done in addition to making wines from the vigor zones. The goal of the model extraction was to have the ability to study extraction in more detail than was possible in the wines. In the 300 g sample used for each rep of the model extractions, there was a trend for a greater number of berries with increasing vine vigor in site A (**Table 4.1**). However, there were no differences in pomace weight. The greater number of berries per 300 g sample was expected in the high vigor zone extraction as this zone had smaller berries in 2004 (Cortell et al. in press).

Juice volume was higher for site A than site B specifically for A-medium and A-low. There were no differences in juice volume for vigor zones in site B. The juice volume and pomace weight are not in agreement with what would generally be expected where a smaller berry size would produce a lower juice volume and higher pomace weight. Although site B had a higher average berry weight in this model extraction, it had a lower juice volume.

Table 4.1. Mean and SEM of fruit composition in the model extraction in 2004 including number of berries in 300 g, pomace weight (g), and juice volume (mL).

Site	Vigor Zone	# Berries in 300 g.	Pomace Wt. (g)	Juice volume (mL)
A	High	480[a]	113.1[a]	447.6[b]
A	Medium	451[ab]	108.9[a]	458.6[a]
A	Low	399[b]	115.1[a]	453.6[ab]
SEM		25.5	3.5	3.4
p-value[a]		0.0886	0.4803	0.1360
B	High	414[a]	106.8[a]	413.8[a]
B	Medium	442[a]	107.0[a]	416.6[a]
B	Low	393[a]	115.5[a]	413.4[a]
SEM		19	2.9	2.0
p-value[a]		0.2525	0.0705	0.3696

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

In addition to the concentration of anthocyanins in skins, much emphasis has been placed on the influence of berry size on wine composition as it can modify the ratio of skin and seed material to pulp and influence the final amount extracted into wine (Matthews & Anderson 1988). This idea of reduced berry size has been

explored in deficit irrigation experiments. Two studies have confirmed two types of response to water deficit, one is an indirect but positive effect on the concentration of anthocyanins due to berry size reduction and the second is a direct influence on biosynthesis (Ojeda et al. 2002; Roby et al. 2004). In another recent study on berry size, in general small berries had a similar skin to fruit ratio and similar juice yield compared to large berries (Walker et al. 2005). This may help explain why we saw no differences in pomace weight and a higher volume of juice produced from the same weight of berries from vigor zones with smaller berries.

In the 2004 model extraction, site A was higher in total anthocyanin concentration as well as all the individual anthocyanins (delphinidin-3-*O*-glucoside (Dp), cyanidin-3-*O*-glucoside (Cy), petunidin-3-*O*-glucoside (Pt), and peonidin-3-*O*-glucoside (Pn)) except for malvidin-3-*O*-glucoside (Mv) than site B (**Table 4.2**). Total anthocyanin concentration in the model extract (mg/L) increased with a reduction in vine vigor in site A while site B the concentrations were similar (**Table 4.3**). In site A, Pt, Pn and Mv increased with a reduction in vine vigor while there was not a significant response in site B for any of the individual anthocyanins. The B-low vigor zone total anthocyanin concentration may have been similar to B-medium due to a similar amount per berry but a larger berry size due to poor fruitset as well as inconsistent ripening as there were pink and green berries in the clusters at harvest. This failure for fruit to mature has been previously described (Hardie & Considine 1976).

The A-low vigor zone model extraction had the highest concentration of anthocyanins. Although A-low had one of the highest average berry weights, it also had the highest anthocyanin concentration per berry and per mg/kg. (Cortell et al. in press). On the other hand, A-medium which had the second highest anthocyanin concentration in the model extraction had the smallest berry size and one of the lowest anthocyanin concentrations per berry. The A-high extraction had a small average berry size and low anthocyanin content per berry and per mg/kg which resulted in the lowest anthocyanin concentration of the vigor zone model extracts.

From these results, it appears that anthocyanin concentration per berry played a more important role than berry size in the anthocyanin concentration in the model extracts except for in A-medium where small berry size may have been an important factor. Other research has also shown that berry size alone does not explain differences in wine as they did not find any indication of a higher anthocyanin content or improved color density in assessing a range of berry sizes except for the very smallest berry category tested (0.3-0.55 g.) (Roby et al. 2004; Walker et al. 2005). The percent extraction of anthocyanins from the fruit was also investigated.

Table 4.2. Mean and SEM of total, delphinidin, cyanidin, petunidin, peonidin and malvidin anthocyanin concentrations (mg/L) in model extractions in 2004 (average of vigor zones) .

Year	Total (mg/L)	Delphinidin (mg/L)	Cyanidin (mg/L)	Petunidin (mg/L)	Peonidin (mg/L)	Malvidin (mg/L)
A	183	5.31	2.81	8.84	39.75	126.64
B	163	3.27	2.12	5.95	31.49	119.84
SEM	7.0	0.30	0.11	0.46	1.39	5.08
p-value[a]	0.0426	<0.0001	0.0002	0.0001	0.0002	0.3515

[a] ANOVA to compare data (*P* indicated), n=10.

Table 4.3. Mean and SEM of total anthocyanin, delphinidin, cyanidin, petunidin, peonidin and malvidin concentrations (mg/L) in the model extractions in 2004.

Site	Vigor Zone	Total (mg/L)	Delphinidin (mg/L)	Cyanidin (mg/L)	Petunidin (mg/L)	Peonidin (mg/L)	Malvidin (mg/L)
A	High	157.6[b]	5.43[a]	2.70[a]	7.97[b]	33.7[b]	107.9[b]
A	Medium	180.5[ab]	4.92[a]	2.67[a]	8.35[b]	38.9[b]	125.7[ab]
A	Low	210.2[a]	6.77[a]	3.45[a]	11.30[a]	46.7[a]	142.0[a]
SEM		9.9	0.69	0.28	0.85	2.32	7.1
p-value[a]		0.0126	0.2056	0.1322	0.0401	0.0089	0.0176
B	High	149.0[a]	3.38[a]	2.17[a]	5.82[a]	28.3[a]	109.3[a]
B	Medium	171.9[a]	3.21[a]	2.07[a]	6.03[a]	32.4[a]	128.2[a]
B	Low	159.3[a]	3.29[a]	2.14[a]	5.91[a]	32.9[a]	115.0[a]
SEM		11.1	0.33	0.16	0.54	2.0	8.4
p-value[a]		0.3829	0.9305	0.9086	0.9615	0.2479	0.3111

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

Although the amount in the fruit and model extract was different in the vigor zones in site A, there was only a mild trend for a higher percent extraction with higher vigor in both sites (**Table 4.4**). There were no differences in the amount in the fruit or the model extracts for the vigor zones in site B. Previous research has shown a higher percent extraction of anthocyanins in less ripe fruit as was seen in the A-high vigor zone (Ribéreau 1972). A-high had approximately 75% extraction and A-low had only ~ 45% extraction. Others have reported higher anthocyanins or color density in wines from vines with greater sun exposure in the fruiting zone (Price et al. 1995; Mazza et al. 1999), from riper fruit (Sims and Bates 1994; Perez-Magarino & Gonzalez-San Jose 2004; Canals et al. 2005), and from deficit irrigation (Hepner, et al. 1985; Sipiora et al. 1998; Salon et al. 2005). However, in many of these studies it has been hard to determine if the differences found in wine were strictly related to berry size, anthocyanin content or if some other variable such as ease of extraction was also playing an important role.

In the present study, extractions done with fruit from medium vigor vines tended to have the highest anthocyanin concentrations but not necessarily related to improved extraction. In a review on winemaking techniques, the ease of which anthocyanins can move from the vacuole and from the hypodermal cell into the wine is important (Sacchi et al. 2005). These results suggest that high vigor or possible less ripe fruit allowed for easier extraction of anthocyanins from the fruit.

Table 4.4. Mean and SEM of anthocyanin amount (mg) in the 300 g. of fruit, amount in the extract volume (mg) and percent extraction in the model extraction experiment.

Site	Vigor Zone	Amount in fruit (mg)	Amount in extract (mg)	Extraction (percent)
A	High	95.4[b]	70.5[b]	74.6[a]
A	Medium	142.1[ab]	82.7[ab]	62.8[a]
A	Low	191.3[a]	95.4[a]	52.8[a]
SEM		19.9	4.4	7.5
p-value[a]		0.0524	0.0093	0.1870
B	High	119.8[a]	61.7[a]	52.4[a]
B	Medium	149.1[a]	71.6[a]	48.6[a]
B	Low	148.3[a]	65.9[a]	45.2[a]
SEM		12.7	4.7	3.0
p-value[a]		0.1850	0.3758	0.1195

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

Anthocyanin concentration in wine. There was a significant difference between vintages for anthocyanin concentration in the wine with 2003 being higher than 2004 (**Table 4.5**). Site A had a higher concentration of anthocyanins in the wines in both years (**Table 4.5**). The average wine anthocyanin concentrations for both years were in a similar range to the amount found in other studies on Pinot noir (Reynolds et al. 1996; Mazza et al. 1999; Parley et al. 2001).

Table 4.5. Mean and SEM of total anthocyanin, delphinidin, cyanidin, petunidin, peonidin and malvidin concentrations (mg/L) in wines for site A and B (average of vigor zones).

Site	Year	Total (mg/L)	Delphinidin (mg/L)	Cyanidin (mg/L)	Petunidin (mg/L)	Peonidin (mg/L)	Malvidin (mg/L)	Vitisin A (mg/L)
A	2003	186.3	5.79	1.24	10.53	20.62	137.39	10.78
B	2003	177.1	5.09	0.96	8.89	16.08	114.96	31.34
A	2004	137.6	2.42	0.92	5.22	16.08	110.10	2.91
B	2004	117.1	1.93	0.77	4.08	11.81	114.96	2.89
SEM		5.9	0.26	0.10	0.41	0.93	5.62	1.99
Site	p-value[a]	0.0180	0.0296	0.0390	0.0018	<0.0001	0.0025	<0.0001
Year	p-value[a]	<.0001	<0.0001	0.0131	<0.0001	<0.0001	0.0002	<0.0001
Site*Year	p-value[a]	0.3388	0.7038	0.5168	0.5448	0.8892	0.4818	<0.0001

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

In 2003 in site A, the medium zone wines had the highest total anthocyanin concentration followed by the low then the high vigor zone wines. In site B, the high vigor zone was highest followed by the low and then the medium vigor zone wines in 2003 (**Table 4.6**).In 2004, the medium and low vigor zone wines in site A were similar while the high vigor zone wines were lower in total anthocyanin concentration (**Table 4.7**). In site B, the medium vigor zone wines were higher in anthocyanin concentration compared to either the high or low vigor zone wines. The A-high vigor zone wines had the lowest anthocyanin concentration in both years (**Table 4.6 & 4.7**). The highest anthocyanin concentration was found in A-medium and B-high in 2003 and in A-medium in 2004. Otherwise, there did not seem to be a definitive pattern related to vigor zones.

In terms of individual anthocyanin concentrations in the wines, there were differences between vigor zones for all anthocyanins in both sites and both years except for only a trend for cyanidin in site A in 2003 and site B in 2004 (**Table 4.6 & 4.7**). In general, the pattern for accumulation of individual anthocyanins paralleled total accumulation. Vitisin A, formation during fermentation increased with a reduction in vine vigor in both sites and both years.

The lower total anthocyanin concentrations found in A-high and B-high wines were consistent with a lower amount of anthocyanins in the fruit compared to the other zones. However, A-medium also had a lower amount in the fruit in 2004 but this did not result in a reduced concentration in the wine. This might have been due to a greater percent extraction as the model experiment between A- medium compared to

Table 4.6. Mean and SEM of total anthocyanin, delphinidin, cyanidin, petunidin, peonidin, malvidin and vitisin A concentration (mg/L) in wines made from vine vigor zones in 2003.

Site	Year	Vigor Zone	Total (mg/L)	Delphinidin (mg/L)	Cyanidin (mg/L)	Petunidin (mg/L)	Peonidin (mg/L)	Malvidin (mg/L)	Vitisin A (mg/L)
A	2003	High	143.90[c]	3.30[b]	0.50[b]	6.66[c]	13.71[c]	114.70[b]	5.04[c]
A	2003	Medium	199.66[a]	6.08[a]	1.29[ab]	11.44[a]	22.21[a]	149.41[a]	9.23[b]
A	2003	Low	159.74[b]	6.14[a]	1.50[a]	9.29[b]	18.57[b]	103.83[c]	20.41[a]
SEM			3.65	0.51	0.31	0.37	0.69	1.82	0.60
p-value[a]			0.0004	0.0025	0.0849	0.0010	0.0020	0.0001	0.0001
B	2003	High	204.81[a]	6.16[a]	1.32[a]	10.95[a]	20.96[a]	150.32[a]	15.11[c]
B	2003	Medium	162.32[c]	4.36[ab]	0.65[b]	7.86[b]	13.58[c]	101.59[b]	34.29[b]
B	2003	Low	177.55[b]	5.38[b]	1.19[a]	8.74[b]	15.84[b]	104.27[b]	42.14[a]
SEM			2.92	0.30	0.06	0.29	0.38	2.25	0.83
p-value[a]			0.0013	0.0327	0.0024	0.0039	0.0004	0.0002	<0.0001

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

Table 4.7. Mean and SEM of total anthocyanin, delphinidin, cyanidin, petunidin, peonidin, malvidin and vitisin A concentration (mg/L) in wines made from vine vigor zones in 2004.

Site	Year	Vigor Zone	Total (mg/L)	Delphinidin (mg/L)	Cyanidin (mg/L)	Petunidin (mg/L)	Peonidin (mg/L)	Malvidin (mg/L)	Vitisin A (mg/L)
A	2004	High	95.70[b]	1.48[b]	0.49[c]	3.26[c]	8.98[c]	79.89[b]	1.61[c]
A	2004	Medium	143.12[a]	2.42[a]	0.95[b]	5.29[b]	16.59[b]	115.04[a]	2.83[b]
A	2004	Low	141.73[a]	3.01[a]	1.06[a]	6.14[a]	18.44[a]	109.06[a]	4.03[a]
SEM			2.35	0.08	0.009	0.11	0.36	1.94	0.08
p-value[a]			0.0001	0.0005	<0.0001	0.0001	<0.0001	0.0002	0.0206
B	2004	High	104.24[b]	1.61[b]	0.63[b]	3.47[b]	9.50[c]	86.79[b]	2.24[b]
B	2004	Medium	125.04[a]	1.94[a]	0.79[ab]	4.16[a]	12.14[b]	102.79[a]	3.04[a]
B	2004	Low	115.44[ab]	2.21[a]	0.86[a]	4.54[a]	13.52[a]	91.05[b]	3.25[a]
SEM			3.65	0.09	0.06	0.17	0.39	2.81	0.16
p-value[a]			0.0357	0.0253	0.0601	0.0300	0.0034	<0.0001	0.0206

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

B-medium as A-medium had a trend for higher percent extraction. Overall, the 2004 model extraction had a higher percent extraction than in the wines. This may have been due to the higher extraction temperature and higher ethanol content used for the model extraction.

The amount of anthocyanins available to be extracted from the fruit (mg/kg) was over twice as much in 2003 compared to 2004 (Cortell et al. in press). This explains the higher levels of anthocyanins found in the 2003 wines. From doing a rough estimate based on anthocyanins in the fruit and the concentration in the wine, the extraction in 2003 was ~ 9-16% and from ~ 10-35% in 2004. In both years, A-medium had the highest percent extraction compared to the other zones (data not included). Determining percent extraction of anthocyanins in finished wines may be of limited usefulness as native anthocyanins are rapidly incorporated into new color pigments in wine.

Since anthocyanin concentrations peak early in extraction, it has been suggested that the limiting factor in extractability is due to a physical barrier of epidermal cells rather than a solubility issue (Sacchi et al. 2005). It has also been suggested that having high levels of total phenolics and specifically copigments such as flavonols may help keep anthocyanins in solution through the phenomenon of copigmentation (Boulton, 2001; Schwarz et al. 2005; Lorenzo et al. 2005). Copigmentation has also been described as the first step toward the formation of more stable pigments (Liao et al. 1992; Brouillard; Boulton 2001). The 2003 wines had double the proanthocyanidin concentration (Cortell et al. 2005) found in the

2004 wines (data not shown). The low vigor wines had a substantially higher flavonol concentration (data not shown) as well as total phenolics than the high vigor zone wines.

Anthocyanin composition in the model extraction. The model extracts from site A were higher in Dp, Cy, Pt and Pn and lower in Mv than from site B (**Table 4.8**). While the model extracts from the sites differed, the vigor zones in site A had similar proportions for all of the anthocyanins (**Table 4.9**). The model extracts from site B showed a pattern of lower Pn and higher Mv for the high and medium vigor zones compared to the low vigor zone extracts. The A-low vigor zone model extract had the highest proportion of Pt, and Pn and the lowest proportion of Mv compared to model extracts from other vigor zones. In the fruit, the A-low vigor zone had the highest content per berry for all of the anthocyanins (Cortell et al. in press).

During the model extraction there was a shift toward a higher proportion of Mv and Pn concomitant with a reduction in the other three anthocyanin glucosides in comparison to the fruit proportional composition (Cortell et al. in press). This shift toward more stable anthocyanins during winemaking has been previously reported (McCloskey & Yengoyan 1981). Anthocyanins were also incorporated into pigmented polymers as discussed later in this paper.

Table 4.8. Mean and SEM of percent composition for delphinidin, cyanidin, petunidin, peonidin and malvidin in model extractions in 2004 in site A and B (average of vigor zones).

Year	Delphinidin (percent)	Cyanidin (percent)	Petunidin (percent)	Peonidin (percent)	Malvidin (percent)
A	2.9	1.5	4.8	21.7	69.0
B	2.0	1.3	3.7	19.4	73.6
SEM	0.1	0.06	0.1	0.3	0.5
p-value[a]	<0.0001	0.0058	<0.0001	<0.0001	<0.0001

[a]ANOVA to compare data (*P* indicated), n=10.

Table 4.9. Mean and SEM of percent anthocyanin composition for delphinidin, cyanidin, petunidin, peonidin and malvidin in the model extractions for vigor zones in site A and B in 2004.

Site	Vigor Zone	Delphinidin (percent)	Cyanidin (percent)	Petunidin (percent)	Peonidin (percent)	Malvidin (percent)
A	High	3.43[a]	1.70[a]	5.05[a]	21.35[a]	68.46[a]
A	Medium	2.74[a]	1.50[a]	4.62[a]	21.67[a]	69.47[a]
A	Low	3.20[a]	1.63[a]	5.35[a]	22.18[a]	67.64[a]
SEM		0.34	0.13	0.32	0.64	1.16
p-value[a]		0.3780	0.5577	0.3261	0.3385	0.4450
B	High	2.28[a]	1.48[a]	3.90[a]	19.04[b]	73.31[ab]
B	Medium	1.86[ab]	1.20[b]	3.50[a]	18.92[b]	74.51[a]
B	Low	2.04[b]	1.34[ab]	3.69[a]	20.75[a]	72.18[b]
SEM		0.12	0.07	0.14	0.44	0.56
p-value[a]		0.0976	0.0656	0.1720	0.0344	0.0547

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

Anthocyanin composition in wine. In addition to vintage variation in anthocyanin concentration in wine, there were also differences in the proportional composition between years (**Table 4.10**). In 2003, Dp and Pt were higher in wines while Mv was lower in comparison to 2004. This reflects the compositional vintage differences in the fruit; however, in the fruit, all of the anthocyanins were higher except for Mv in 2003 compared to 2004 (Cortell et al. in press). The Mv proportion was about 5% higher in the 2004 wines than in the 2003 wines. There were large reductions in the percent composition of Dp, Cy, Pt, and Pn and an increase in the percent Mv that occurred during the winemaking process resulting in ~ 83% malvidin in 2004.

In comparing the wines to the model extraction anthocyanin proportions, there was a greater proportion of Mv, no difference in Pt, minimal reduction in Dp and large reductions in Cy and Pn in the wines. The large reduction in the proportion of Pn in the wines compared to the model extractions may be due to yeast cell wall adsorption in the wines as the model extracts did not contain yeast. Pn has been reported to be strongly adsorbed to yeast cell walls compared to other anthocyanins (Morata et al. 2003).

Table 4.10. Weighted mean and SEM of percent composition for delphinidin, cyanidin, petunidin, peonidin, malvidin and vitisin A in wines from site A and B in 2003 and 2004.

Site	Year	Delphinidin[b] (percent)	Cyanidin[b] (percent)	Petunidin[b] (percent)	Peonidin[b] (percent)	Malvidin[b] (percent)	Vitisin A[c] (percent)
A	2003	3.12	0.71	5.99	11.76	78.22	6.0
B	2003	3.51	0.65	6.12	11.01	78.72	18.2
A	2004	1.79	0.68	3.86	11.85	81.82	2.1
B	2004	1.68	0.67	3.57	10.33	83.74	2.5
SEM		0.14	0.06	0.13	0.32	0.59	1.3
Site	p-value[a]	0.7632	0.5744	0.5460	0.0013	0.0480	<0.0001
Year	p-value[a]	<0.0001	0.8761	<0.0001	0.3719	<0.0001	<0.0001
Site*Year	p-value[a]	0.3151	0.6479	0.1259	0.2430	0.2370	<0.0001

[a]ANOVA to compare data (*P* indicated), n=9. [b]Percent calculated on total of native anthocyanins excluding vitisin A. [c] Percent calculated on total anthocyanins including vitisin A.

There were also some differences in the anthocyanin proportion in wines between vineyard sites (**Table 4.10**). In 2003, Pn was higher in site A than in site B. Vitisin A formed in the wine during fermentation was higher in site A compared to site B in 2003 but not in 2004. In 2004, Pt and Pn were higher and Mv was lower in site A compared to site B.

In 2003, the major change between the fruit and wine proportional composition was in site B having much greater formation of Vitisin A (18.2%). In site A, there was only about 6% Vitisin A production and Mv went from ~ 59% in the fruit to ~ 78% of the total proportion in the wines when excluding vitisin A from the percent calculation. Since the formation of vitisin A involves a reaction involving Mv and pyruvic acid produced from yeast (Bakker & Timberlake 1997; Fulcrand et al. 1998), this result is likely related to differences in fermentation processes rather than a direct effect of vine vigor. It is possible that the greater formation of vitisin A in site B in 2003 had to do with the lower pH and also possible higher levels of pyruvic acid as a pH of 2.7 - 3.0 has been reported to favor vitisin A formation (Romero & Bakker 1999). In 2004, there were no differences in pH or Vitisin A formation between sites.

Wines made from vine vigor zones also resulted in proportional variations in anthocyanin composition (**Table 4.11 & 4.12**). In both years and both sites, there was a higher proportion of Dp and Pt and lower Mv in wines made from fruit from low vigor zones. For Cy, there was a higher proportion in the wines made from fruit from low vigor vines in both sites and vintages except for only a trend for site A in 2003. The Pn proportion increased in wines with a reduction in vine vigor in site A in 2003

and in site A and B in 2004. This reflects the variation in the fruit reasonable well except that A-high had the highest proportion of Pn in the fruit (Cortell et al. in press) and one of the lowest proportions in the wine. The Mv proportion increased in wines made from fruit with increasing vine vigor in both years. The Mv proportion was also higher in high vigor fruit (Cortell et al. in press). Vitisin A production increased in wines made from fruit with decreasing vine vigor.

Overall, wines from low vigor fruit had a greater diversity of anthocyanins where as high vigor wines were predominated by Mv. Mv was the major contributing anthocyanin in all the wines as has been previously reported (Gao et al. 1997). In comparing the composition in the wine and the fruit, the patterns were reasonably consistent. In 2003, low vigor fruit was higher in Dp and lower in Mv (Cortell et al. in press). This same pattern was expressed in the wine. In a study by Sims and Bates (1994), wine made from more mature grapes had higher levels of Mv and Pn and lower levels of Dp, Cy and Pt. Although there were some differences in ripening as determined by soluble solids, the composition in these wines appears to be more directly related to environmental influences rather than ripening as the high vigor (lowest °Brix) had the highest proportion of Mv.

Table 4.11. Mean and SEM of percent composition for delphinidin, cyanidin, petunidin, peonidin, malvidin and vitisin A in wines made from vine vigor zones in 2003.

Site	Year	Vigor Zone	Delphinidin[b] (percent)	Cyanidin[b] (percent)	Petunidin[b] (percent)	Peonidin[b] (percent)	Malvidin[b] (percent)	Vitisin A[c] (percent)
A	2003	High	2.37[c]	0.37[a]	4.79[b]	9.86[c]	82.62[a]	3.50[c]
A	2003	Medium	3.19[b]	0.68[a]	6.01[a]	11.66[b]	78.46[b]	4.62[b]
A	2003	Low	4.39[a]	1.08[a]	6.66[a]	13.31[a]	74.57[c]	12.77[a]
SEM			0.29	0.22	0.23	0.23	0.68	0.15
p-value[a]			0.0040	0.1027	0.0047	0.0012	0.0012	<0.0001
B	2003	High	3.24[b]	0.69[b]	5.77[b]	11.05[ab]	79.24[a]	7.38[c]
B	2003	Medium	3.39[b]	0.51[c]	6.13[ab]	10.61[b]	79.36[a]	21.15[b]
B	2003	Low	3.98[a]	0.88[a]	6.46[a]	11.69[a]	77.01[b]	23.74[a]
SEM			0.14	0.04	0.13	0.19	0.29	0.54
p-value[a]			0.0401	0.0035	0.0548	0.0366	0.0075	<0.0001

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

Table 4.12. Mean and SEM of percent composition for delphinidin, cyanidin, petunidin, peonidin, malvidin and vitisin A in wines made from vine vigor zones in 2004.

Site	Year	Vigor Zone	Delphinidin[b] (percent)	Cyanidin[b] (percent)	Petunidin[b] (percent)	Peonidin[b] (percent)	Malvidin[b] (percent)	Vitisin A[c] (percent)
A	2004	High	1.57[b]	0.52[c]	3.47[c]	9.55[c]	84.89[a]	1.68[c]
A	2004	Medium	1.72[b]	0.68[b]	3.77[b]	11.82[b]	82.01[b]	1.98[b]
A	2004	Low	2.19[a]	0.77[a]	4.45[a]	13.39[a]	79.20[c]	2.84[a]
SEM			0.05	0.01	0.07	0.14	0.23	0.05
p-value[a]			0.0019	<0.0001	<0.0001	0.0001	0.0002	0.0003
B	2004	High	1.58[b]	0.62[b]	3.40[b]	9.31[c]	85.09[a]	2.15[c]
B	2004	Medium	1.59[b]	0.65[b]	3.41[b]	9.95[b]	84.40[b]	2.43[b]
B	2004	Low	1.97[a]	0.76[a]	4.04[a]	12.06[a]	81.16[c]	2.81[a]
SEM			0.03	0.03	0.05	0.05	0.10	0.06
p-value[a]			0.0020	0.0119	0.0011	<0.0001	<0.0001	0.0034

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

Changes in anthocyanin concentration and composition during winemaking. The fermentation curve through pressing for total anthocyanin extraction by reversed-phase HPLC most closely resembles the extraction curve for Mv since Mv accounts for around 80% of the total anthocyanins (**Figure 4.1 & 4.2**). In site A, the Dp and Pt concentrations peaked on day 3 of fermentation (**Figure 1a, c)** while Cy, Pn and Mv peaked on day 4 (**Figure 4.1b, d, e)**. Dp, Pt, and Pn peaked on day 3 (**Figure 4.2a, c, d)**, Cy (**Figure 4.2b)** on day 2 and Mv (**Figure 4.2e)** on day 5 in site B. Cy and Pn were at or close to their maximal levels at the end of the first day of fermentation as day 1 was the end of the cold-soak prior to the start of fermentation. The high levels of Cy and Pn at the end of the first day of fermentation is hard to explain based on water solubility alone as it would be expected that delphinidin would respond in a similar way as cyanidin based on hydroxyl groups. However, it is possible this was related to molecule size as cyanidin and peonidin both have only a hydrogen R_2 functional group.

The high concentration of individual anthocyanins found on day three or four specifically in the medium and low vigor zones dropped substantially by pressing so that differences in monomeric anthocyanins between vigor zones in the finished wines were minimized (**Figure 4.1 & 4.2**). Previous studies have shown that anthocyanin extraction usually reaches a maximum in the first couple days of fermentation and then the concentration drops and this was found in the present study as well (Sacchi et al. 2005).

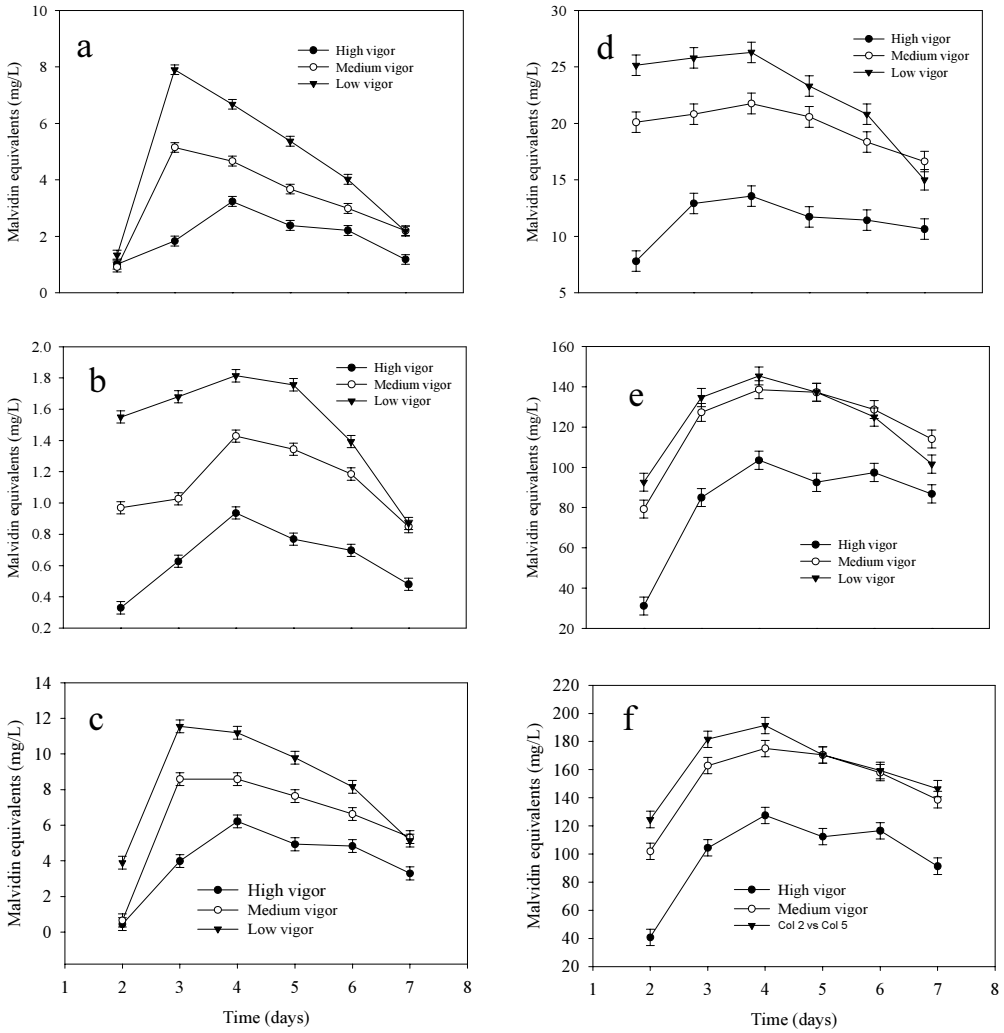


Figure 4.1. Site A anthocyanin evolution during fermentation for a) delphinidin, b) cyanidin, c) petunidin, d) peonidin, e) malvidin and f) total glucosides from the start of fermentation (Day 2) through pressing (Day 7). Post cold-soak data not included (Day 1). Analyzed by reversed-phase HPLC.

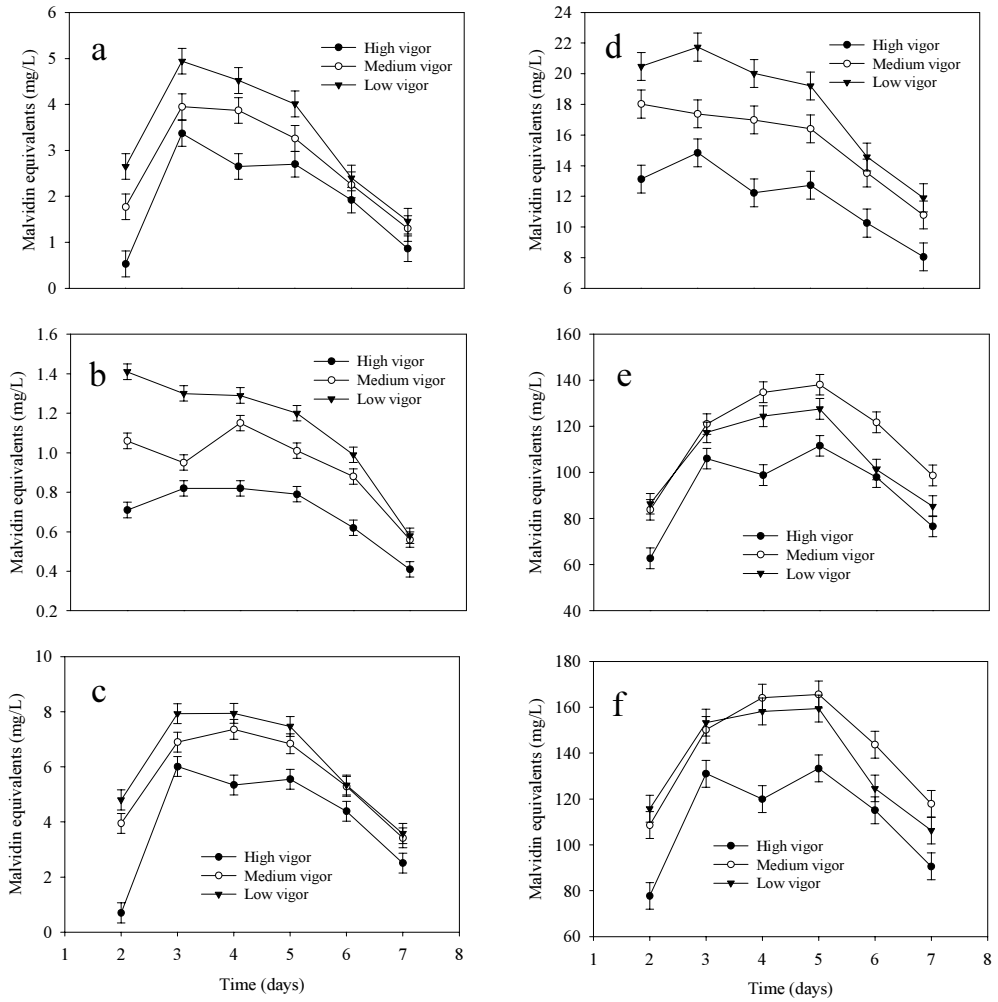


Figure 4.2. Site B anthocyanin evolution during fermentation for a) delphinidin, b) cyanidin, c) petunidin, d) peonidin, e) malvidin and f) total glucosides from the start of fermentation (Day 2) through pressing (Day 7). Post cold-soak data not included (Day 1). Analyzed by reversed-phase HPLC.

From these data, it is not possible to assess what percent of the anthocyanins were incorporated into pigmented polymers during fermentation and the amount lost due to degradation or precipitation.

Although pressing did not have an effect on the anthocyanin concentration in

wines, there were some compositional changes (data not shown). The proportion of less stable pigments including Dp (p=0.0397) and Cy (p=0.0025) in particular were reduced with pressing.

In comparing the anthocyanin levels in the wine fermentation to the model extraction, the wine extraction concentration on Day 3 in site A and Day 4 in site B was comparable although still somewhat lower than the model extraction particularly in the A-high and B-high zones. From doing a rough estimate of percent extraction in the wine, the model extract had greater extraction than found in the wines likely in response to the higher temperature and ethanol used.

Formation of pigmented polymers during winemaking. Numerous studies have shown a strong relationship between pigmented polymers and color density (Somers 1971; Mazza 1995; Gao et al 1997). This is due to incorporation of monomeric anthocyanins into several classes of pigmented polymers. Peng et al. (2002) reported that pigmented polymers accounted for 50% of the color after two years of aging and Lee et al. (2004) found that pigmented polymers accounted for close to 70% of color after one year. However, much of the formation is thought to occur rapidly and early during fermentation (Harbertson et al. 2002; Morel-Salmi et al. 2006). The fermentation process has been described as a decrease in free anthocyanins with a concomitant increase in pigmented polymers (Somers 1971; Gao et al, 1997; Parley et al. 2001).

Consequently, due to the importance of pigmented polymers in red wine color, in addition to monitoring monomeric anthocyanins by reversed-phase HPLC, we also measured pigmented polymers. During wine fermentation, the simultaneous extraction of monomeric 520nm absorbing material and the formation of pigmented polymers were determined by a GPC method (**Figure 4.3**). The monomeric anthocyanins reached maximal levels on Day 4 in site A (**Figure 4.4**) and on Day 3 in site B (**Figure 4.5**). The maximal levels were quantified to be higher with GPC than by RP although the concentration determined at pressing was consistent with the RP numbers for site A and reasonable consistent for site B. Pigmented polymer formation showed a rapid increase on Day 2 and 4 in site A (**Figure 4.6**) and on Day 2 in site B (**Figure 4.7**). A decrease in monomeric anthocyanins was seen on Day 2 and may represent monomeric anthocyanins being rapidly incorporated into new products. After Day 2 in site B, the pigmented polymer concentration remained stable through pressing.

As samples were not pulled during fermentation in 2003 it was not possible to compare extraction profiles between the two years. In addition, the wines were not analyzed at different time point to assess changes during aging. Based on the observed leveling off during fermentation and the lower anthocyanin and proanthocyanidin concentration in wines, it is unlikely the 2004 wines would achieve the levels found in the 2003 wines even with longer aging.

In a study on Syrah, Eglinton et al. (2004) found that pigmented polymers formed at high rate until pressing and then continued to form for the following 90 days at a much reduced rate and then formation leveled off.

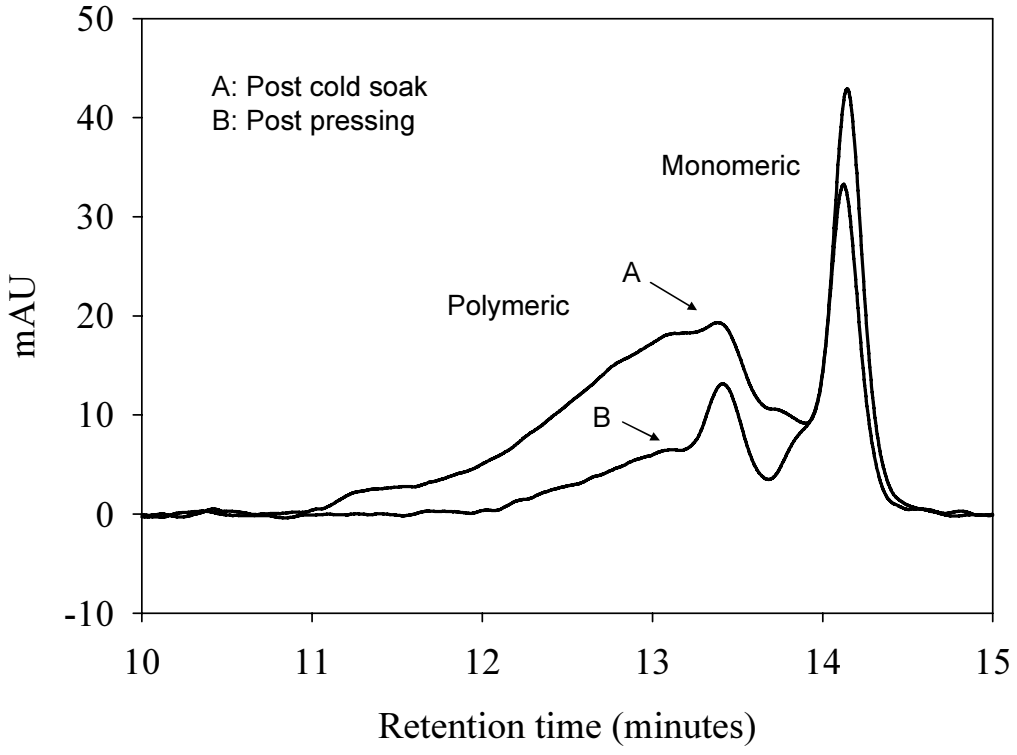


Figure 4.3. GPC chromatogram showing monomeric and polymeric peaks after cold soak and after pressing in a medium vigor zone wine.

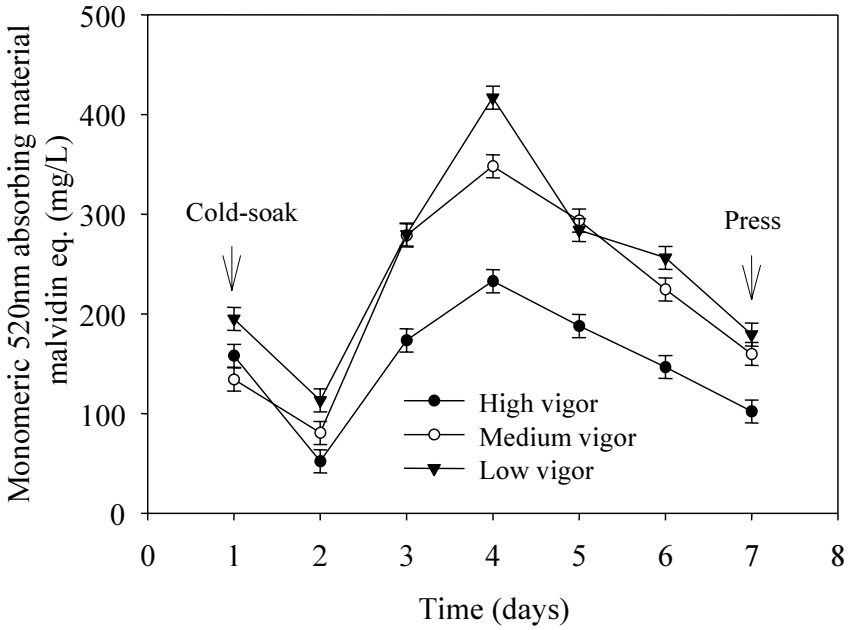


Figure 4.4. Site A monomeric 520nm absorbing material (mg/L) evolution during fermentation quantified as malvidin equivalents. Analyzed by GPC.

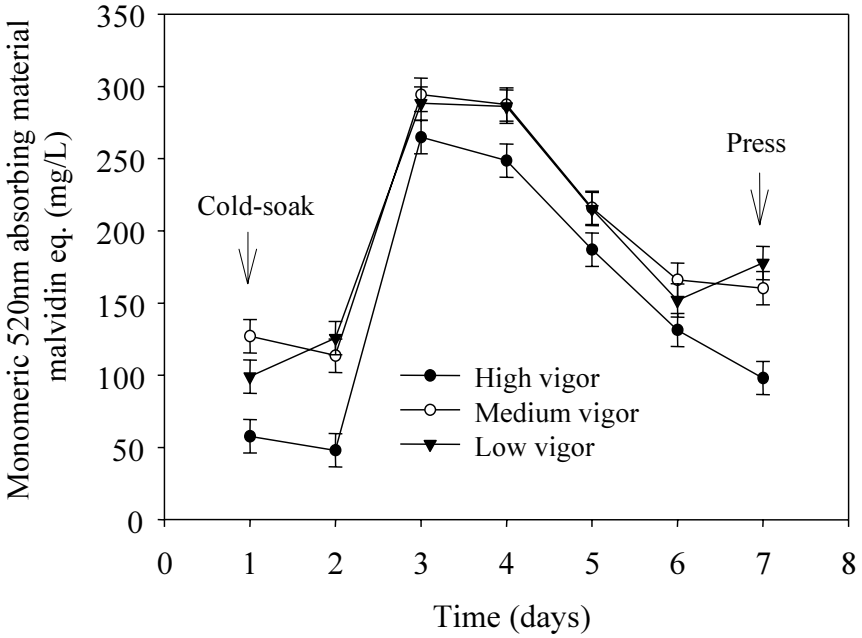


Figure 4.5. Site B monomeric 520nm absorbing material (mg/L) evolution during fermentation quantified as malvidin equivalents. Analyzed by GPC.

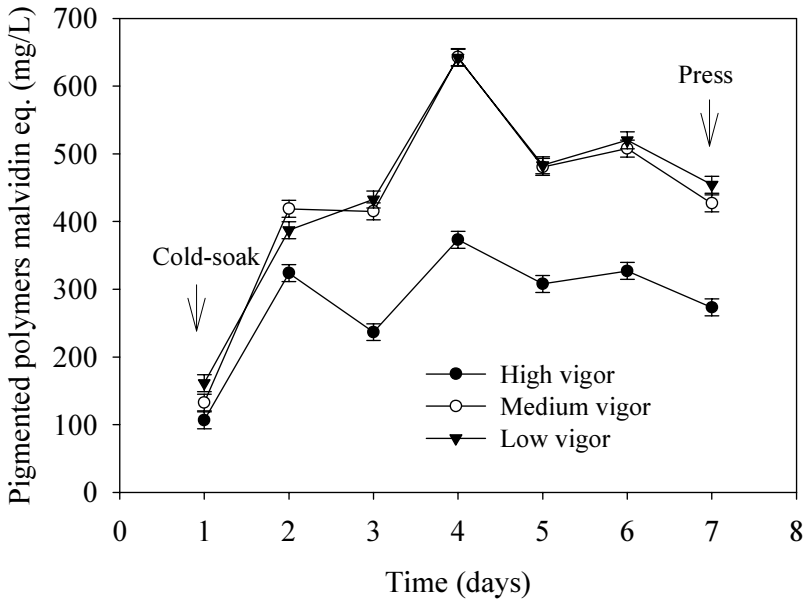


Figure 4.6. Site A pigmented polymer (mg/L) evolution during fermentation quantified as malvidin equivalents at 520nm. Analyzed by GPC.

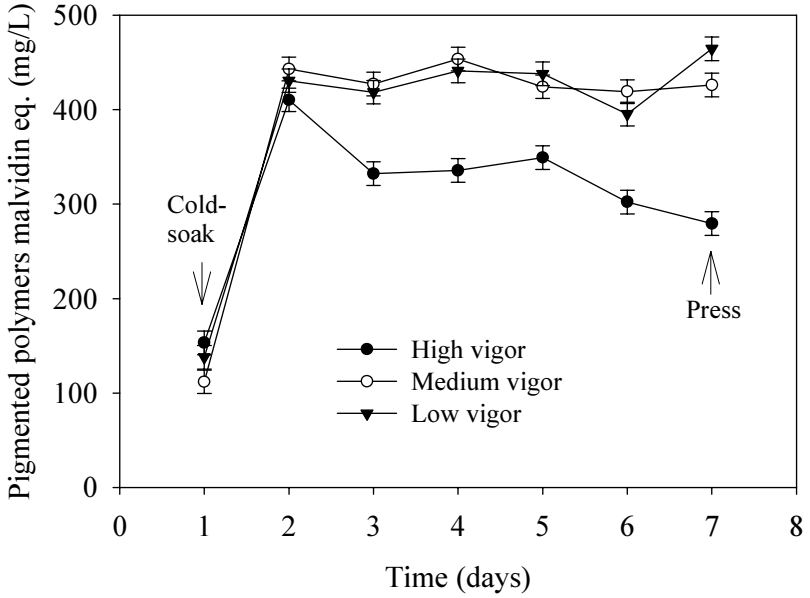


Figure 4.7. Site B pigmented polymer (mg/L) evolution during fermentation quantified as malvidin equivalents at 520nm. Analyzed by GPC.

In the model extraction experiment, there was a high level of pigmented polymer formation (data not included). The experiment was done using 20% ethanol that is 5-8% higher than most red wine fermentations, for a shorter period of time (48 hours), and at a higher than normal fermentation temperature. This might explain the rapid formation of pigmented polymers in this system.

In analyzing the finished wines, there was around three-fold more pigmented polymers (mg/L) in wine in 2003 compared to 2004 vintage (**Table 4.13**). No site differences were found in 2004. In both vintages and sites, there was a strong relationship between high pigmented polymers concentration in the wine and a reduction in vine vigor (**Table 4.14 & 4.15).** The pigmented polymer concentration in wine was close to double in the lowest vigor zone (B-high) compared to the highest vigor zone (A-high) in 2004 and over twice as much in 2003.

Our results are in agreement with a previous study on Pinot noir where wines made from highly exposed clusters had 40% greater polymeric anthocyanins compared to the less exposed treatments (Price et al. 1995). The low vigor zones within the vineyard have greater sun exposure in the fruiting zone and site B is overall lower in vigor compared to site A. It is difficult to compare the amount of pigmented polymers in wine when different methods are used that may under or overestimate the true value. For, example Eglinton et al. (2004) reported concentrations of 50-100 mg/L where we have reported values of 200-1500 mg/L by GPC.

The values reported here may be overestimated as previously discussed (Cortell et al. 2005); however, it is still possible to compare results between years, vineyard sites and vigor zones.

As many of the classes of pigmented polymers have resistance to sulfite bleaching, this test was done to confirm the presence of pigmented polymers. The ~ 3-fold higher percent of sulfite resistant pigments found in 2003 compared to 2004 is in agreement with the higher amount of pigmented polymers in the wine (**Table 4.13**). No differences in sulfite resistance were found between vineyard sites in either year. Sulfite resistance increased in wines made from vines with reduced vine vigor in both years and vineyard sites similar to what was observed for pigmented polymer concentration in wines (**Table 4.14 & 4.15**).

Several scientists have suggested the formation of pigmented polymers is concentration driven and can be predicted by anthocyanin to proanthocyanidin ratios (Mazza & Miniata 1993; Fulcrand et al. 2004; Morel-Salmi et al. 2006). Brossaud et al. (1999) reported that vineyard sites usually yielding intense well-balanced Cabernet franc wines were produced from grapes with higher anthocyanin content and higher anthocyanin to tannin ratios. Consequently, the relationships between anthocyanins and proanthocyanidins in fruit and wine and the formation of pigmented polymers were investigated.

Table 4.13. Mean and SEM of color density (420nm + 520nm), hue (420/520), red pigment (%) and sulfite resistant pigments (%) of wines averaged for Site A and B.

Site	Year	Color Density (420nm + 520nm)	Hue (420nm/520nm)	Red Pigment (percent)	Sulfite Resistant Pigments (percent)	Pigmented polymer (mg/L)
A	2003	6.2	0.75	26.7	38.3	865
B	2003	9.8	0.64	35.3	41.2	1223
A	2004	7.2	0.55	33.9	10.8	391
B	2004	7.3	0.54	23.3	9.3	360
SEM		0.4	0.02	1.5	1.0	41
Site p-value[a]		<0.0001	0.0004	0.5233	0.4911	0.0004
Site *Year p-value[a]		<0.0001	0.0031	<0.0001	0.0393	<0.0001

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

Table 4.14. Mean and SEM of color density (420nm + 520nm), hue (420/520), red pigment (%) and sulfite resistant pigments (%) of wines made from vine vigor zones in 2003.

Site	Year	Vigor Zone	Color Density (420nm + 520nm)	Hue (420nm/520nm)	Red Pigment (percent)	Sulfite Resistant Pigments (percent)	Pigmented polymer (mg/L)
A	2003	High	4.54[a]	0.79[a]	26.39[b]	36.86[b]	632[c]
A	2003	Medium	6.00[b]	0.77[a]	25.37[b]	37.70[b]	844[b]
A	2003	Low	8.24[c]	0.68[b]	31.95[a]	41.63[a]	1090[a]
SEM			0.13	0.01	0.60	0.86	14.8
p-value[a]			<0.0001	0.0082	0.0014	0.0341	<0.0001
B	2003	High	7.95[c]	0.67[a]	29.79[b]	33.32[b]	989[c]
B	2003	Medium	9.60[b]	0.64[ab]	35.11[b]	43.74[a]	1223[b]
B	2003	Low	12.07[a]	0.62[b]	41.21[a]	44.31[a]	1459[a]
SEM			0.24	0.01	1.73	0.80	46.0
p-value[a]			0.0007	0.1244	0.0140	0.0007	0.0050

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

Table 4.15. Mean and SEM of color density (420nm + 520nm), hue (420/520), red pigment (%) and sulfite resistant pigments (%) of wines made from vine vigor zones in 2004.

Site	Year	Vigor Zone	Color Density (420nm + 520nm)	Hue (420nm/520nm)	Red Pigment (percent)	Sulfite Resistant Pigments (percent)	Pigmented polymer (mg/L)
A	2004	High	6.07[c]	0.75[a]	25.73[b]	6.21[c]	227[c]
A	2004	Medium	7.11[b]	0.53[b]	37.44[a]	11.16[a]	399[b]
A	2004	Low	8.49[a]	0.48[b]	24.82[b]	12.04[a]	425[a]
SEM			0.25	0.02	2.22	0.22	6.0
p-value[a]			0.0023	0.0005	0.0277	0.0001	0.0005
B	2004	High	6.62[b]	0.58[a]	20.83[b]	7.29[c]	286[b]
B	2004	Medium	7.69[a]	0.53[b]	24.45[a]	9.31[b]	386[a]
B	2004	Low	7.25[ab]	0.52[b]	23.81[ab]	11.35[a]	388[a]
SEM			0.21	0.01	0.97	0.47	8.7
p-value[a]			0.0201	0.0066	0.0259	0.0055	0.0009

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

In this study, a strong positive correlation between the formation of pigmented polymer and the proanthocyanidin concentration in the wine was found (R^2 =0.92) using proanthocyanidin data from phloroglucinolysis and pigmented polymer date from GPC (**Figure 4.8)**. The correlation between anthocyanin and pigmented polymer concentrations in wines had an R^2 of 0.56, a ratio of anthocyanins to tannin to pigmented polymers was similar with R^2 =0.58, and a ratio of proanthocyanidins to anthocyanins was a stronger correlation (R^2 =0.72) than the others.

The same relationships were also assessed to see if they could be extended to proanthocyanidin or anthocyanin levels found in the fruit. In 2003, there was a good relationship between proanthocyanidin concentrations in the fruit (mg/kg) and pigmented polymer concentration in the wine (R^2 =0.70) (**Figure 4.9**) and a moderate relationship between the anthocyanin in the fruit (mg/kg) and pigmented polymers in the wine (R^2 =0.47). However, there did not appear to be similar relationships in the 2004 data. The relationships with the fruit were not expected to be as strong since the formation of pigmented polymers occurs primarily during the winemaking process. It has been suggested that increases in pigmented polymer formation appeared to be due more to an increase in proanthocyanidin rather than anthocyanin extraction (Harbertson et al. 2002). In the present study, our data also suggests an important role of proanthocyanidins in forming pigmented polymers that have a major contribution to wine color density.

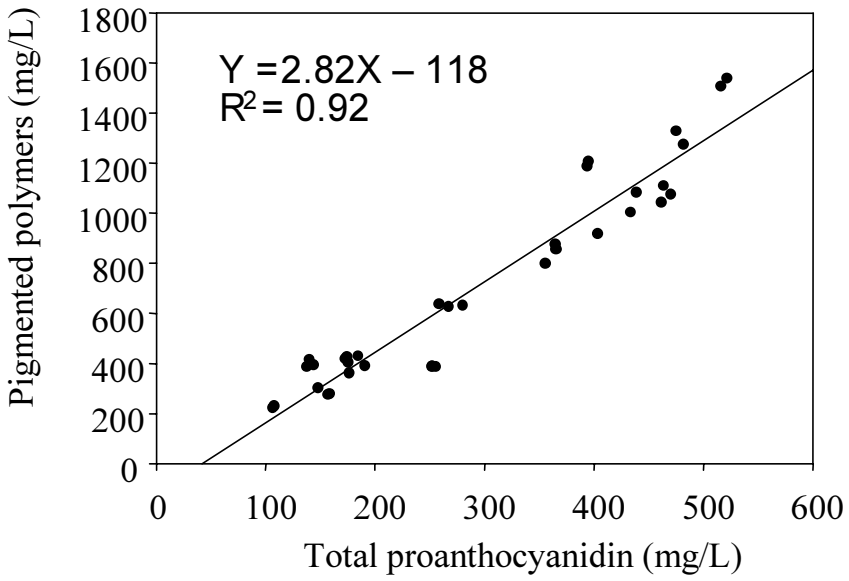


Figure 4.8. Relationship between total proanthocyanidin in wine (mg/L) determined by phloroglucinolysis against pigmented polymer concentration (mg/L) determined by GPC.

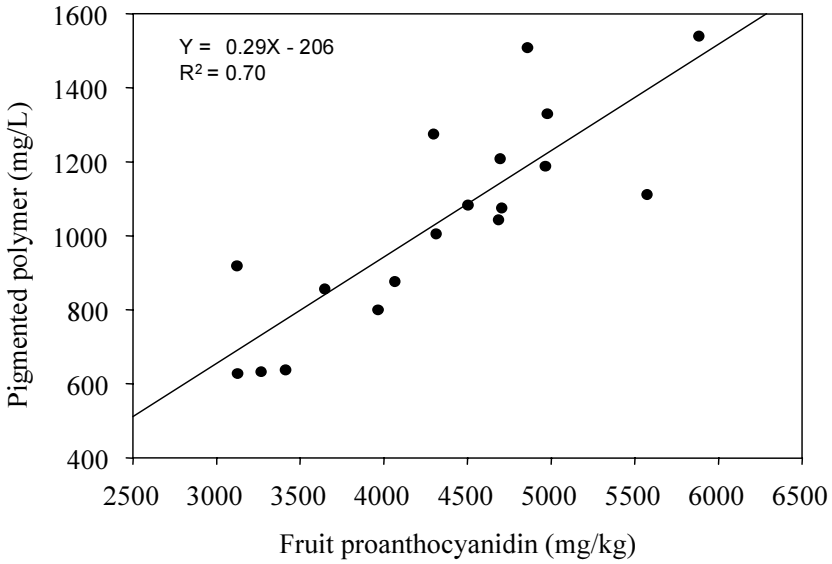


Figure 4.9. Relationship in 2003 between total proanthocyanidin in fruit (mg/kg) determined by phloroglucinolysis against pigmented polymer concentration (mg/L)

determined by GPC.

Wine color density. Although vintage spectrophotometric color measurements differences were observed, they were not included as the wines were analyzed at different time points. In comparing the two sites, color density was substantially higher in Site B compared to Site A in 2003 while there were no differences in 2004 (**Table 4.13**). Although there were minimal differences in monomeric anthocyanins in the fruit in 2003, color density was substantially higher in wines made from low vigor fruit in both sites (**Table 4.14**). In 2004, color density increased with a reduction in vine vigor in both sites except for the B-low wines were similar to the B-medium wines. In both years and particularly in 2003, there was a good correlation between pigmented polymer concentration and wine color density (**Figure 4.10**). This agrees with previous results in Pinot noir where all the polymeric pigments were important in wine color intensity (Gao et al. 1997). In another study on several varieties including Pinot noir, viticultural practices that increased cluster sun exposure resulted in wines with higher phenolics and color density of wines (Mazza et al. 1999).

Wine hue and percent red pigments. Hue (yellow/brown color) was higher in wines from Site A compared to Site B in 2003 and there were no differences in 2004 (**Table 4.13**). Percent red pigment was higher in Site B in 2003 and higher in Site A in 2004. In both years, hue was lower in wines made from vines with lower levels of vine vigor meaning that wines from high vigor zones had greater yellow-brown color (**Table 4.14 & 4.15**). In 2003, percent red pigment was significantly

higher in wines made from low vigor zones than from high vigor zones.

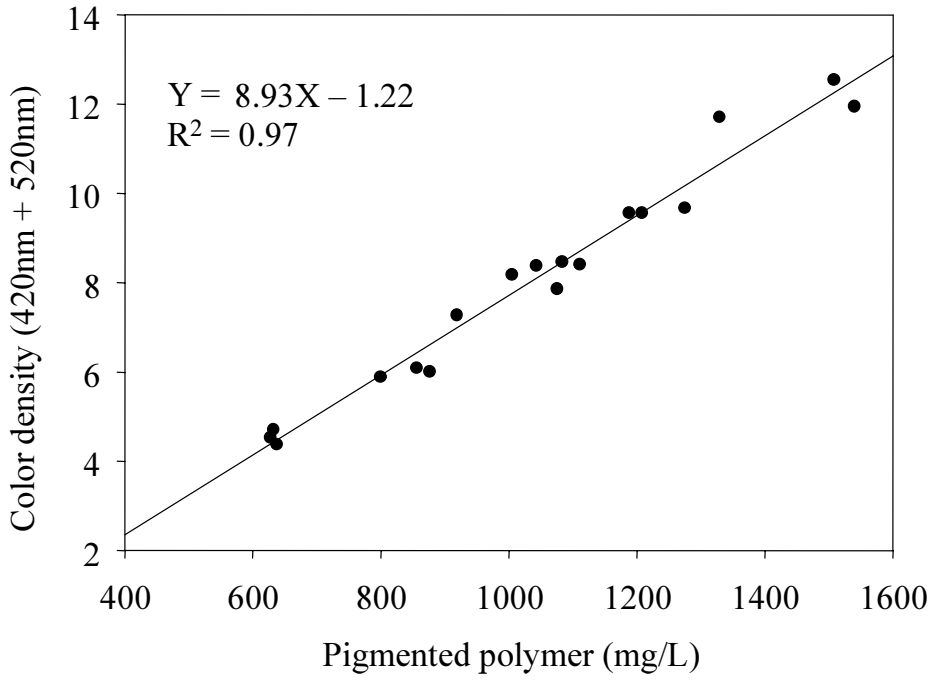


Figure 4.10. Relationship between pigmented polymer in wine (mg/L) analyzed by GPC against wine color density (420nm + 520nm)) determined by spectrophotometry in 2003.

Percent red pigment was higher in the medium vigor zone wines of site A compared to the other wines in 2004. There also appeared to be a relationship between wine pigmented polymer and hue although this relationship could be a result of other colored compounds in the wine as well. High pigmented polymer concentration was associated with a lower hue and higher percent red pigments.

During wine fermentation, several types of pigmented polymers can form through different mechanisms. In wines with a lower proanthocyanidin to

anthocyanin ratio, it is more likely a greater proportion of derived pigments that do not involve proanthocyanidins will be formed (Morel-Salmi et al. 2006). These pigments could be pyranoanthocyanins such as vitisin A that are more orange colored and sulfite resistant than anthocyanin proanthocyanidin adducts. In this study, there were not remarkable differences in percent sulfite resistant pigments in the wines and the low vigor zones had a greater formation of vitisin A. However, the high vigor zone wines had a lower proanthocyanidin concentration and a substantially lower concentration of pigmented polymer formation than wines from low vigor zones so the proportion of orange colored compounds in the wine contributing to color could be greater. This might be one possible explanation for the higher hue values and reduction in the percent red color in the high vigor wines particularly in 2003. However, pyranoanthocyanins have been reported to contribute only 3-5% to the color of aged red wines (Schwarz et al. 2003).

On the other hand, as seen in **Figure 4.9**, there was a strong correlation between the proanthocyanidins concentration (mg/L) in wine and the formation of pigmented polymers. Pigmented polymers formed through reactions involving anthocyanins and proanthocyanidins are thought to be major contributors to wine color. In Cortell et al. (2005) we showed that wines from the low vigor zones had substantially higher skin proanthocyanidin and pigmented polymer concentration than wines made from high vigor zones. As previously mentioned, the concentration of pigmented polymers in 2004 was much lower than in 2003. High levels of proanthocyanidins would be expected to encourage proanthocyanidin- anthocyanin

(T-A) adducts which have similar color properties to anthocyanins (Salas et al. 2004). In this study, we showed that wines from low vigor vines had a higher pigmented polymer concentration and also greater formation of Vitisin A. This shows that the low vigor wines had a greater propensity to form a large number of different pigments during winemaking that is in part, a reflection of the anthocyanin and phenolic profile of the fruit.

In summary, we have shown that although there is variation in anthocyanin accumulation in fruit in response to variations in vine vigor within a site, the variation in the fruit appears to be magnified in wines due to variations in berry size, differences in percent extraction of anthocyanins and also the formation of pigmented polymers in wine. In this study, reduced vine vigor produced wines with a higher anthocyanin concentration, greater diversity of anthocyanins, greater pigmented polymer formation, increased Vitisin A formation, more sulfite resistant pigments, greater color density, higher percent red pigments and reduced hue. However, the high variability in anthocyanin content in berries and differences in extraction make it difficult to use fruit anthocyanin content alone as a predictor of red wine color. There appears to be a stronger relationship between reduced vine vigor and formation of pigmented polymers in wine (Cortell et al. 2005). Consequently, further investigation is needed on factors that influence extraction of anthocyanins from the berry and how phenolic concentration and composition effect the formation of pigmented polymers.

ABBREVIATIONS USED

TA, titratable acidity (mg/L); Dp, delphinidin-3-*O*-glucoside; Cy, cyanidin-3-*O*-glucoside; Pt, petunidin-3-*O*-glucoside; Pn, peonidin-3-*O*-glucoside; CI, 95% confidence interval.

ACKNOWLEDGMENTS

We thank Archery Summit Winery for the use of their vineyards and fruit for wine production as well as Leigh Bartholomew and Anna Matzinger specifically for their support. In addition, we would like to thank students: Jason Bell, Fiorella Cerpa, Jose Luis Pastor del Rio and Chris Zielinski for their help. Funding for this project was provided by the USDA Northwest Center for Small Fruit Research and the American Society for Enology and Viticulture.

LITERATURE CITED

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

Bishop, P. D.; Nagel, C. W. Characterization of the condensation product of Malvidin3,5-diglucoside and catechin. *J. Agric. Food Chem.* **1984,** 32, 1022-1026.

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

Brossaud, F.; Cheynier, V.; Asselin, C.; Moutounet, M. flavonoid compositional differences of grapes among site test plantings of Cabernet franc. *Am. J. Enol. Vitic.* **1999,** 50, 277-284.

Bravdo, B.; Hepner, Y.; Loinger, C; Cohen, S; Tabacman, H. Effect of irrigation and crop level on growth, yield and wine quality of cv. Cabernet Sauvignon. *Am. J. Enol. Vitic.* **1985,** 36, 132-139.

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

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

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

Cortell, J. M.; Halbleib, M.; Gallagher, A. V.; Righetti, T.; Kennedy, J. A. Influence of vine vigor on grape (*Vitis vinifera* L. Cv. Pinot noir) anthocyanins: Part I. anthocyanin concentration and composition in fruit. *J. Agric. Food Chem.* **2006,** in press.

Eglinton, J.; Griesser, M.; Henschke, P.; Kwiatkowski, M.; Parker, M.; Herderich, M. Yeast mediated formation of pigmented polymers in red wine. In *Red wine color: revealing the mysteries;* Waterhouse, A. L., Kennedy, J., Eds.; ACS Symposium series 886; American Chemical Society: Washington, DC, 2004; pp 125-142.

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

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

Fulcrand, H.; Atanasova, V.; Salas, E.; Cheynier, V. The fate of anthocyanins in wine: Are there determining factors?. In *Red wine color: revealing the mysteries;* Waterhouse, A. L., Kennedy, J., Eds.; ACS Symposium series 886; American Chemical Society: Washington, DC, 2004; pp 68-88.

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

Gómez-Cordovés, C. New pigments in red wines via different enological processes. In *Red wine color: revealing the mysteries;* Waterhouse, A. L., Kennedy, J., Eds.;

ACS Symposium series 886; American Chemical Society: Washington, DC, 2004; pp 89-124.

Harbertson, J. F.; Picciotto, E. A.; Adams, D. O. Measurement of polymeric pigments in grape berry extracts and wine using a protein precipitation assay combined with bilsulfite bleaching. *Am. J. Enol. Vitic.* **2003,** 54, 301-306.

Hardie, W. J.; Considine, J. A. Response of grapes to water-deficit stress in particular stages of development. *Am. J. Enol. Vitic.* **1976,** 27, 55-61.

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

Kennedy, J. A.; Taylor, A. W. (2003) Analysis of proanthocyanidins by high-performance gel permeation chromatography. *J. Chromatogr. A.* **2003,** 995, 99-107.

Lamuela-Raventos, R. M.; Waterhouse, A. L. A direct HPLC separation of wine phenolics. *Am. J. Enol. Vitic.* **1994,** 45, 1-5.

Lee, D. F.; Ewald, E. S.; Asenstorfer, R. E.; Jones, G. P. Factors affecting the formation of red wine pigments. In *Red wine color: revealing the mysteries;* Waterhouse, A. L., Kennedy, J., Eds.; ACS Symposium series 886; American Chemical Society: Washington, DC, 2004; pp 125-143.

Liao, H.; Cai, Y.; Haslam, E. Polyphenolic interactions. Anthocyanins: copigmentation and colour changes in red wines. *J. Sci. Food Agric.* **1992,** 59, 299-305.

Lorenzo, C.; Pardo, F.; Zalacain, A.; Alonso, L. G.; Salinas, M. R.; Effect of red grapes co-winemaking in polyphenols and color of wines. *J. Agric. Food Chem.* **2005,** 53, 7609-7616.

Matthews, M. A.; Anderson, M. M. Fruit ripening in *Vitis vinifera* L.: Response to seasonal water deficits. *Am. J. Enol. Vitic.* **1988,** 39, 313-320.

McCloskey, L.P.; Yengoyan, L.S. Analysis of anthocyanins in *Vitis vinifera* wines and red color versus aging by HPLC and spectrophotometry. *Am. J. Enol. Vitic.* **1981,** 32, 257-261.

Mazza, G.; Miniata, E. Grapes. In *Anthocyanins in Fruits, Vegetables, and Grain;* G., Miniata, E., Eds.; CRC Press: Boca Raton, 1993; pp 149-199.

Mazza, G. Anthocyanins in grapes and grape products. *Crit. Rev. Food Sci. Nutr.* **1995,** 35, 341-371.

Mazza, G.; Fukumoto, L.; Delaquis, P.; Girard, B.; Ewert, B. Anthocyanins, phenolics, and color of Cabernet franc, Merlot, and Pinot noir wines from British Columbia. *J. Agric. Food Chem.* **1999,** 47, 4009-4017.

Morata, A.; Gómez-Cordovés, M. C.; Suberviola, J.; Bartolomé, B.; Colomo, B.; Suárez, J. A. Adsorption of anthocyanins by yeast cell walls during the fermentation of red wines. *J. Agric. Food Chem.* **2003,** 51, 4084-4088.

Morel-Salmi, C.; Souquet, JM.; Magali, B.; Cheynier, V. Effect of flash release treatment on phenolic extraction and wine composition. *J. Agric. Food Chem.* **2006,** 54, 4270-4276.

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

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

Ojeda, H.; Andary, C.; Kraeva, E.; Carbonneau, A.; Deloire, A. Influence of pre- and post-veraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. *Am. J. Enol. Vitic.* **2002,** 53, 261-267.

Parley, A.; Vanhanen, L.; Heatherbell, D.; Effects of pre-fermentation enzyme maceration on extraction and colour stability in Pinot noir wine. *Aust. J. Grape Wine Res.* **2001,** 7, 146-152.

Peng, Z.; Iland, P.G.; Oberholzer, A.; Sefton, M. A.; Walters, E. J. Analysis of pigmented polymers in red wine by reverse phase HPLC. *Aust. J. Grape Wine Res.* **2002,** 8, 70-75.

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

Price, S. F.; Breen, P. J.; Valladao, M.; Watson, B. T. Cluster exposure and quercetin in Pinot noir grapes and wine. *Am. J. Enol. Vitic.* **1995,** 46, 187-194.

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

Reynolds, A. G.; Yerle, S.; Watson, B.; Price, S. F.; Wardle, D. A. Fruit environment and crop level effects on Pinot noir. III. Composition and descriptive analysis of Oregon and British Columbia wines. *Am. J. Enol. Vitic.* **1996,** 47, 329-339.

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

Ribéreau-Gayon, P. Évolution des composés phénoliques au cours de la maturation du raisin. II. Discussion des resultants obtenus en 1969, 1970 et 1971. *Connaiss. Vigne Vin.***1972,** 6, 161-175.

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

Romero, C.; Bakker, J. Interactions between grape anthocyanins and pyruvic acid with effect of pH and acid concentration on anthocyanin composition and color in model solutions.

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

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

Salas, E.; Le Guernevé, C.; Fulcrand, H.; Poncet-Legrand, C.; Cheynier, V. Structure determination and color properties of a newly synthesized direct-linked flavanol-anthocyanin dimer. *Tetrahedron Lett.* **2004,** 45, 8725-8729.

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

Salon, J. L.; Chirivella, C.; Castel, J. R. Response of cv. Bobal to timing of deficit irrigation in Requena, Spain: water relations, yield and wine quality. *Am. J. Enol. Vitic.* **2005,** 56, 1-8.

Saucier, C.; Little, D.; Glories, Y. First evidence of acetaldehyde-flavanol condensation products in red wine. *Am. J. Enol. Vitic.* **1997,** 48, 370-373.

Schwarz, M; Quast, P.; von Baer, D.; Winterhalter, P. Vitisin A content in Chilean wines from *Vitis vinifera* cv. Cabernet Sauvignon and contribution to the color of aged red wines. *J. Agric. Food Chem.* **2003,** 51, 6261-6267.

Schwarz, M.; Picazo-Bacete; J. J.; Winterhalter, P.; Hermosín-Gutiérrez, I. Effects of copigments and grape cultivar on the color of red wine fermented after the addition of copigments. *J. Agric. Food Chem.* **2005,** 53, 8372-8381.

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

Sipiora, M. J.; Gutierrez-Granda, M. J. Effects of pre-véraison irrigation cutoff and skin contact time on composition, color and phenolic content of young Cabernet Sauvignon wines in Spain. *Am. J. Enol. Vitic.* **1998,** 49, 152-162.

Somers, T. C. Polymeric nature of wine pigments. *Phytochemistry* **1971,** 10, 2175-2186.

Somers, T. C.; Evans, M. E. spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO_2, 'chemical age'. *J. Sci. Food Agric.* **1977,** 28, 42-54.

Timberlake, C. F.; Bridle, P.; Interactions between anthocyanins, phenolic compounds, and acetaldehyde and their significance in red wines. *Am. J. Enol. Vitic.* **1976,** 27, 97-105.

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

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

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

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

Effect of shading on accumulation of phenolic compounds in (*Vitis vinifera* L.) Pinot noir fruit and extraction in a model system

Jessica M. Cortell & James A. Kennedy

Journal of Agricultural Food Chemistry
PO Box 3337,
Columbus, OH, 43210, USA
In press

ABSTRACT

Accumulation and compositional changes of flavonols, proanthocyanidins and anthocyanins were measured in *Vitis vinifera* L. cv Pinot noir in shaded and exposed fruit. 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, skin proanthocyanidin and minimal differences in anthocyanins. In analyzing seed proanthocyanidins by phloroglucinolysis, shaded and exposed fruit were similar at véraison; however, by harvest shaded fruit had higher extension and terminal subunits (nmol/seed) compared to exposed fruit. 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, shaded fruit 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. Skin proanthocyanidin percent extraction was found to be ~ 17% higher in the exposed model extraction than the shaded treatment.

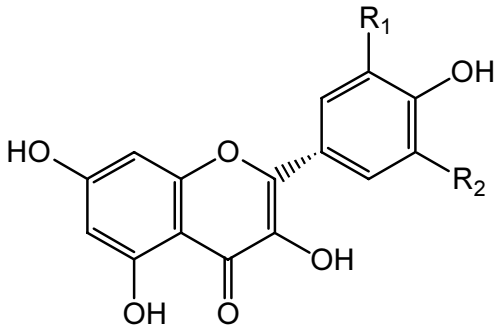
KEYWORDS: shading; UV exposure; flavonoids; flavonols; anthocyanins; proanthocyanidins; flavan-3ol monomers; HPLC; GPC; model wine extraction

INTRODUCTION

Phenolic compounds provide a range of functions in plants such as attracting pollinators and seed dispersers, providing UV-light protection and resisting pathogens and herbivores (Winkel-Shirley 2001). Three major classes of phenolic compounds found in grapes (*Vitis vinifera* L.) include proanthocyanidins (condensed tannins), anthocyanins and flavonols (Souquet et al. 1996) (**Figure 5.1**). Phenolics are important in wine because of their color, astringency and potential role in human health (Santos-Buelga and Scalbert 2000).

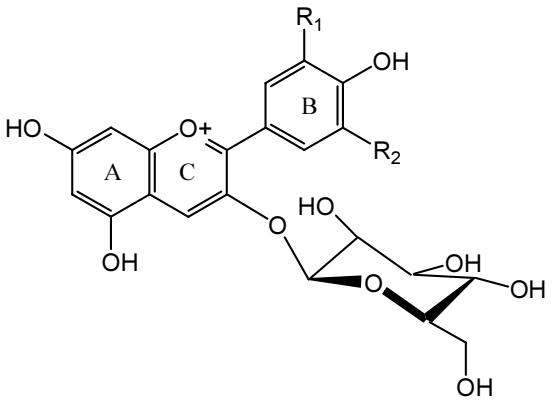
Flavonols are found in grape skins as glycosides of kaempferol, quercetin, myricetin and isorhamnetin (**Figure 5.1a**). Grape seed flavan-3-ols include (+)-catechin (C), (-)-epicatechin (EC) and (-)-epicatechin-3-*O*-gallate (ECG) which exist as both monomers and/or polymeric proanthocyanidins (**Figure 5.1b**) (Prieur et al. 1994; Romeyer et al. 1986; Czochanska et al. 1979). 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 5.1b**). Anthocyanins exist as 3-*O*-monoglucosides and their acylated derivatives. Pinot noir fruit is distinct in having no acylation (**Figure 5.1c**) (Fong et al. 1971).



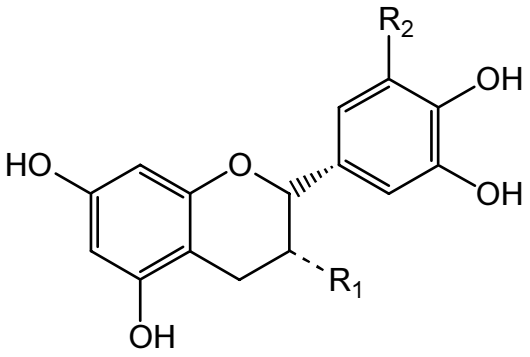
Flavonol	R_1	R_2
kaempferol	H	H
quercetin	OH	H
myricetin	OH	OH

Figure 5.1a. Structures of flavonols based on substitution pattern commonly found in *V. vinifera*.



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 5.1b. Structures of anthocyanins based on substitution pattern commonly found in *V. vinifera* cv. Pinot noir.



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

Figure 5.1c. Structures of flavan-3-ol monomers based on substitution pattern commonly found in *V. vinifera*.

Grapes are a non-climacteric fruit and have two stages of berry growth separated by a lag phase (Coombe 1976). Flavonols, flavan-3ol monomers and proanthocyanidins are biosynthesized during the first phase of berry growth, whereas anthocyanins are biosynthesized during fruit ripening (Bogs et al. 2006; Bogs et al. 2005; Burger and Botha 2004; Boss et al. 1996a; Boss et al. 1996b, **Figure 5.2**). Phenolic accumulation can also respond to external factors such as UV radiation, drought and cold temperatures (Chalker-Scott 1999; Winkel-Shirley 2002). 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 (Bieza & Lois 2001; Li et al. 1993; Nagata et al. 2003).

Flavonols are highly responsive to light exposure and appear to function as UV protectants (Winkel-Shirley 2001; Price et al. 1995; Flint et al. 1985; Spayd et al. 2002; Downey et al. 2004). Proanthocyanidin amount has been observed to decrease slightly with respect to exposure (Downey et al. 2004) and vine water status (Kennedy et al. 2002). However, an increase in skin proanthocyanidin amount with a reduction in vine vigor has also been reported (Cortell et al. 2005). Anthocyanins have been found to have a variable response to light exposure (Flint et al. 1985; Spayd et al. 2002; Kennedy et al. 2002; Cortell et al. 2005; Smart et al. 1988; Bergqvist et al. 2001; Dokoozlian and Kliewer 1996; Gao and Cahoon 1994). Various viticultural practices also influence anthocyanin accumulation (Jackson and Lombard 1993; Reynolds et al. 2005; Hilbert et al. 2003; Ojeda et al. 2002; Yokotsuka et al. 1999).

Compositional shifts in response to UV-B have been found in flavonol biosynthesis in Arabidopsis (Ryan et al. 2002). Anthocyanins change toward a higher proportion of B-ring trihydroxylation in response to UV light (Downey et ak, 2004; Ryan et al. 2002; Jaakola et al. 2004). Recent results on grape skin proanthocyanidins suggest that light exposure can also result in higher B-ring trihydroxylation (Downey et al. 2004; Cortell et al. 2005).

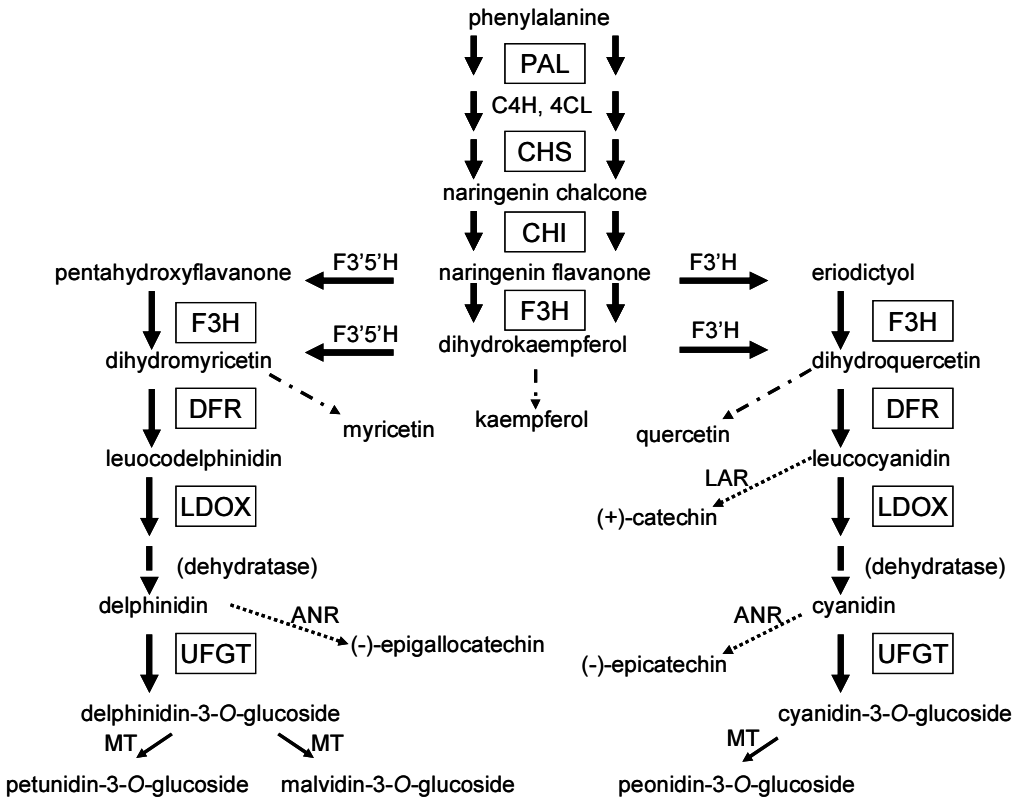


Figure 5.2. Simplified phenolic biosynthetic pathway showing products from flavonoid 3'-hydroxylase (F3'H) and flavonoid 3',5'-hydroxylase (F3'5'H) activity, flavonols = dashed arrows, flavan-3-ols = dotted arrows, and anthocyanins = solid arrows, PAL, phenylalanine ammonia lyase; CHS, chalcone synthase; CHI, chalcone isomerase; DFR, dihydroflavonol-4-reductase; LDOX, leucoanthocyanidin dioxygenase; UFGT, UDP glucose: flavonoid-3-*O*-glucosyltransferase; LAR, leucoanthocyanidin reductase; ANR, anthocyanin reductase; MT, methyltransferase

In addition to the phenolic amount in the fruit, the rate of extraction is an important parameter that determines wine phenolic concentration. Fruit ripeness, ethanol content (Canals et al. 2005) and perhaps berry size have been reported to influence extraction of phenolics (Walker et al. 2005). An increase in skin proanthocyanidin extraction with a reduction in vine vigor has been reported (Cortell et al. 2005).

In an initial study assessing the impact of vine vigor on phenolic accumulation, substantial differences were found in skin proanthocyanidin accumulation and composition (Cortell et al. 2005). Since 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, nutrient uptake). Our first objective was to investigate the relative importance of sunlight exposure on fruit phenolic accumulation and composition. The second objective was to determine if fruit exposure influenced phenolic extractability in a model wine system.

MATERIALS AND METHODS

Vineyard. This study was conducted in 2004 within an 8-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, USA. Vines were planted at a spacing of 1 m X 2.8 m with ~ 5113 vines per hectare. The training system was vertical shoot position (VSP) with each vine pruned to 10-12 nodes. Vine vigor zones within the study site were determined as previously described (Cortell et al. 2005). For this experiment, data vines were randomly selected within the low vigor zone in Site 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 5.3**) (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 (Downey et al. 2004). The temperature within the box was found to be within 0.5°C of the ambient canopy temperature (Downey et al. 2004). In this experiment, temperature was also monitored with dataloggers (Onset; Bonrne, 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).

Figure 5.3. Light exclusion boxes installed over fruit clusters in the vineyard.

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 one day prior to commercial harvest (September 9). Harvested clusters were randomly divided into sub-samples for juice composition (soluble solids, titratable acidity and pH), HPLC analysis and model extraction. For juice composition (5-6 replicates) and HPLC analysis (8-10 replicates), a replicate consisted of all the berries from 1-2 clusters. For HPLC analysis frozen berries were removed from the rachis, and prepared as previously described (Kennedy et al. 2000).

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, (+)-catechin, (-)-epicatechin 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 was purchased from Extrasynthése (Genay, France).

Model extraction. For the model extraction, 10 replicates (~6 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 6 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. 300 ml of the ethanol solution was added to the 300g 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 hours at 38 °C. After 48 hours, 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 (Lamuela-Raventos and Waterhouse 1994). Aqueous extracts were filtered using Teflon filters (0.45µm; Acrodisc CR13) before injection. (+)-Catechin, quercetin and malvidin-3-*O*-glucoside were used as quantitative standards for flavan-3-ols, flavonols and anthocyanins, respectively.

Proanthocyanidin isolates were characterized by phloroglucinolysis (Kennedy and Jones 2001) under modified HPLC conditions (Kennedy and Taylor 2003). Phloroglucinolysis provided information on subunit composition, conversion yield and mean degree of polymerization (mDP). Seed and skin extracts were prepared as previously described (Cortell et al. 2005). Skin and seed proanthocyanidin extraction into model extracts was calculated as described (Peyrot des Gachons and Kennedy 2003).

Gel Permeation Chromatography (GPC) was used to analyze proanthocyanidins while still intact (Kennedy and Taylor 2003). Proanthocyanidins were considered to be 280 nm absorbing material over 500 molecular weight units. Samples were prepared as previously described (Cortell et al. 2005); however, after freeze drying, samples were dissolved in mobile phase. C and Mv were used as quantitative standards at 280 nm 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 5.1)**. Average seeds per berry were the same at véraison; however at harvest the exposed treatment was higher than the shaded treatment. No differences were observed for average dry seed

or skin weight (mg) in ripe fruit except for the shaded treatment had a lower dry skin weight compared to the exposed treatment at véraison.

Table 5.1. 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,

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 versus 0.049 mg/berry, p=0.0001). By harvest, the shaded treatment concentration was slightly more than 8 times lower than the exposed treatment (0.012 versus 0.10 mg/berry, p=0.0002). Due to 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 (+)-catechin (C) and (-)-epicatechin (EC) (**Table 5.2a**). 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.

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 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 difference 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 5.2b)**. The shaded treatment extension proanthocyanidin subunits decreased ~7% while the exposed treatment decreased ~15% from véraison to harvest. For the terminal proanthocyanidin subunits, they were about the same 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 lower seed mDP at véraison but by harvest the treatments were similar.

The composition of proanthocyanidins was determined by phloroglucinolysis (**Table 5.2c**). 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 (-)-epicatechin-3-*O*-gallate (ECG) 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.

Table 5.2. Mean (± SEM) a) seed flavan-3-ol monomer concentration (nmol/seed) and molar proportion and b) extension, terminal and total subunit concentration (nmol/seed) and mDP 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	<.0001	<.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

Table 5.2 (continued). Mean (± SEM) c) proanthocyanidin molar extension and terminal subunit proportions by phloroglucinolysis from shaded and exposed treatments at véraison and commercial harvest.

c) Proanthocyanidin composition

Treatment	Time	Extension C (%)	Extension EC (%)	Extension ECG (%)	Terminal C (%)	Terminal EC (%)	Terminal 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	<.0001	<.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	<.0001	0.0002	0.0062	0.0012

[a]ANOVA to compare data (*P* indicated), n=8-10, with the following subunit abbreviations: C: (+)-catechin, EC: (-)-epicatechin, ECG: (-)-epicatechin-3-*O*-gallate.

Skin flavan-3-ols. 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 compared to 1.20 ± 0.10 mg/berry skin proanthocyanidin in the exposed treatment (p=0.0116).

By phloroglucinolysis, 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 the shaded treatment (**Table 5.3a**). 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 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 compared to the exposed treatment.

Skin proanthocyanidin extension subunits consisted of C, EC, ECG and (-)-epigallocatechin (EGC) (**Table 5.3b**). 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.

Table 5.3. 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	<.0001	<.0001	<.0001	<.0001	<.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	<.0001	<.0001	.9342	<.0001	<.0001

[a]ANOVA to compare data (*P* indicated), n=8-10, with the following subunit abbreviations: C: (+)-catechin, EC: (-)-epicatechin, EGC: (-)-epigallocatechin, ECG: (-)-epicatechin-3-*O*-gallate.

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 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 compared to the exposed treatment.

Skin anthocyanins. On a per berry basis, there was a trend towards a reduced anthocyanin concentration in the shaded treatment of ~38% (**Table 5.4**). 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 malvidin-3-*O*-glucoside (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.

At véraison and harvest, the individual phenolic classes were compared on a per berry basis. Although, the total amount of phenolics was similar at véraison (p=0.9300), skin phenolics (skin proanthocyanidins and flavonols) were higher in the exposed treatment compared to the shaded treatment (Figure **4a;** p=0.0004).

Table 5.4. 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	Total	Delphinidin	Cyanidin	Petunidin	Peonidin	Malvidin	3'4'-OH	3'4'5'-OH
	(mg/berry)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
Shaded	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	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	<.0001	0.0982	<.0001	<.0001	<.0001	<.0001	<.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).

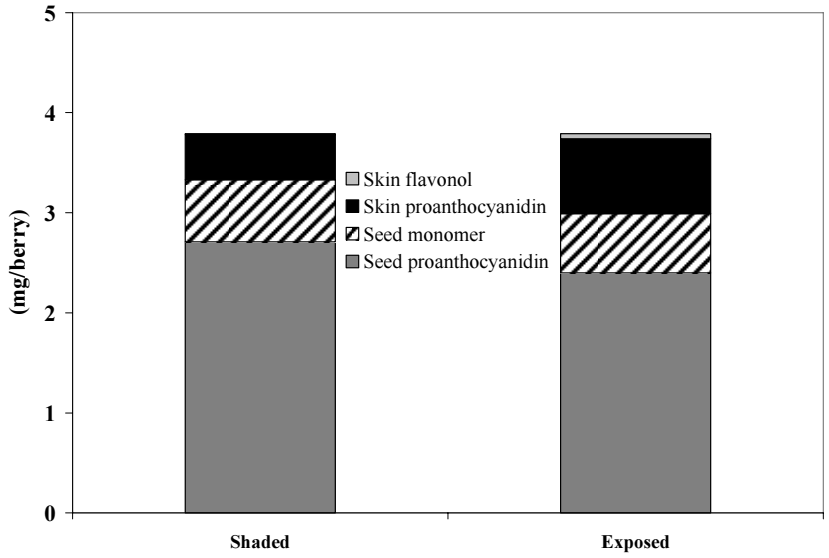


Figure 5.4a. Total accumulation of seed flavan-3-ol monomers, seed proanthocyanidins, skin proanthocyanidins and skin flavonols (mg/berry) in shaded and exposed fruit at véraison (n=10). Seed and skin proanthocyanidin were determined by phloroglucinol.

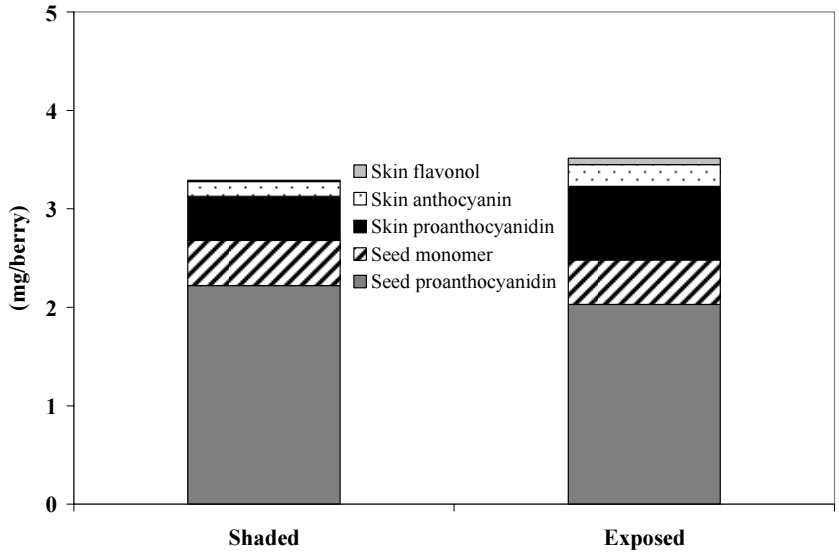


Figure 5.4b. Total accumulation of seed flavan-3-ol monomers, seed proanthocyanidins, skin proanthocyanidins, skin flavonols and skin anthocyanins (mg/berry) in shaded and exposed fruit at commercial harvest (n=8-9). Seed and skin proanthocyanidin were determined by phloroglucinol.

Similarly, at harvest, total phenolic amount for the three classes was similar on a per berry basis (p=0.1549); however, the relative accumulation of specific classes differed between treatments (**Figure 5.4b**). The shaded treatment had a similar proportion of seed phenolics (monomers and proanthocyanidins) and a reduction of 0.49 mg/berry ± 0.09 (p=0.0035) in accumulation of skin phenolic compounds compared to the exposed treatment. In comparing the percent of skin phenolic content per berry, at véraison, the shaded treatment contained 12% and the exposed had 21% skin phenolics (p=<0.0001). With the additional accumulation of anthocyanins at harvest, the shaded treatment was 17% and the exposed treatment was 30% skin phenolics 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 phenolic 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 berry sample was not statistically significant at p≤0.05 (**Table 5.1 and 5.5**). Pomace weight (g), juice volume (mL) did not differ between treatments.

Model extract flavonols. 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 (~2.5x versus ~8x, respectively).

Table 5.5. 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
Number 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.

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 5.6a**). 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 5.6b**).

The proanthocyanidin composition of model extracts (**Table 5.6c**) 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 5.2b**). The C and EC terminal subunit ratio in model extracts was similar to the relationship found between treatments in

seed terminal units (**Table 5.2b**). For EC and ECG, the variations seen in the model extracts (**Table 5.6c**) are consistent with the treatment differences seen in the skin extension subunits (**Table 5.3b**). An increase in extension subunit concentration (53%) and no increase in terminal subunits indicated a small reduction in mDP for the shaded treatment extract. The exposed treatment had a higher concentration of skin proanthocyanidin **(Table 5.7).** In addition, there was a greater percent of skin proanthocyanidin extraction in the exposed compared to the shaded extracts. However, no differences were observed between treatments for seed proanthocyanidin concentration in the extracts.

Model extract anthocyanins. The anthocyanin concentration (mg/L) was higher in the exposed treatment model extract than the shaded treatment (**Table 5.8**). The extract treatment difference in anthocyanin concentration was 66.7 % which was greater than the treatment difference observed in the fruit (**Table 5.4**). 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 5.8**). The anthocyanin compositional treatment differences found in the fruit (**Table 5.4**) were reflected in the model extracts (**Table 5.8**).

Table 5.6. 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	<.0001	<.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]	<.0001	0.3100	<.0001	0.0826

c) Proanthocyanidin Composition

Treatment	Extension subunits				Terminal subunits	
	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	<.0001	<.0001	<.0001	0.0036	0.0036

[a]ANOVA to compare data (*P* indicated), n=8-10, with the following subunit abbreviations: C: (+)-catechin, EC: (-)-epicatechin, EGC: (-)-epigallocatechin, ECG: (-)-epicatechin-3-*O*-gallate.

However, in both treatments there was a reduction in the proportion of Dp and Pt in model extracts compared to the proportions found in the fruit.

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 5.8**). The anthocyanin compositional treatment differences found in the fruit (**Table 5.4**) were reflected in the model extracts (**Table 5.8**). However, in both treatments there was a reduction in the proportion of Dp and Pt in model extracts 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 (Chaves and Escudero 1999). Ultraviolet radiation and water stress have been shown in numerous studies to be the most relevant factors in the induction of phenolic biosynthesis (Chaves and Escudero 1999). 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 phenolic compounds in Pinot noir grapes (Cortell et al. 2005). In this study, we compare 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.

Table 5.7. 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	Skin Extracted	Skin	Seed
	(mg/L)	(%)	(mg/L)	(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.

Table 5.8. Mean (± SEM) anthocyanin concentration (mg/L) calculated in malvidin equivalents and proportional analysis in shaded and exposed model extractions.

Treatment	Time	Total	Delphinidin	Cyanidin	Petunidin	Peonidin	Malvidin
		(mg/L)	(%)	(%)	(%)	(%)	(%)
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]		<.0001	<.0001	0.0002	<.0001	<.0001	<.0001

[a]ANOVA to compare data (*P* indicated), n=8-10.

Berry composition. The lack of differences observed in soluble solids accumulation (**Table 5.1**) 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 (Gao and Cahoon 1994) while others found no effect from shading (Spayd et al. 2002; Downey et al. 2004). In this experiment, average number of seeds per berry was determined to be similar at véraison but was lower in the shaded treatment at harvest (**Table 5.1**). It is possible this was due to high sample variability. Based upon previous work (Harbertson et al. 2002), 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 (Roby and Matthews 2004) can affect average berry size and subsequent skin, seed and pulp proportions (Coombe et al. 1987). In this experiment, berry size was similar and no differences were observed in percent skin, seed or pulp (**Table 5.1**). Higher skin tissue mass has been found in berries from vines grown under a low vine water status (Roby et al. 2004).

Skin Flavonols. The phenolic 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 5.2**). 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 (Downey et al. 2003a; Downey et al. 2003b). 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 (Cortell et al. 2005; Spayd et al. 2002; Downey et al. 2004) and apple (Solovchenko and Schmitz-Eiberger 2003b); 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 (Harbourne 1997) and also to protect developing fruit from fungal pathogens (Mercier 1987). Bogs et al. (Bogs et al. 2005) 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 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 due to the higher number of seeds per berry in the exposed treatment at harvest (**Table 5.1**). 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 (Cortell et al. 2005).

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 (Kennedy et al. 2000; Roby et al. 2004) and light exclusion (Downey et al. 2004).

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 (Downey et al. 2003b; Kennedy et al. 2000). In the present experiment, total flavan-3-ol monomer concentrations were similar between treatments at véraison and harvest **Table 5.2a**). Downey et al. (Downey et al. 2004) found higher levels of monomers in exposed clusters at véraison but no differences between shaded and exposed fruit at harvest. In other results, total flavan-3-ol monomers were found to be lower in minimally irrigated Cabernet Sauvignon vines (Kennedy et al. 2000) and in low vigor vines (Cortell et al. 2005). 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 (Pastor del Rio and Kennedy 2006).

There were differences in the flavan-3-ol monomer proportion of EC and C (**Table 5.2a**). 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 (Cortell et al. 2005). 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 (Kennedy et al. 2000; Downey et al. 2003b; Kennedy et al. 2000). 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 (Pastor del Rio and Kennedy 2006)

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 5.2b**). 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 (Cortell et al. 2005). In other studies, extension proanthocyanidin subunits were highest at véraison (Kennedy et al. 2000; Pastor del Rio and Kennedy 2006) or two weeks post véraison (Downey et al. 2003b) 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 (Pastor del Rio and Kennedy 2006).

Shading resulted in a lower proportion of C and higher proportion of ECG in the seed extension subunits at véraison and harvest (**Table 5.2c**). For terminal subunits, the shaded treatment had lower C and higher EC and ECG proportions

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. (Kennedy et al. 2000) 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 (Cortell et al. 2005). 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 non-covalent associations with anthocyanins (Kennedy et al. 2001). To date, it is still unclear whether pigmented proanthocyanidins (Somers 1971) 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 showed that skin proanthocyanidin concentration peaked near véraison and then declined with increasing maturity (Kennedy et al. 2002; Downey et al. 2003b; Pastor del Rio and Kennedy 2006). Recently we found vines with low vigor had a substantially higher skin proanthocyanidin concentration than high vigor vines (Cortell et al. 2005). 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 5.1**), 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 (Kennedy et al. 2002). 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 (Downey et al. 2004). 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 total proanthocyanidin concentration (mg/berry) was analyzed by GPC (Cortell et al. 2005). 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 (Pastor del Rio and Kennedy 2006) while Downey et al. (Downey et al. 2003b) reported that skin mDP increased during the early phase of berry development then decreased after véraison. Our observations in both treatments agree with a reduction in skin mDP between véraison and harvest (**Table 5.3a**). The mDP values found in the present study at véraison were consistent with reported values in Pinot noir, however, our harvest values were lower (Pastor

del Rio and Kennedy 2006). Downey et al. (Downey et al. 2004) found a similar reduction in skin mDP with shading. Previously, a higher mDP with a reduction in irrigation (Kennedy et al. 2002) and in fruit from low vigor vines was reported (Cortell et al. 2005). 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 5.3)** was substantially greater than the increase of 6.4% previously reported in fruit from low vigor compared to high vigor vines (Cortell et al. 2005). 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 compared to shaded clusters (Downey et al. 2004). 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 (Harbourne 1997; Kennedy et al. 2001). As EGC has the highest rate of degradation due to oxidation (Jorgensen et al. 2004), 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 5.2**) (Boss et al. 1996a). For most grape varieties,

UFGT is only found in red grape skins and is expressed at the time of anthocyanin accumulation (Boss et al. 1996b). While many grape varieties have very

complex anthocyanin profiles with up to 20 different anthocyanins (Wulf and Nagel 1976), Pinot noir has only five anthocyanins: Dp, Cy, Pt, Pn and Mv.

Shading reduced 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 5.4**). Downey et al. (Downey et al. 2004) did not find a difference in two out of three years for anthocyanin accumulation (with three replicates) in Shiraz using identical boxes for cluster shading. Price et al. (Price et al. 1995) 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, anthocyanin content was found to be higher in exposed fruit (Spayd et al. 2002; Smart et al. 1988; Bergqvist et al. 2001; Dokoozlian and Kliewer 1996).

For anthocyanin composition, shading resulted in lower proportions of Dp, Cy, Pt, and Mv with only an increase in Pn (**Table 5.4**). 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 (Gao and Cahoon 1994). In Shiraz, shading was found to have no effect on 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 (Downey et al. 2004). 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 (Spayd et al. 2002). In the same study, the use of a UV barrier that sites 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 (Spayd et al. 2002).

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 (Spayd et al. 2002). It is not possible to determine whether this increase was from greater accumulation of Dp at the cooler temperature, a higher conversion rate of Dp to Mv by methyltransferase or a higher oxidative degradation rate of Dp in the sun exposed warmer fruit. Dp is more susceptible to oxidation than Mv (Skrede et al. 2000). Mv has been previously reported to be less sensitive to light intensity compared to the other four anthocyanins (Keller and Hrazdina 1998). Owing to their phenolic B-ring substitution, Pn and Mv are relatively stable and represent the major anthocyanin pools in mature grapes (Roggero et al. 1986).

When investigating anthocyanin F3′5′H products compared to the F3′H products (**Figure 5.2**), the shaded treatment had a much lower proportion of trioxygenated (Dp, Pt and Mv) anthocyanins (**Table 5.4**). The anthocyanin accumulation was consistent with increased F3′5′H activity. Downey et al. (Downey et al. 2004) found between a 3-10% increase (depending on the year) in trioxygenated anthocyanins in exposed clusters compared to shaded clusters. In

Reliance, 95% shading resulted in an approximate 5% decrease in trioxygenated compared to dioxygenated anthocyanins (Gao and Cahoon 1994). However, in Merlot, no increases were noted in percent trioxygenated anthocyanins with greater light exposure (Spayd et al. 2002). 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 5.4a, 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 (Bogs et al. 2005) 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 phenolics (flavonols, anthocyanins and proanthocyanidins) were higher in the exposed treatment at harvest. The increase in skin phenolics likely plays a role in UV protection. These differences in fruit composition seen between shaded and exposed treatments are also likely to affect phenolic concentration in a wine system.

Model extracts. The ratio of skin and seed material to pulp was thought to influence the concentration of phenolics found in wine although a recent study suggests berry size may be of limited importance (Walker et al. 2005). Although there were no differences in percent fresh skin, seed or pulp in the berry sample (**Table 5.1**) or in the pomace weight and juice volume (**Table 5.5**), 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 (Kennedy et al.

2002; Ojeda et al. 2002; Roby and Matthews 2004). However, reduced berry growth was reported when shading occurred in the initial stages of berry growth (Dokoozlian and Kliewer 19996). 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 phenolics 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 (Price et al. 1995). The amount found in the exposed model extractions was higher than the levels reported in Pinot noir (Price et al. 1995) wine and this could be due to the higher temperature, higher ethanol content or shorter length of time used in the model extraction system compared to standard winemaking.

Shading fruit reduced the skin proanthocyanidin concentration in both the fruit and in the model extraction (**Table 5.3a, 5.6b**). 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 (Cortell et al. 2005). Interestingly, the shaded treatment percent skin proanthocyanidin extraction of 54% (**Table 5.7**) 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%) (Cortell et al. 2005). Due to 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 5.8**) than from the shaded treatment even though differences in the fruit were not apparent due to high variability (**Table 5.4**). 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 compared to shaded fruit (Price et al. 1995). 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 phenolic compounds into wine (Canals et al. 2005). In this case, there were no obvious differences in ripeness between treatments as determined by soluble solids (**Table 5.1**). This suggests that there was improved extractability of anthocyanins associated with the exposed treatment.

The anthocyanin composition in the model extractions (**Table 5.8**) was similar to the pattern found between treatments in the fruit (**Table 5.4**) 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 (Sims and Bates 1994). The rate of reaction for pigmented polymers is related to both the concentration and composition of anthocyanins, proanthocyanidins and other

cofactors (Zimman et al. 2002). In this model extraction, the pigmented polymer concentration was substantially higher in the exposed treatment 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 phenolic 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 (Cortell et al. 2005). However, skin proanthocyanidin concentration and percent skin EGC were lower in the high vigor zone (characterized by a dense, shady canopy) 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 is similar to what was observed in high vigor vines (unpublished data). This suggests these responses in phenolic 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 phenolic 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 compared to seed derived proanthocyanidins while flavan-3-ol monomers are reported to have a negative attribute of increasing the bitterness of wine (Cheynier et al. 1998). The concentration and composition of anthocyanins are important in color stability in wines and flavonols also play a role in co-pigmentation (Boulton 2001). 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;

ACKNOWLEDGMENTS

We thank Archery Summit Winery for the use of their vineyards and fruit for wine production as well as Leigh Bartholomew and Anna Matzinger specifically for their support. In addition, we would like to thank Dr. Mark Downey for allowing us to use his light exclusion boxes as well as students: Jason Bell, Fiorella Cerpa, Jose Luis Pastor del Rio and Chris Zielinski for their help. Funding for this project was provided by the USDA Northwest Center for Small Fruit Research and the American Society for Enology and Viticulture.

LITERATURE CITED

Bergqvist, J.; Dokoozlian, N.; Ebisuda, N. Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the central San Joaquin Valley of California. *Am. J. Enol. Vitic.* **2001,** 52, 1-7.

Bieza K.; Lois, R. An Arabidopsis mutant tolerant to lethal ultraviolet-B levels shows constitutively elevated accumulation of phenolics and other phenolics. *Plant Physiol.* **2001,** 126, 1105-1115.

Bogs, J.; Downey, M. O.; Harvey, J. S.; Ashton, A. R.; Tanner, G. J.; Robinson, S. P. Proanthocyanidin synthesis and expression of genes encoding leucoanthocyanidin reductase and anthocyanidin reductase in developing grape berries and grapevine leaves. *Plant Physiol.* **2005,** 139, 652-663.

Bogs, J.; Ebadi, A.; McDavid, D.; Robinson, S. P. Identification of the flavonoid hydroxylases from grapevine and their regulation during fruit development. *Plant Physiol.* **2006,** 140, 279-291.

Boss, P. K.; Davies, C.; Robinson, S. P. Analysis of the expression of anthocyanin pathway genes in developing Vitis vinifera L. cv Shiraz grape berries and the implications for pathway regulation. *Plant Physiol.* **1996a,** 111, 1059-1066.

Boss, P. K.; Davies, C.; Robinson, S. P. Expression of anthocyanin biosynthesis pathway genes in red and white grapes. *Plant Mol. Biol.* **1996b,** 32, 565-569.

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

Burger, A. L.; Botha, F. C. Ripening-related gene expression during fruit ripening in *Vitis vinifera* L. cv. Cabernet sauvignon and Clairette blanche. *Vitis* **2004,** 43, 59-64.

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

Chalker-Scott, L. Environmental significance of anthocyanins in plant stress responses. *Photochem. Photobiol.* **1999,** 70, 1-9.

Chaves, N.; Escudero, J. C. Variation of flavonoid synthesis induced by ecological factors. In *Principles and Practices of Plant Ecology, Allelochemical Interactions.* (Eds Inderjit, K.M.M. Dakshini & C.L. Foy) CRC Press, Boca Raton, Florida, USA. **1999;** pp267-285.

Cheynier, V.; Fulcrand, H.; Brossaud, F.; Asselin, C.; Moutounet, M. (**1998**) Phenolic composition as related to red wine flavor. In *Chemistry of Wine Flavor.* (Eds A.L. Waterhouse & S.E. Ebeler) pp. 125-141. Oxford University Publishing, New York, USA.

Coombe, B. G. The development of fleshy fruits. *Ann. Rev. Plant Physiol.* **1976,** 27, 507-528.

Coombe, B. G.; Bovio, M.; Schneider, A. Solute accumulation by grape pericarp cells V. Relationship to berry size and the effect of defoliation. *J. Exp. Biol.* **1987,** 38, 1789-1798.

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

Czochanska, Z.; Foo, L. Y.; Lawrence, J. P. Compositional changes in lower molecular weight flavans during grape maturation. *Phytochemistry* **1979,** 18, 1819-1822.

Dokoozlian, N. K; Kliewer W. M. Influence of light on grape berry growth and composition varies during fruit development. *J. Am. Soc. Hort. Sci.* **1996,** 121, 869-874.

Downey, M. O.; Harvey, J. S.; Robinson, S. P. Synthesis of flavonols and expression of flavonol synthase genes in the developing berries of Shiraz and Chardonnay (*Vitis vinifera* L.) *Aust. J. Grape Wine Res.* **2003a,** 9, 110-121.

Downey, M. O.; Harvey, J. S.; Robinson, S. P. The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. *Aust. J. Grape Wine Res.* **2004,** 1, 55-73.

Downey, M. O.; Harvey, J. S.; Robinson, S. P. Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. *Aust. J. Grape Wine Res.* **2003b**, 9, 15-27.

Flint, S. D.; Jordan, P. W.; Caldwell, M. M. Plant protective response to enhanced UV-B radiation under field conditions: leaf optical properties and photosynthesis. *Photochem. Photobiol.* **1985,** 41, 95-99.

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

Gao, Y.; Cahoon, G. A. Cluster shading effects on fruit quality, fruit skin color, and anthocyanin content and composition in Reliance (*Vitis* hybrid). *Vitis* **1994,** 33, 205-209.

Harbertson, J. F.; Kennedy, J. A.; Adams, D. O. Tannin in skins and seeds of Cabernet Sauvignon, Syrah, and Pinot noir berries during ripening. *Am. J. Enol. Vitic.* **2002,** 53, 54-59.

Harbourne J. Phytochemistry of fruits and vegetables: an ecological overview. In *Phytochemistry of Fruits and Vegetables;* Tomas-Barberan, F., Eds; Oxford University Press; New York, **1997**; pp 353-367.

Hilbert, G.; Soyer, J. P.; Molot, C.; Giraudon, J.; Milin, G. S.; Gaudillere, J. P. Effects of nitrogen supply on must quality and anthocyanin accumulation in berries of cv. Merlot. *Vitis* **2003,** 42, 69-76.

Jaakola, L.; Määttä-Riihinen, K.; Kärenlampi, S.; Hohtola, A. Activation of flavonoid synthesis by solar radiation in bilberry (*Vaccinium myrtyillus* L.) leaves. *Planta* **2004,** 218, 721-728.

Jackson, D. I.; Lombard, P. B. Environmental and management practices affecting grape composition and wine quality – A review. *Am. J. Enol. Vitic.* **1993,** 44, 409-430.

Jorgensen, E. M.; Marin, A. B.; Kennedy, J. A. Analysis of the oxidative degradation of proanthocyanidins under basic conditions. *J. Agric. Food Chem.* **2004,** 52, 2292-2296.

Keller, M. K.; Hrazdina, G. Interaction of nitrogen availability during bloom and light intensity during véraison. II. Effects on anthocyanin and phenolic development during grape ripening. *Am. J. Enol. Vitic.* **1998,** 49, 341-349.

Kennedy, J. A., Hayasaka, Y., Vidal, S., Waters, E. J. & Jones, G. P. Composition of grape skin proanthocyanidins at different stages of berry development. *J. Agric. Food Chem.* **2001,** 49, 5348-5355.

Kennedy, J. A.; Jones, G. P. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J. Agric. Food Chem.* **2001,** 49, 1740-1746.

Kennedy, J. A.; Matthews, M. A.; Waterhouse, A. L. Changes in grape seed polyphenols during fruit ripening. *Phytochemistry* **2000,** 55, 77-85.

Kennedy, J. A.; Matthews, M. A.; Waterhouse, A. L. Effect of maturity and vine water status on grape skin and wine flavonoids. *Am. J. Enol. Vitic.* **2002,** 53, 268-274.

Kennedy, J. A.; Taylor, A. W. (2003) Analysis of proanthocyanidins by high-performance gel permeation chromatography. *J. Chromatogr. A.* **2003,** 995, 99-107.

Kennedy, J. A.; Troup, G. J.; Pilbrow, J. R.; Hutton, D. R.; Hewitt, D.; Hunter, C. R.; Ristic, R.; Iland, P. G.; Jones, G. P. Development of seed polyphenolics in berries from *Vitis vinifera* L. cv Shiraz. *Aust. J. Grape Wine Res.* **2000,** 6, 244-254.

Lamuela-Raventos, R. M.; Waterhouse, A. L. A direct HPLC separation of wine phenolics. *Am. J. Enol. Vitic.* **1994,** 45, 1-5.

Li, J.; Ou-Lee, T. M.; Raba, R.; Amundson R. G.; Last, R. L. Arabidopsis mutants lacking phenolic sunscreens exhibit enhanced ultraviolet-B injury and oxidative damage. *Plant Cell* **1993,** 5,171-179.

Mercier, J. Role of phytoalexins and other antimicrobial compounds from fruits and vegetables in post harvest disease resistance. In *Phytochemistry of Fruits and Vegetables.* Tomas-Barberan, F., Eds; Oxford University Press; New York, **1987**; pp 221-241.

Nagata, T.; Todoriki, S.; Masumizu, T.; Suda, I.; Furuta, S.; Du, Z.; Kikuchi, S. Levels of active oxygen species are controlled by ascorbic acid and anthocyanin in *Arabidopsis*. *J. Agric. Food Chem.* **2003,** 51, 2992-2999.

Ojeda, H.; Andary, C.; Kraeva, E.; Carbonneau, A.; Deloire, A. Influence of pre- and post-veraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. *Am. J. Enol. Vitic.* **2002,** 53, 261-267.

Pastor del Rio, J.; Kennedy, J. A. Development of proanthocyanidins in *Vitis vinifera* L. cv. Pinot noir grapes and extraction into wine. *Am. J. Enol. Vitic.* **2006,** 57, 125-131.

Peyrot des Gachons, C.; Kennedy, J.A. Direct Method for Determining seed and skin proanthocyanidin extraction in red wine. *J. Agric. Food Chem.* **2003,** 51, 5877-5881.

Price, S. F.; Breen, P. J.; Valladao, M.; Watson, B. T. Cluster exposure and quercetin in Pinot noir grapes and wine. *Am. J. Enol. Vitic.* **1995,** 46, 187-194.

Prieur, C.; Rigaud, J.; Cheynier, V.; Moutounet, M. Oligomeric and polymeric proanthocyanidins from grape seeds. *Phytochemistry* **1994,** 36, 781-784.

Reynolds, A. G.; Molek, T.; De Savigny, C. Timing of shoot thinning in *Vitis vinifera*: Impacts on yield and fruit composition Variables. *Am. J. Enol. Vitic.* **2005,** 56, 343-356.

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

Roby, G.; Matthews, M. A. Relative proportions of seed, skin and flesh, in ripe berries from Cabernet Sauvignon grapevines grown in a vineyard either well irrigated or under water deficit. *Aust. J. Grape Wine Res.* **2004,** 10, 74-82.

Roggero, J. P.; Coen, S.; Ragonnet, R. High performance liquid chromatography survey on changes in pigment content in ripening grapes of Syrah. An approach to anthocyanin metabolism. *Am. J. Enol. Vitic.* **1986,** 37, 77-83.

Romeyer, F.M.; Macheix, J.J.; Sapis, J.C. Changes and importance of oligomeric proanthocyanidins during maturation of grape seeds. *Phytochemistry* **1986,** 25, 219-221.

Ryan, K. G.; Swinny, E. E.; Markham, K. R.; Winefeld, C. Flavonoid gene expression and UV photoprotection in transgenic and mutant Petunia leaves. *Phytochemistry* 59, **2002,** 23-32.

Santos-Buelga, C.; Scalbert, A. Review: Proanthocyanidins and tannin-like compounds – nature, occurrence, dietary intake and effects on nutritional health. *J. Sci. Food Agric.* **2000,** 80, 1094-1117.

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

Skrede, G.; Wrolstad, R. E.; Durst, R. W. Changes in anthocyanins and polyphenolics during juice processing of highbush blueberries (*Vaccinium corymbosum* L.). *J. Food Sci.* **2000,** 65, 357-364.

Smart, R. E.; Smith, S. M.; Winchester, R. V. Light quality and quantity effects on fruit ripening for Cabernet sauvignon. *Am. J. Enol. Vitic.* **1988,** 39, 250-258.

Solovchenko, A.; Schmitz-Eiberger, M. Significance of skin flavonoids for UV-B protection in apple fruits. *J. Exp. Biol.* **2003b,** 54, 1977-1984.

Somers, T. C. The polymeric nature of wine pigments. *Phytochemistry* **1971,** 10, 2175-2186.

Souquet, J. M.; Cheynier, V.; Brossaud, F.; Moutounet, M. Polymeric proanthocyanidins from grape skins. *Phytochemistry* **1996,** 43, 509-512.

Spayd, S. E.; Tarara, J. M.; Mee, D. L.; Ferguson, J.C. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *Am. J. Enol. Vitic.* **2002,** 53, 171-182.

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

Winkel-Shirley, B. It takes a Garden. How work on diverse plant species has contributed to an understanding of flavonoid metabolism. *Plant Physiol.* **2001,** 127, 1399-1404.

Winkel-Shirley. Biosynthesis of flavonoids and effects of stress. Current Opinions in Plant Biology. **2002,** 5:3:218-223.

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

Yokotsuka, K.; Nagao, A.; Nakazawa, K.; Sato, M. Changes in anthocyanins in berry skins of Merlot and Cabernet sauvignon grapes grown in two soils modified with limestone or oyster shell versus a native soil over two years. *Am. J. Enol. Vitic.* **1999,** 50, 1-12.

Zimman, A.; Joslin, W. S.; Lyon, M. L.; Meier, J.; Waterhouse, A. L. Maceration variables affecting phenolic composition in commercial-scale Cabernet sauvignon winemaking trials. *Am. J. Enol. Vitic.* **2002,** 53, 93-98.

Influence of Vine Vigor on Pinot noir Fruit Composition, Wine Chemical Analysis and Wine Sensory Attributes

Jessica M. Cortell, Hanne K. Sivertsen,
Hildegarde Heymann, James A. Kennedy

American Journal of Enology and Viticulture
P.O. Box 1855
David, California 95617
To be submitted on October 15, 2006

ABSTRACT

The relationships between grapevine (*Vitis vinifera*) vigor variation and resulting fruit composition, fruit chemical analysis, wine chemical analysis and sensory attributes were investigated. The study was conducted in a commercial vineyard consisting of the same clone, rootstock, age, and vineyard management practices. In 2003, vine vigor parameters were used to designate vigor zones within two vineyard sites to produce research wines. Similar winemaking practices were used. Descriptive sensory analysis and Partial Least Squares (PLS) modeling was done on two-year-old wines. Wines and model extracts were analyzed by HPLC. Significant sensory attributes included earthy, chemical, heat, sweet, sour, bitter and astringent. Astringency was the most important attribute used to differentiate the wines. Low vigor wines had much higher astringency than high vigor wines. Positively correlated variables included number of seeds per berry, fruit total PA (mg/kg), and skin PA (mg/berry) while total monomers per seed, total monomers per berry, fruit titratable acidity and berry weight had negative correlations with the sensory attributes. Interestingly, seed tannin was not an important variable in the model. There was a strong relationship between fruit total tannin and wine astringency and this was related to differences in skin rather than seed derived tannin. There was a strong relationship between measured and predicted astringency.

KEYWORDS: astringency; sweet; sour; bitter; heat; earthy chemical; tannin; flavan-3-ol monomers; berry weight, seeds; skin.

INTRODUCTION

Astringency and bitterness in wine is provided primarily by flavan-3ols and proanthocyanidins phenolic compounds originating from the fruit (Noble 1994). This sensory attribute is an important aspect of wine quality. Flavan-3-ols and proanthocyanidins (tannins) are also important in wine because of their role in the formation of pigmented polymers, long-term color stability (Somers, 1971) and human health benefits (Santos-Buelga & Scalbert 2000; Dixon et al. 2004).

Due to the large number of chemical compounds in wine (Rapp 1990), interactions of chemical compounds in wine (Lesschaeve & Noble 2005) and the complex nature of the human response to gestation (Thorngate 1997), there is interest in understanding the relationship between the chemical analyses of a wine and the perception of taste. In addition, there is interest in understanding how various vineyard factors influence fruit composition and the sensory characteristics of the resulting wine. Astringency in particular has been difficult to study because of its long persistence and carry over effects in sensory studies (Lee & Lawless 1991; Valentová et al. 2002).

Astringency is a tactile sensation that can be described sensorially as mouth drying and puckering. Tannins are chemically defined as having molecular weights between 500 and 3000 and having the ability to precipitate proteins. In the case of wine, tannins precipitate salivary proteins. While monomeric flavan-3-ols are primarily bitter, as molecular weight increases with polymerization; astringency becomes predominate over bitterness (Noble 1994; Peleg et al. 1999).

Consequently, large polymeric tannins from skins and seeds are the major contributors to wine astringency.

There are differences in grape skin and seed tannin composition and these are thought to have different astringent qualities in wine. Skin differs from seed tannin in having a higher mean degree of polymerization (mDP), the trihydroxylated flavan-3-ol (-)-epigallocatechin (EGC) and lower galloylation. Typical mDP for Pinot noir seeds is in the range of 6-9 while skins were found to be from 27-42 flavan-3-ol units (Pastor del Rio & Kennedy 2006). Studies have shown there are also differences in sensory properties related to the identity of the monomeric unit, the specific linkages, degree of galloylation and formation of derivatives (Peleg et al. 1999; Vidal et al. 2003; Lesschaeve & Noble 2005). Although it had been suggested that skin tannin played an important role in wine tannin (Meyer & Hernandez 1970), a recent method has made it easier to determine the percent of seed and skin tannin in wine (Peyrot des Gachons & Kennedy 2003).

Other taste factors modify the intensity of astringency through enhancement or suppression. Increasing the ethanol content increased the intensity of bitterness but had no effect on astringency (Fischer et al. 1994). Lowering the pH of wines increased the sourness (Fischer & Noble 1994) while adding acid increased the astringency of wines (Kallithraka et al. 1997). Increasing sweetness or viscosity has been reported to decrease bitterness in vermouth. In wine, the addition of sucrose (Ishikawa & Noble 1995) and increasing the viscosity with carboxymethylcellulose reduced the astringency (Smith et al. 1996).

Consequently, the mouth feel of wine can be modified by a number of compounds and interactions.

In addition, sensory studies have been used to investigate the effect of vintage (Boselli et al. 2004), growing region (Heymann & Noble 1987; Boselli et al. 2000; Kallithraka et al. 2001), grape cultivar (Boselli et al. 2000), and various viticultural practices (Reynolds et al 1996) on the relationship between the wine chemical profile and sensory characterization. Principle component analysis (PCA) has been used to determine relevant biological parameters for fruit and wine (Kallithraka et al. 2001) and partial least squares (PLS) regression analysis has been used to evaluate the ability to predict a response (Frank & Kowalski 1984).

Although there have been studies comparing growing regions, there have been few studies on site environment or vine vigor variation within vineyards and the influence on the resulting fruit and wine. In premium wine growing regions, specific sites of fruit often sell for a higher price as they are perceived to be of higher quality and go into top tiered wines. In the present study, vigor zones were delineated in two sites of vines in order to investigate differences in fruit and wine chemical analyses (Cortell et al. 2005). Although the two vineyard site had vines that were the same age, rootstock, clone, and under similar management practices, the fruit was used for wines with distinctly different price points. The objective of this study was to compare sensory perception to biological and chemical analyses of the fruit and wine with an emphasis on astringency. A second objective was to evaluate the ability to predict astringency based on chemical analyses with the use of PLS.

VINEYARD AND CHEMICAL ANALYSES MATERIAL 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, USA. Vines were planted at a spacing of 1 m (within row) X 2.8 m (between rows) with approximately 5113 vines per hectare. The training system was a vertical shoot position with each vine pruned to 10-12 buds. Two vineyard sites (A and B) were selected for study based upon historic evidence for phenolic variation, and were 1.28 and 0.21 hectares, respectively. These sites were under similar management practices. The vineyard received minimal irrigation post véraison (<150 mm). This research was initiated in April 2003 starting with budbreak.

Vine Measurements. Data vines were established on a grid pattern in each site and measurements on average shoot length (June, prior to hedging), estimated leaf chlorophyll content (SPAD-502 meter, Minolta, USA) (one month prior to véraison) and cross sectional trunk area were collected. A vigor index was calculated as previously described (Cortell et al. 2005).

Fruit Sampling and Extraction. Fruit samples were collected across each vigor zone (3 replicates/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 (Kennedy et al. 2000).

Winemaking. Triplicate wines were produced from each vigor zone (**Figure 6.1**). For each replicate, 35 kg fruit was destemmed with a Velo DPC 40 crusher/stemmer operated without the crusher, underwent a 2.5 day pre-fermentation cold maceration (10 °C) and then inoculated with Lalvin RC 212 yeast according to the manufacturer's guidelines. On day two of fermentation, wines were transferred to a water bath maintained at 32°C. Wines were punched down 2 times per day and pressed 6 days after inoculation (bladder-type press, Wilmes, Germany), to a maximum pressure of 2 bars. Wines were transferred into 5-gallon 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, 35 ppm SO_2 was added followed by 4 weeks cold stabilization and then bottled. The same time/temperature profile was maintained during all fermentations in order to reflect vineyard derived differences.

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). Phloroglucinol, (+)-catechin and (-)-epicatechin were purchased from Sigma (St. Louis, MO).

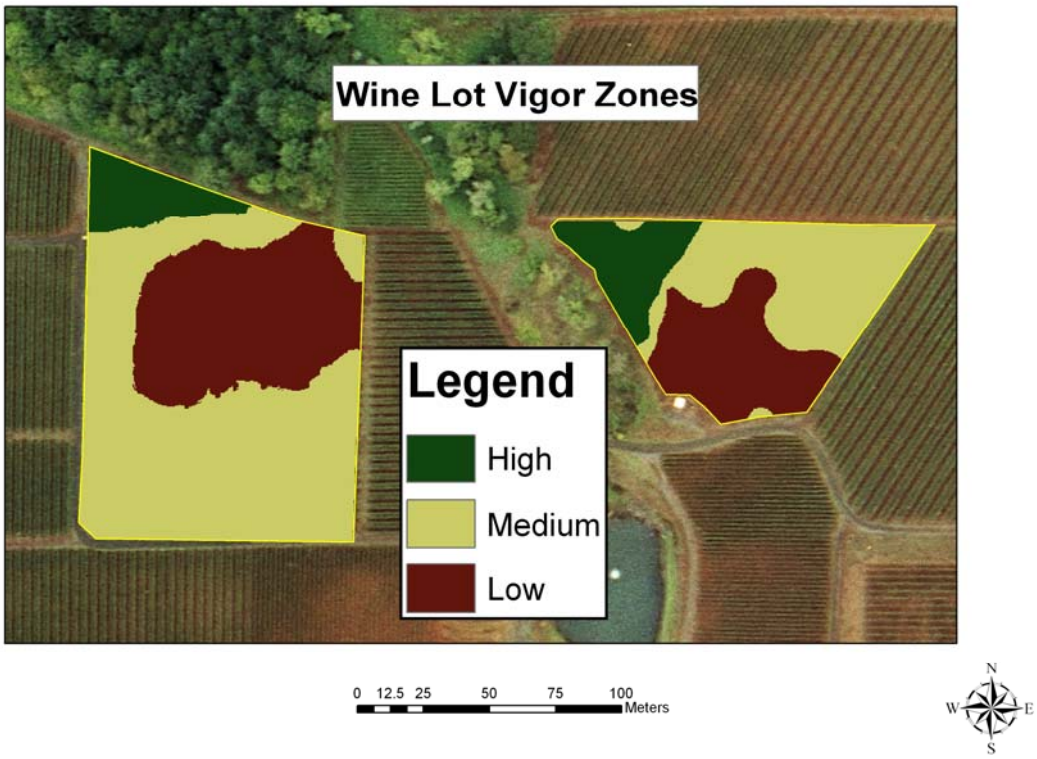


Figure 6.1. High resolution image with delineation of wine production vigor zones.

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, 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 (Lamuela-Raventos &Waterhouse 1994). Aqueous extracts and wines were filtered using Teflon filters (0.45µm, Acrodisc CR13) before injection. Eluting flavan-3-ol monomers were identified and their 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 (Kennedy & Jones 2001) under modified HPLC conditions (Kennedy and Taylor, 2003). Phloroglucinolysis provided information on subunit composition, conversion yield and mean degree of polymerization (mDP). To prepare seed and skin extracts for analysis, 3 mL aqueous extract was freeze dried and then dissolved in 5 mL (seed) or 2 mL (skin) 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 water, and then applied to a C18-SPE column (1 g Alltech) after activation with 10 mL methanol followed by 15 mL water. After sample was applied, the column was washed with 15 mL water and eluted with 10 mL methanol. The methanolic solution was divided into two 5 mL samples. One sample was prepared for phloroglucinolysis and the other for GPC. For phloroglucinolysis, the methanolic sample was evaporated under reduced pressure

and 40 °C, reconstituted into 1 mL 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 (Peyrot des Gachons & Kennedy 2003). The percent skin proanthocyanidin extracted from the fruit into the wine was calculated based on the ratio of EGC/EC in the fruit and wine for each vigor zone/rep combination.

Gel Permeation Chromatography. Gel Permeation Chromatography (GPC) was used to analyze intact Tannin. 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 (Kennedy & Taylor 2003). Samples were prepared as described above; however, after freeze drying they were dissolved in mobile phase. Malvidin-3-glucoside was obtained from Polyphenols Labs (Sandness, Norway) and used as a standard for GPC analysis at 520 nm, while (+)-catechin was used as the quantitative standard at 280 nm.

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. All statistical analyses were performed using (SAS version 8e, SAS Institute, Cary, N.C., 2002).

SENSORY ANALYSIS MATERIALS AND METHODS

Wines. The bottled wines were stored horizontally at room temperature during the time of the experiment. The wines were also served at room temperature and poured about 30 minutes before being evaluated. The 15 samples are listed below:

Table 6.1. List of wines, site, zone, replicate, and codes used in the sensory experiment.

Sample No.	Site	Zone	Rep	Code
1	A	High	1	AhighV-1
2	A	High	2	AhighV-2
3	A	High	3	AhighV-3
4	A	Medium	1	AmediumV-1
5	A	Medium	2	AmediumV-2
6	A	Medium	3	AmediumV-3
7	A	Low	1	AlowV-1
8	A	Low	2	AlowV-2
9	A	Low	3	AlowV-3
10	B	Medium	1	BmediumV-1
11	B	Medium	2	BmediumV-2
12	B	Medium	3	BmediumV-3
13	B	Low	1	BlowV-1
14	B	Low	2	BlowV-2
15	B	Low	3	BlowV-3

Panelists. Nine panelists, all students, mainly from the Department of Viticulture and Enology, participated in the study. The panelists were experienced wine tasters. Most had been participating in sensory descriptive analysis before. Three students were inexperienced in using sensory descriptive analysis.

Training. Six training sessions were run. During the first two sessions of training they tasted all samples included in this study, to get familiar with the wines

and to make a list of attributes that would include the variability among them. This list could be changed until all panelists agreed on a final list of attributes and how to define them. References were made to help them understand how to describe each attribute. The references were used during the training sessions, and during the real taste sessions. The list below (Table 2) includes written definitions and standards.

The list of attributes was used to evaluate each wine quantitatively on an unstructured scale. The panelists were trained to rate the attributes in the samples relative to the rest of the samples of wines in the study.

Taste sessions. The samples were evaluated in individual sensory booths, under red light. 15 samples were served in three replicates. In each of nine sessions, seven or eight samples were evaluated in a completely randomized order. The panelists rated the samples based on the list of attributes on an unstructured scale by marking it with the mouse. Water was used to rinse in between each sample. A 30 second rest in between each sample was included as part of the taste program. All 7 or 8 samples were evaluated continually, without other breaks than the compulsory 30 sec. The data was collected using the Fizz for Windows (version 2.00 E, BIOSYSTEMS).

Table 6.2: Sensory attributes, their definitions and respective standards. All standards were added the base wine: "05 CS Base HGH" – 30 mL VE-wine cellar.

Sensory attribute	Definition	Standard
Fresh fruit aroma	Aroma of all kinds of fresh fruit, dark or light	Raspberries – 2 cut into small pieces Driscolls, USA Blueberries – 3 cut into small pieces Hurst's Berry farm, Mexico Blackberry – 1 cut into small pieces VBM, Chile
Processed fruit aroma	Aroma of stewed, cooked, jammed fruit any kind	1 teaspoon of blackberry, raspberry and strawberry jams each. Smucker's
"Sweet" aroma	Aroma of chocolate, Tamarind, vanilla, cocoa powder	½ teaspoon of cocoa powder, Hershey's cocoa powder for baking 3 drops of Pure Vanilla Extract: Spice islands
"Green" aroma	Aroma of both cooked, and canned vegetables like peas, beans and asparagus. This aroma could also include fresh green smells cut grass, snow peas.	Asparagus, canned, ¼ of an asparagus, cut into small pieces Green beans, canned, ½ green bean, cut into pieces
		One fresh snow pea cut into small pieces
Spicy aroma	Aroma of spices like black pepper, nutmeg and cloves, Could also include vanilla aroma	Nutmeg: 1 drizzle of McCormick ground nutmeg Black pepper, coarse ground, 1 drizzle Clove, 1 piece, McCormick
Earthy aroma	Aroma of Mushroom, soil, musty (dusty, cellar, cardboard), leather, barnyard	Potting soil, 1 big table spoon, Champignon, 3 slices cut into small pieces

Table 6.2 (continued): Sensory attributes, their definitions and respective standards. All standards were added the base wine: "05 CS Base HGH" – 30 mL VE-wine cellar.

Chemical aroma	An off odor of sulphur, plastic or acetone (nail polish)	Five drops of Acetone, five drops of vinegar
Heat	Aroma or feeling of heat caused by alcohol or VA (vinegar)	1mL of denatured 95% ethanol
Sweet taste	Taste of sweet as in sugar (sucrose)	No standard
Sour taste	Taste of sourness or fresh fruit acidity	0.5 g/L citric acid in water
Bitter taste	Taste of bitter like caffeine	0.5 g/L caffeine in water
Astringent	A mouth feel of dryness in the mouth. If you feel with the tongue against the upper palate, if should feel like it meets resistance.	0.5 g/L Alum (Aluminum Sulphate) g/L in water

Sensory data analysis. Data were exported from the FIZZ data collection program into an Excel spread sheet. A mixed-model analysis of variance (using judge*wines interaction as the error term) and lsd-values were calculated for each sample. Canonical Variate Analysis (CVA) was run on significant attributes (SAS version 8e, SAS Institute, Cary, N.C., 2002).

RESULTS

Fruit composition. The vineyard zones were different except for A-low and B-medium were similar (**Table 6.3**). The range of vigor variation was large with A-high being highest at 0.82 and B-low was lowest at 0.09. Berry weight was lower with a reduction in vine vigor while number of seeds per berry was higher. Soluble

solids (°brix) was higher and titratable acidity was lower in the medium and low vigor zones compared to A-high. Site B was lower for pH than Site A.

Fruit chemical analyses. There was a trend for higher flavan-3-ol monomers per seed in A-high compared to A-low and B-low (**Table 6.4**). A-high and A-medium vigor zone seed monomers had a higher proportion of EC to C compared to the zones with lower vigor. Seed proanthocyanidin (mg/seed) was similar among vigor zones; however skin proanthocyanidin (mg/berry) increased as vine vigor decreased.

Wine chemical analyses. Wine pH decreased with decreasing vine vigor (**Table 6.5**). Titratable acidity was lower in a-high and A-medium compared to the other vigor zone wines. The flavan-3-ol monomer concentration was higher in wines from Site A than Site B. The proportion of monomers was similar to the fruit in that wines from Site A had a higher proportion of EC and lower C compared to Site B. Wine total proanthocyanidin, pigmented polymers and percent skin proanthocyanidin increased in the wines as vine vigor decreased. The fruit and wine data were previously reported in more detail in Cortell et al (2005).

Sensory attributes. The intensities of significant sensory attributes for all wines in the study are presented in **Table 6.6**. The mean intensities of significant sensory attributes for the vigor zones wines are presented in **Table 6.7**. Other sensory attributes assessed in the wines included fresh fruit, processed fruit, green and spicy; however, they were not included as they were not found to be significant.

Table 6.3. Vigor zone ratings and vine vigor zone fruit composition.

Site	Zone	Vigor index	Berry Weight (g)	Number of seeds per berry	°Brix	Titratable Acidity (g/L)	pH
A	High	0.82 a	0.99 a	1.31 c	23.5 c	5.7 a	3.5 a
	Medium	0.64 b	0.91 ab	1.37 bc	24.3 a	4.9 b	3.5 a
	Low	0.44 cd	0.87 bc	1.56 a	24.1 b	4.7 c	3.5 a
B	Medium	0.35 d	0.87 bc	1.50 ab	24.0 b	4.9 b	3.3 b
	Low	0.09 e	0.78 c	1.59 a	24.4 a	4.7c	3.3 b
p value		<0.0001	0.0079	0.0040	<0.0001	<0.0001	<.0001

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

Table 6.4. Chemical analyses of the fruit from the vine vigor zones.

Site	Zone	Flavan-3-ol monomers (mg/seed)	Monomer (+) Catechin (%)	Monomer (-)-epicatechin (%)	Seed tannin ((mg/berry)	Skin tannin (mg/berry)
A	High	0.47 a	62.3 b	37.7 a	3.6 a	1.15 c
	Medium	0.42 ab	63.1 b	36.9 a	3.5 a	1.34 bc
	Low	0.38 b	67.2 ab	32.8 ab	3.4 a	1.79 ab
B	Medium	0.43 ab	71.6 a	28.5 b	3.4 a	1.69 ab
	Low	0.37 b	71.5 a	28.5 b	3.2 a	1.94 a
p value		0.1484	0.0040	<0.0001	0.8896	0.0026

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

Table 6.5. Chemical analyses of the wines from the vine vigor zones.

Site	Zone	pH	Titratable acidity (g/L)	Total monomers (mg/L)	Monomer (+) Catechin (%)	Monomer (-)-epicatechin (%)	Total tannin (mg/L)	Pigmented polymers (mg/L)	Skin tannin (percent)
A	High	3.85 a	4.8 bc	53.6 a	77.3 b	22.7 a	1040 e	632 e	53 d
	Medium	3.75 b	5.2 a	50.5 ab	75.7 b	24.3 a	1340 d	844 d	64 c
	Low	3.64 d	5.2 a	46.1 b	77.6 b	22.4 a	1586 c	1090 b	68 bc
B	Medium	3.65 c	4.6 c	36.2 c	86.6 a	13.4 c	1792 b	1223 b	75 ab
	Low	3.64 cd	5.0 ab	35.6 c	88.0 a	12.0 c	2051 a	1459 a	79 a
p value		<0.0001	0.0006	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

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

Table 6.6. Wine intensity of sensory attributes for all wines included in the sensory study.

Wine ID	**Wine Intensities of Sensory Attributes**						
Wine	**Earthy**	**Chemical**	**Heat**	**Sweet**	**Sour**	**Bitter**	**Astringent**
AhighV-1	0.9 e	1.0 ef	2.1 cd	2.5 c	3.1 cde	2.8 def	2.0 g
AhighV-2	1.5 cde	0.9 f	2.1cd	2.6 c	2.8 de	2.3 f	1.8 g
AhighV-3	1.1 bcde	1.2 def	1.9 d	2.2 c	2.8 e	2.4 ef	2.3 fg
AmediumV-1	1.0 de	1.9 abcde	2.8 abc	3.6 ab	3.2 bcde	3.8 abc	3.1 ef
AmediumV-2	1.4 bcde	1.0 ef	2.3 abcd	2.9 bc	3.7 abcde	3.0 cde	3.2 e
AmediumV-3	1.6bcd	2.3 abc	1.9 d	2.9 bc	4.0 ab	3.6 abc	3.4 e
AlowV-1	2.3 a	1.9 abcdef	2.5 abcd	2.4 c	3.8 abc	3.8 abc	4.5 d
AlowV-2	1.3 bcde	2.6 ab	2.6 abcd	2.5 c	3.7 abcde	3.5 abcd	4.8 d
AlowV-3	1.4 bcde	1.6 cdef	2.6 abcd	2.8 bc	3.2 bcde	4.2 a	4.9 cd
BmediumV-1	1.7 bcde	2.1 abcd	3.0 a	3.1 bc	3.7 abc	3.8 ab	5.3 bcd
BmediumV-2	1.6 bcde	1.9 abcdef	2.5 abcd	2.9 bc	3.4 abcde	3.5 abcd	5.1 cd
BmediumV-3	1.3 abc	1.7 abcdef	2.2 bcd	2.2 c	3.5 abcde	3.6 abc	5.2 bcd
BlowV-1	1.7 bcde	2.6 a	2.8 abc	3.8 ab	4.2 a	3.4 bcd	5.7 abc
BlowV-2	1.8 ab	1.7 bcdef	1.9 d	2.8 bc	3.8 abc	3.6 abc	6.0 ab
BlowV-3	1.7 abcd	2.5 abc	3.0 ab	4.3 a	3.7 abcd	3.9 ab	6.2 a
p value	0.0215	0.0039	0.0030	0.0007	0.0525	0.0006	<0.0001

Table 6.7. Wine intensity of sensory attributes for the means of wines from each vigor zone included in the sensory study.

	Mean Intensities of Sensory Attributes						
Wine	**Earthy**	**Chemical**	**Heat**	**Sweet**	**Sour**	**Bitter**	**Astringent**
A-high	1.2 c	1.0 b	2.1 a	2.4 c	2.9 c	2.5 b	2.0 d
A-medium	1.4 bc	1.7 ab	2.3 a	3.1 ab	3.6 ab	3.5 a	3.2 c
A-low	1.7 a	2.0 a	2.5 a	2.6 bc	3.6 b	3.8 a	4.7 b
B-medium	1.5 ab	1.9 ab	2.6 a	2.7 bc	3.6 b	3.6 a	5.2 b
B-low	1.7 a	2.3 a	2.6 a	3.6 a	3.9 a	3.6 a	6.0 a
SEM	0.1	0.3	0.2	0.2	0.2	0.1	0.2
p-value (type III)	0.0186	0.0399	0.0603	0.0019	0.0019	0.0019	<0.0001

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

Table 6.8. Pearson correlation coefficients and p-values for fruit and wine chemical analyses and sensory attributes.

	Skin tannin (mg/berry)	Total fruit tannin (mg/kg)	Skin tannin (percent)	Pigmented polymers (mg/L)	Wine tannin (mg/L)	Total wine monomers (mg/L)
Earthy	0.68396	0.73704	0.70503	0.78481	0.77106	-0.57392
	0.0049	0.0017	0.0033	0.0005	0.0008	0.0253
Chemical	0.59928	0.72475	0.56895	0.71157	0.72273	-0.58454
	0.0182	0.0022	0.0269	0.0029	0.0023	0.0221
Heat - alcohol	0.47425	0.63522	0.49619	0.58262	0.58102	-0.56864
	0.0741	0.0109	0.0599	0.0227	0.0231	0.027
Sweet	0.32984	0.33332	0.46722	0.56694	0.59235	-0.43643
	0.2299	0.2247	0.0791	0.0275	0.02	0.1039
Sour	0.73224	0.70394	0.66916	0.7777	0.78009	-0.62979
	0.0019	0.0034	0.0064	0.0006	0.0006	0.0119
Bitter	0.68966	0.70911	0.79353	0.7088	0.70923	-0.58128
	0.0044	0.0031	0.0004	0.0031	0.0031	0.023
Astringent	0.89383	0.89583	0.9009	0.94568	0.93283	-0.87543
	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Table 6.8 shows Pearson correlation coefficients for fruit, wine and sensory attributes. The astringent sensory attribute had a strong positive correlation with all of the included fruit and wine variables except it was negatively correlated with total wine monomers. Total wine monomers were negatively correlated with all of the sensory attributes including bitterness. Both pigmented polymers and wine tannin were highly correlated with astringency.

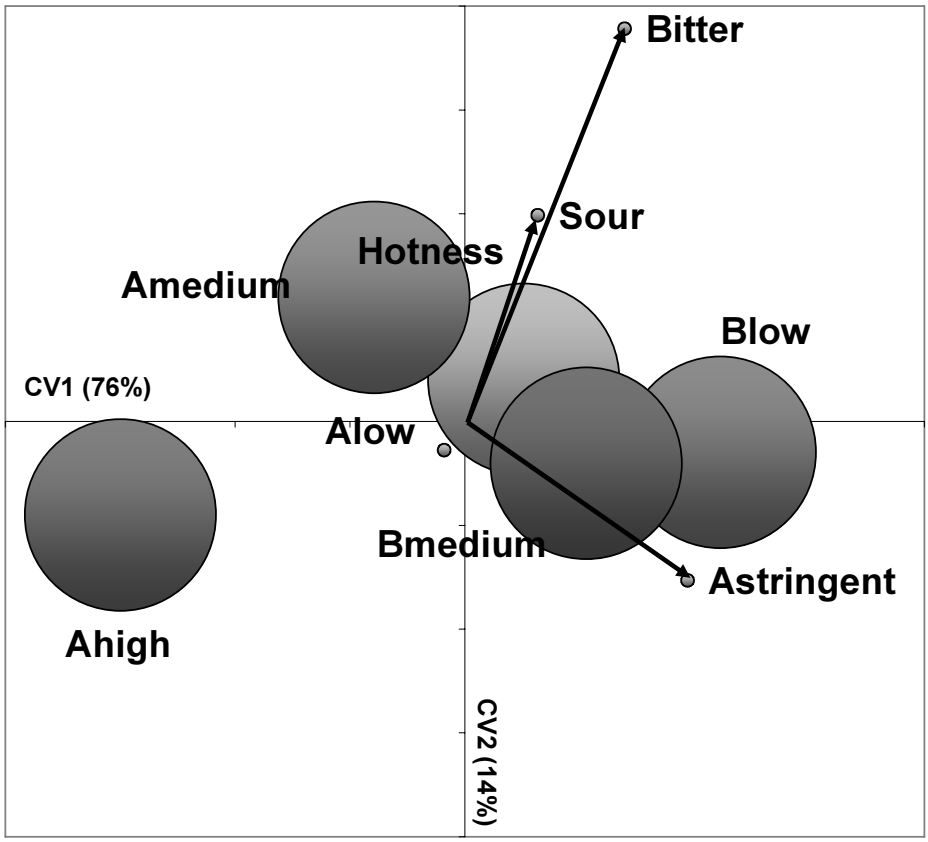


Figure 6.2. Canonical variate plot of the wines. Circles indicate 95% confidence intervals.

The canonical variate analysis showed significant differences between the wines as seen in **Figure 6.2**. The first two variates were significant ($P > 0.05$) accounting for 76% and 14% of the variance ratio, respectively. The A-high vigor zone wines were very different from the other wines primarily in having much lower astringency. The other wines showed increasing astringency with a reduction in vine vigor. The A-low and B-medium wines were similar in astringency although A-low tended to be slightly more sour and bitter although not significant.

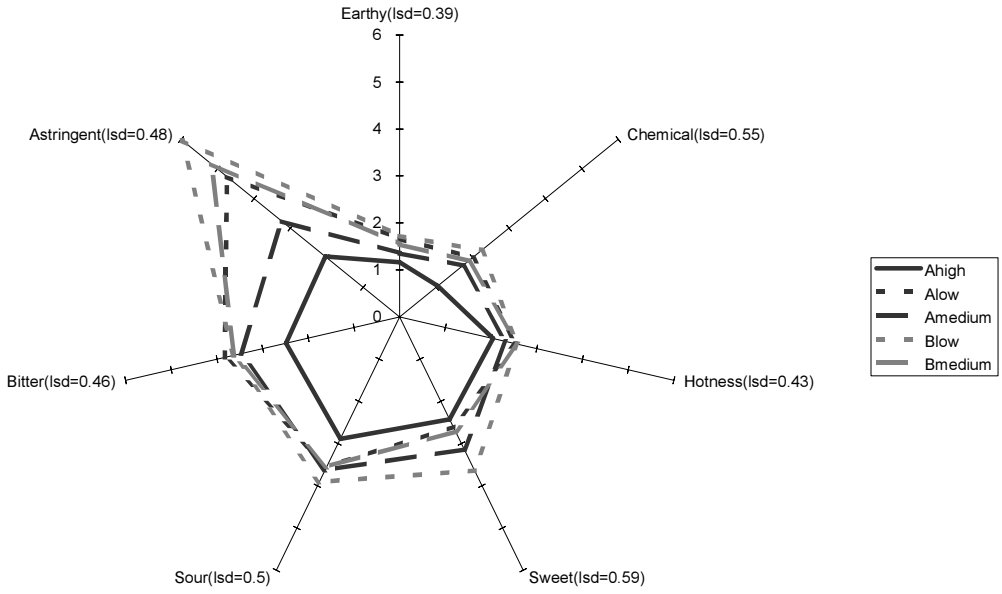


Figure 6.3. Cobweb showing variation in attribute intensity for all significant attributes for the means of vine vigor zone wines.

The B-medium and B-low wine were not perceived to be different in astringency due to the overlapping 95% confidence intervals. **Figure 6.3** shows the mean attribute intensities for all significant sensory terms for the vine vigor zone wines. The greatest difference between wines was found in astringency followed by bitterness and sourness. Below the sensory attributes are discussed in more detail.

Sensory attributes. The wines from the low vigor zones (A-low and B-low) had a higher intensity of earthy and chemical attributes than in wines from zones with higher vigor. There was a trend for higher heat or ethanol intensity in medium and low vigor zones wines compared to the A-high wine. This is in agreement with

the initial °Brix levels found in the fruit (**Table 6.3**) where A-high was lower than the other zones. The detection of sweetness was higher in the A-medium and B-low wines compared to the other wines. There was a higher intensity of sour taste in wines from B-low compared to A-high vigor zones. This did not appear to be highly related to titratable acidity in the wines or in the fruit (**Table 6.3 & 6.5**) and may have been due to differences in pH instead. The intensity of bitterness was perceived to be higher in wines from the low and medium vigor zones compared to A-high. The intensity of astringency increased in wines going from high vigor to low vigor zones. A-low and B-medium were similar in intensity.

PLS predictions of chemical and sensory attributes. The importance of the sensory and fruit variables can be seen in the correlations loadings plot (**Figure 6.4**). Variables in the outer ring were important in contributing to the predictive model. On the right side of the axis, significant positively correlated variables included number of seeds per berry, fruit total PA (mg/kg), and skin PA (mg/berry). On the left side, total monomers per seed, total monomers per berry, fruit titratable acidity and berry weight had significant negative correlations with the sensory attributes. Interestingly, seed tannin was not an important variable in the model. Dimension 1 explained 95% of the differences in fruit terms and 66% of the sensory attributes. Dimension 2 explained 5% of the fruit variables and 2 % of the sensory attributes.

As astringency was the most important wine sensory attribute, the correlation loadings plot in **Figure 6.5** shows the correlation of the fruit variables with the sensory attribute astringency. The number of seeds per berry, fruit total tannin and skin tannin were again positively correlated with astringency while berry weight, fruit titratable acidity, total monomers per seed and per berry were negatively correlated with astringency. Fruit total tannin was strongly correlated with the sensory attribute astringency in the wines as seen in **Figure 6.6**.

In **Figure 6.7**, PLS was used to predict astringency in wine based on fruit variables found to be important in measuring astringency. The fruit variables included in the PLS regression analysis were the number of seeds per berry, total berry monomers, skin tannin, berry weight, seed tannin and total tannin. In this set of wines, there was a strong correlation (0.894) between measured astringency and predicted astringency. Astringency was the most important attribute found to explain differences in wines made from vigor zones within the two vineyard sites.

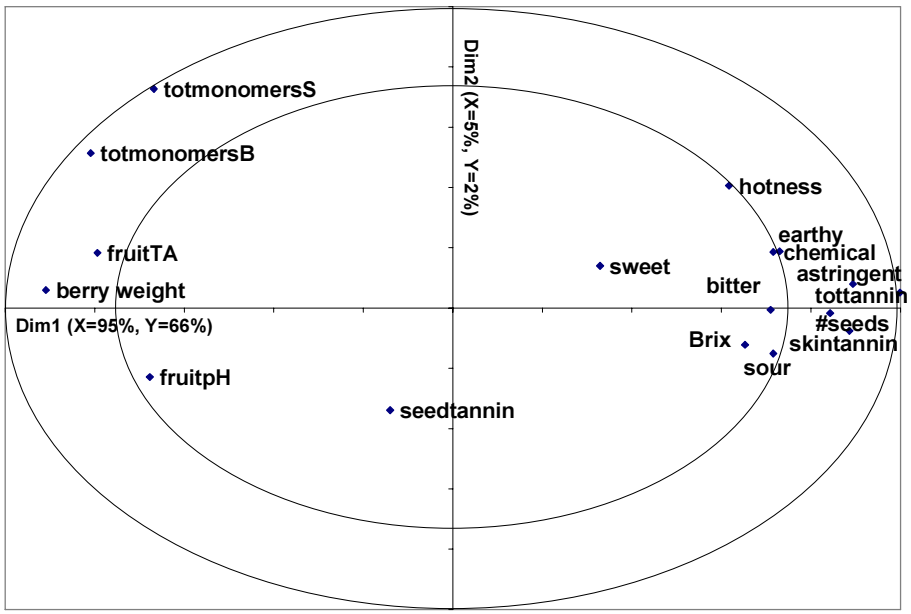


Figure 6.4. Correlation loadings of fruit characteristics for wine sensory attributes.

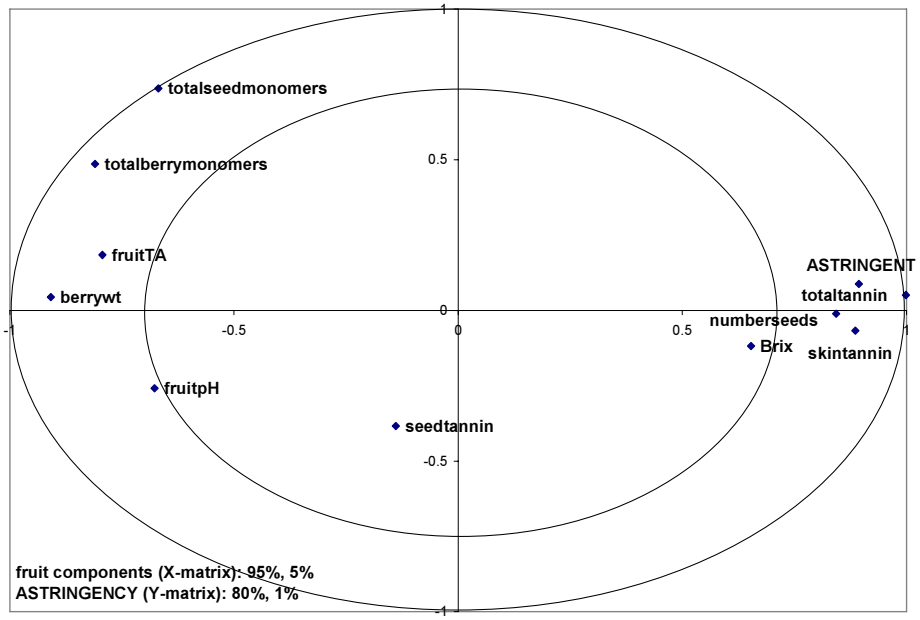


Figure 6.5. Correlation loadings of fruit characteristics for wine stringency.

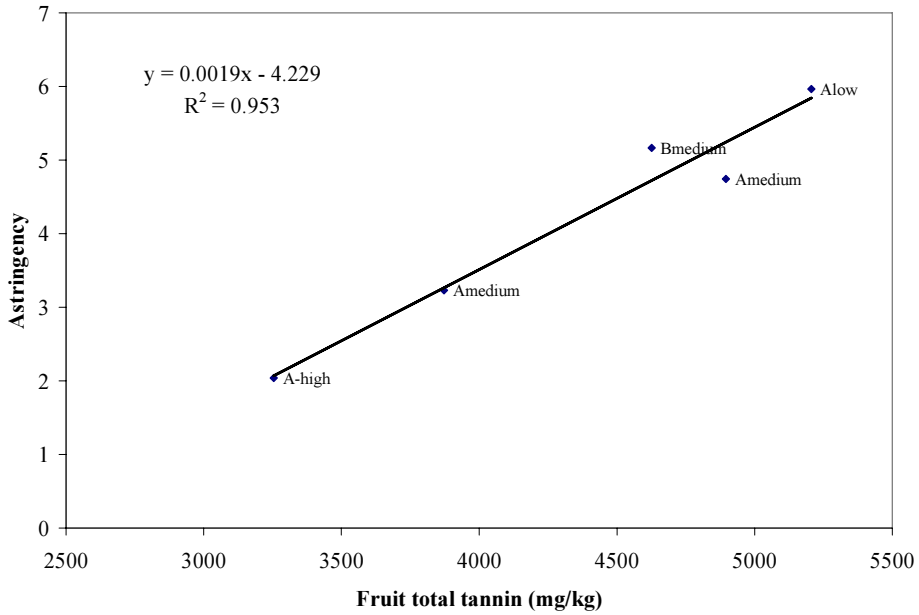


Figure 6.6. Relationship between fruit total tannin (mg/kg) and astringency in vigor zone wines.

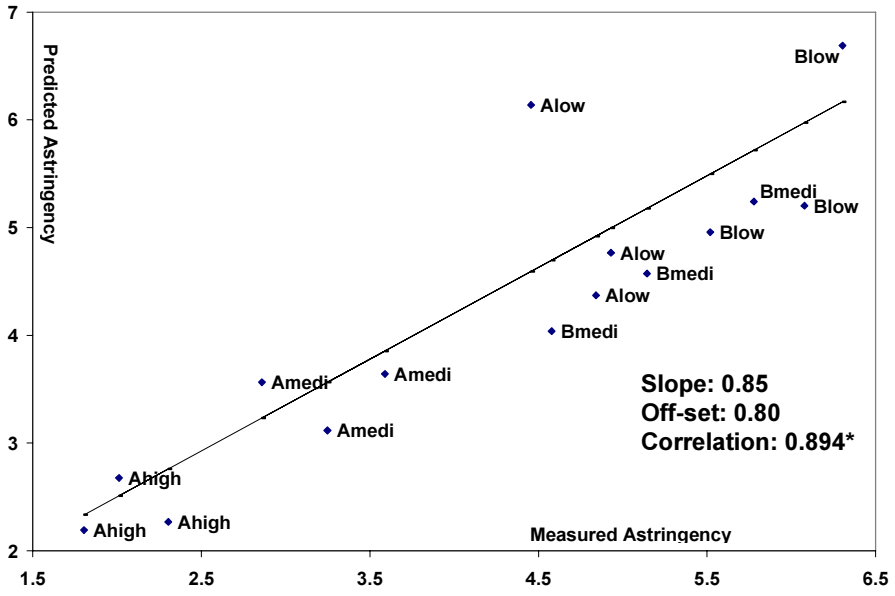


Figure 6.7. PLS prediction of wine astringency based on fruit parameters including number of seeds, berry monomers, skin tannin, berry weight, seed tannin and total tannin.

DISCUSSION

The higher intensity of sensory attributes of earthy and chemical in the low vigor zone wines (**Table 6.7**) are likely in response to differences in sulfur containing compounds found in the high versus the low vigor zone wines (data not included).These attributes probably developed from stress during fermentation due to low nitrogen and nutrient levels in the low vigor wines. The differences in sweet taste may have been from reducing sugars or glycerol (Ough & Amerine 1988). Glycerol production has been reported to be higher in stressed fermentations. Both sugars and polysaccharides that increase sweetness or viscosity have been reported to reduce astringency (Ishikawa & Noble 1995; Smith et al. 1996).

Sourness in wine is primarily from tartaric acid with some contribution from malic and lactic acids (Ough & Amerine 1988). Sour intensity (**Table 6.7**) was lowest in the A-high wine which had the lowest titratable acidity and the highest pH (**Table 6.5**). The B-low vigor zone wines had the highest intensity of sour taste with an intermediate titratable acidity and the lowest pH. There was not similar pattern in the wines as in the fruit (**Table 6.4**) because the high vigor zone wines completed malolactic fermentation without difficulty while some of the low vigor zones did not complete malolactic fermentation. A sour taste in wine can be increased by either lowering the pH or by adding acid (Fischer & Noble 1994; Kallithraka et al. 1997).

Bitter taste is elicited by many structurally diverse compounds and the mechanisms for the perception of bitterness are poorly understood (Thorngate 1997). In the present study, the high vigor wine (A-high) was found to be lower in bitterness

than the medium or low vigor wines. Flavan-3ol monomers have been reported to be bitter in a number of studies (Lea and Arnold 1978; Arnold et al. 1980; Noble 1994). In addition, (-)-epicatechin was found to be significantly more bitter and to have a longer duration of bitterness compared to (+)-catechin (Noble 1994; Thorngate & Noble 1995; Kallithraka et al. 1997). The A-high wine was higher in total monomers and also had a higher proportion of (-)-epicatechin to (+)-catechin compared to the medium and low vigor wines (Table 5) but was perceived to have lower bitterness. The higher bitterness in the medium and low vigor zones may have been due to interactions in the wine or the substantially higher astringency in these wines may have masked or contributed to the perception of bitterness. While large polymeric tannins are thought to be primarily astringent they also contribute bitterness to wine (Robichaud & Noble 1990). The higher ethanol concentrations in the medium and low vigor zone wines could enhance the perception of bitterness. Enhancement of bitterness with an increase in ethanol levels has been reported in wine (Fischer et al. 1994). Higher acidity was also found to increase the astringency of grape phenolic compounds (Peleg et al. 1998).

Astringency in wine is primarily from large molecular weight tannins (Robichaud & Noble 1990; Noble 1994). Astringency increases with increasing tannin polymerization (Arnold et al. 1980); however, variations in tannin composition, the extent of galloylation, and formation of derivatives can affect both bitterness and astringency (Lesschaeve & Noble 2005).

In wines, tannin comes from both seed and skin material. Skin tannin has greater polymerization than seed tannin (Labarbe et al. 1999). In the present study, total tannin was about twice as high in B-low compared to the A-high vigor zone wines; however, the increase was from higher skin tannin rather than seed tannin (Cortell et al. 2005). There was a corresponding increase in tannin molecular size in the low vigor wines associated with the increase in skin tannin. In addition, skin tannin contain (-)-epigallocatechin which is a trihydroxylated flavanol subunit. The higher number of hydroxyl groups could modify the perception of astringency. Consequently, the concentration, composition and mean degree of polymerization of tannins are important in the intensity of astringency.

In the present study, fruit tannin (skin + seed) and skin tannin were strongly correlated with astringency in the wine (**Table 6.8; Figure 6.6**). Skin tannin in the fruit was more important in differentiating the wines as there was higher skin tannin in the low vigor fruit compared to the high vigor fruit while seed tannin was similar across vigor zones (**Table 6.4**). The wines from low vigor zones also had a much higher concentration of total tannin and a higher percent of skin tannin than the high vigor wines (**Table 6.5**). The B-low wine was ~ 20% and the A-high wine was ~ 50% seed tannin. It could be expected that astringency would be higher in the low vigor wines because of the higher concentration of tannin but also because of the higher percent skin tannin which have higher molecular weight than seed tannin.

Although skin tannin is thought to have a preferred mouth feel in wines (Cheynier et al. 1998), few studies have characterized the differences in skin and seed tannin astringency perception.

The other major difference between high and low vigor zone wines was that the low vigor zones wines had much greater formation of pigmented polymers (**Table 6.5**). In the present study, the pigmented polymer and tannin concentration were both found to be highly correlated with astringency (**Table 6.8**). Few sensory studies have been done specifically on the contribution of pigmented polymers to wine astringency. Although pigmented polymers can include a wide diversity of derived compounds, they are thought to be primarily tannin -anthocyanin adducts. The formation of pigmented polymers was thought to reduce astringency of wine (Somers 1971); however, the taste of reaction products and the effect on astringency of incorporating anthocyanin units into a tannin structure remain to be investigated (Cheynier 2005). A recent paper on micro-oxygenation found higher ethyl bridged anthocyanin-flavanol pigments and combined anthocyanins, found no effect on the total proanthocyanidin concentration, a slightly higher mean degree of polymerization and a drastically lower astringency (del Carmen Llaudy et al. 2006). However, they did not investigate specifically the concentration of tannin-anthocyanin adducts or the effect on astringency as these adducts may differ from ethyl-linked pigments. Another study reported a decrease in astringency and an increase in bitterness by modifying tannin structure with an ethyl bridge (Vidal et al. 2003).

In this study, it was possible to predict astringency based on measured fruit chemical analyses (**Figure 6.7**). The fruit variables included the number of seeds per berry, berry weight, berry monomers, skin tannin, seed tannin and total tannin. The importance of skin and seed tannins in astringency perception have already been discussed. Berry monomers can contribute to astringency although they are considered to be primarily bitter (Thorngate & Noble; 1995).

Extraction of tannin from seeds is generally quite low although seeds contain a large amount of flavan-3-ols and tannin. The number of seeds per berry is more important in extraction than the amount of tannin per seed due to the increased surface area for extraction (Harbertson et al. 2002). In this study, the number of seeds per berry was positively correlated with astringency because fruit from low vigor zones had a higher number of seeds per berry than in the high vigor zone (**Table 6.3**). Since berry weight decreased with decreasing vine vigor (**Table 6.3**), berry weight was negative correlated with astringency. An increase in berry weight has been previously reported in shaded fruit (Reynold et al 1986; Crippen & Morrison 1986) and higher shade would be expected in high vigor vines also. The ratio of seed, skin and pulp in berries is thought to influence the extraction and concentration of phenolic compounds in wine (Coombe et al. 1987; Matthews & Anderson 1988). However, a recent study showed that berry size alone did not have a major impact on the concentration of phenolic compounds (Walker et al. 2005).

It is difficult to understand relationships between fruit composition, fruit and wine chemical analyses and sensory perception due to the complexity of wine,

number of reactions occurring in wine, interactions among compounds in wine and variability in human perception. In this experiment, differences in fruit and wine chemical analyses were found that played an important role in sensory perception particularly for astringency in wines from high, medium and low vigor zones. In summary, the low vigor zone wines were differentiated primarily by differences in astringency in addition to the attributes of earthy, chemical, heat, sweet, sour, and bitter. The fruit and wine chemical sensory attributes that were significantly correlated with the sensory attributes included skin tannin, total fruit tannin, percent wine skin tannin, total wine tannin, pigmented polymers and wine monomers. The measurable differences in the fruit and wine, for example, in skin tannin and pigmented polymers may have been contributing factors for the fruit being targeted for different wine price levels. Improving our understanding of the vine-fruit-wine continuum is important in being able to make decisions in the vineyard and winemaking techniques to achieve a desired wine style. I

ABBREVIATIONS USED

DMF, N,N-dimethylforamide; GPC, gel permeation chromatography, CI, 95% confidence interval; mDP, mean degree of polymerization;

ACKNOWLEDGMENTS

We thank Archery Summit Winery for the use of their vineyards and fruit for wine production as well as Leigh Bartholomew and Anna Matzinger specifically for their support. We would like to thank students: Jason Bell, Fiorella Cerpa, Jose Luis

Pastor del Rio and Chris Zielinski for their help. In addition, we thank the staff and students at Davis who participated in the sensory study. Funding for this project was provided by the USDA Northwest Center for Small Fruit Research and the American Society for Enology and Viticulture.

LITERATURE CITED

Arnold, R. A.; Noble, A. C.; Singleton, V. L. Bitterness and astringency of phenolic fractions in wine. *J. Agric. Food Chem.* **1980,** 28, 675-678.

Boselli, E.; Boulton, R. B.; Thorngate, J. H.; Frega, N. G. chemical and sensory characterization of DOC red wines from Marxhe (Italy) related to vintage and grape cultivars. *J. Agric. Food Chem.* **2004,** 52, 3843-3854.

Cheynier, V.; Fulcrand, H.; Brossaud, F.; Asselin, C.; Moutounet, M. Phenolic composition as related to red wine flavor. In *Chemistry of Wine Flavor*. A.L. Waterhouse and S.E. Ebeler Eds. Oxford University Publishing, New York, NY. **1998,** pp. 125-141.

Cheynier, V. Polyphenols in food are more complex than often thought. *Am. J. Clin. Nutr.* **2005,** 81, (suppl), 223S-229S.

Coombe, B. G.; Bovio, M.; Schneider, A. Solute accumulation by grape pericarp cells V. Relationship to berry size and the effect of defoliation. *J. Exp. Biol.* **1987,** 38, 1789-1798.

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

Crippen, D. D.; Morrison, J. C. The effects of sun exposure on the compositional development of Cabernet Sauvignon berries. *Am. J. Enol. Vitic.* **1986,** 38, 235-242.

Del Carmen-Llaudy, M.; Canals, R.; González-Manzano, S.; Canals, J. M.; Santos-Bulega, C.; Zamora, F. Influence of micro-oxygenation treatment before oak aging on phenolic compounds composition, astringency, and color of red wine. *J. Agric. Food Chem.* **2006,** 54, 4246-4252.

Dixon, R. A.; xie, D. Y.; Sharma, S. B. PAs – a final frontier in flavonoid research? *New Phytologist.* **2005**, 165, 9-28.

Frank, I. E.; Kowalski, B. R. Prediction of wine quality and geographical origin from chemical measurements by partial least square regression modeling. *Anal. Chim. Acta* **1984,** 162, 241-251.

Fischer, U.; Boulton, R. B.; Noble, A. C. Physiological factors contributing to the variability of sensory assessments: relationship between salivary flow rate and temporal perception of gustatory stimuli. *Food Qual. Pref.* **1994,** 5, 55-64.

Fischer, U.; Noble, A. C. The effect of ethanol, catechin concentration and pH on sourness and bitterness of wine. *Am. J. Enol. Vitic.* **1994,** 45, 6-10.

Harbertson, J. F.; Kennedy, J. A.; Adams, D. O. Tannin in skins and seeds of Cabernet Sauvignon, Syrah, and Pinot noir berries during ripening. *Am. J. Enol. Vitic.* **2002,** 53, 54-59.

Heymann, H.; Noble, A. C. Descriptive analysis of commercial Cabernet Sauvignon wines from California. *Am. J. Enol. Vitic.* **1987,** 38, 41-44.

Ishikawa, T.; Noble, A. C. Temporal perception of astringency and sweetness in red wine. *Food Qual. Pref.* **1995,** 6, 27-33.

Kallithraka, S.; Bakker, J.; Clifford, M. N. Evaluation of bitterness and astringency of (+)-catechin and (-)-epicatechin in red wine and in model solution. *J. Sensory Stud.* **1997,** 12, 25-37.

Kallithraka, S.; Arvanitoyannis, I. S.; Kefalas, P.; El-Zajouli, A.; Soufleros, E.; Psarra, E.; Instrumental and sensory analysis of Greek wines: implementation of principle component analysis (PCA) for classification according to geographical origin. *Food Chem.* **2001,** 73, 501-514.

Kennedy, J.A.; Matthews, M.A.; Waterhouse, A.L. Changes in grape seed polyphenols during fruit ripening. *Phytochemistry.* **2000.** 55, 77-85.

Kennedy, J.A.; Jones, G.P. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J. Agric. Food Chem.* **2001,** 49, 1740-1746.

Kennedy, J.A.; Taylor, A.W. Analysis of proanthocyanidins by high-performance gel permeation chromatography. *J. Chromatogr. A.* **2003,** 995, 99-107.

Labarbe, B.; Cheynier, V.; Brossaud, F.; Souquet, J. M.; Moutounet, M. Quantitative fractionation of grape proanthocyanidins according to their degree of polymerization. *J. Agric. Food Chem.* **1999,** 47, 2719-2723.

Lamuela-Raventos, R.M.; Waterhouse, A.L. A direct HPLC separation of wine phenolics. *Am. J. Enol. Vitic.* **1994,** 45, 1-5.

Lee, C. B.; Lawless, H. T. Time-course of astringent sensations. *Chem Senses* **1991,** 16, 225-238.

Lesschaeve, I.; Noble, A. C.; Polyphenols: factors influencing their sensory properties and their effects on food and beverage preferences. *A. J. Clin. Nutr.* **2005,** 81 (suppl):330s-335s.

Matthews, M. A.; Anderson, M. M. Fruit ripening in *Vitis vinifera* L.: Response to seasonal water deficits. *Am. J. Enol. Vitic.* **1988,** 39, 313-320.

Meyer, J.; Hernandez, R. Seed tannin extraction in Cabernet Sauvignon. *Am. J. Enol. Vitic.* **1970,** 21, 184-188.

Noble, A. C. Bitterness in wine. *Physiol. Behav.* **1994,** 6, 1251-1255.

Ough, C. S.; Amerine, M. A. Methods for Analysis of Musts and Wines (2nd ed.) **1988,** John wile and sons, 377 pp.

Pastor del Rio, J.; Kennedy, J. A. Development of proanthocyanidins in *Vitis vinifera* L. cv. Pinot noir grapes and extraction into wine. *Am. J. Enol. Vitic.* **2006,** 57, 125-131.

Peleg, H.; Bodine, K. K.; Noble, A. C. The influence of acid on astringency of alum and phenolic compounds. *Chem. Senses* 1998, 23, 371-378.

Peleg, H.; Gacon, K.; Schlich, P.; Noble, A. C. Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. *J. Sci. Food Agric*. **1999,** 79, 1123-1128.

Peyrot des Gachons, C.; Kennedy, J.A. Direct Method for Determining seed and skin proanthocyanidin extraction in red wine. *J. Agric. Food Chem.* **2003,** 51, 5877-5881.

Rapp, A. Natural flavours of wine: correlation between instrumental analysis and sensory perception. *Fres. J. analyt. Chem..* **1990,** 337, 777-785.

Reynolds, A. G.; Pool, R. M.; Mattick, L. R. Influence of cluster exposure on fruit composition and wine quality of Seyval blanc grapes. *Vitis* **1986,** 25, 85-95.

Reynolds, A. G.; Yerle, S.; Watson, B.; Price, S. F.; Wardle, D. A. Fruit environment and crop level effects on Pinot noir. III. Composition and descriptive analysis of Oregon and British Columbia wines. *Am. J. Enol. Vitic.* **1996,** 47, 329-339.

Robichaud, J. L.; Noble, A. C. Astringency and bitterness of selected phenolics in wine. *J. Sci. Food Agric.* **1990,** 53, 343-353.

Santos-Buelga, C.; Scalbert, A. Review: Proanthocyanidins and tannin-like compounds – nature, occurrence, dietary intake and effects on nutritional health. *J. Sci. Food Agric.* **2000,** 80, 1094-1117.

Smith, A. K.; June, H.; Noble, A. C. effects of viscosity on the bitterness and astringency of grape seed tannin. *Food Qual. Pref.* **1996, *7, 161-166.***

Somers, T. C. The polymeric nature of wine pigments. *Phytochemistry* **1971,** 10, 2175-2186.

Thorngate, J. H.; Noble, A. C. Sensory evaluation of bitterness and astringency of 3*R*(-)-epicatechin and 3*S*(+)-catechin. *J. Sci. Food Agric.* **1995,** 67, 531-535.

Thorngate, J. H. The physiology of human sensory response to wine: A review. *Am. J. Enol. Vitic.* **1997,** 48, 271-279.

Valentová, H.; Skrováková, S.; Panovská, Z.; Pokorny, J. Time intensity studies of astringent taste. *Food Chem.* **2002,** 78, 29-37.

Vidal, S.; Cheynier, V.; Waters, E.; Noble, A. c. effect of tannin composition and wine carbohydrates on astringency and bitterness. In: Meiselman H., Ed. 5th Pangborn Sensory Science Symposium. Boston, MA., Elsevier, **2003.**

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

Conclusions

We knew the fruit from different parts of the sites were going into different priced wines, it was not known what to expect in terms of compositional differences or the extent of variation in the accumulation of phenolic compounds. At one point in this research before the wines were analyzed, the question was would there be any detectable differences in the wines from different vigor zones? In fact the differences in accumulation of flavonoid compounds in the fruit and its effect on wine flavonoid composition were substantial and much greater than found in a number of studies on vineyard management practices or deficit irrigation.

In the fruit, the most important finding was the large increase in accumulation of skin proanthocyanidin in low vigor compared to high vigor zones and limited influence on seed proanthocyanidin. We had anticipated investigating seed proanthocyanidins in more depth as much importance had been placed on their influence in wine particularly in Pinot noir. However, the focus quickly turned to skin proanthocyanidin as there was ~ 70% more skin proanthocyanidin in the low vigor zone (B-low) compared to A-high. The low vigor fruit also had a greater proportion of skin proanthocyanidin EGC extension subunits arising from the 3' 5' H branch of the flavonoid biosynthetic pathway compared to high vigor fruit.

In the wine, there was a much higher concentration of proanthocyanidin in the low vigor compared to high vigor wines and it was due to the difference in skin not seed proanthocyanidin. There was also a higher percent extraction of skin proanthocyanidin in the low vigor compared to the high vigor wines.

The percent composition of seed proanthocyanidin was similar in all wines. Several studies reported that wine proanthocyanidin is ~ 50% seed derived by the end of fermentation. However, in this study, the A-high vigor zone was ~ 50% while B-low was only ~ 20% seed proanthocyanidin. This confirmed previous reports that skin proanthocyanidin plays an important role in total proanthocyanidins in wine. Skin proanthocyanidin has also been reported to have preferred sensory characteristics (Cheynier 1998).

Proanthocyanidins are important in red wine in providing astringency. Astringency is a tactile sensation that can be described sensorially as mouth drying and puckering. While monomeric flavan-3-ols are primarily bitter, as molecular weight increases with polymerization; astringency becomes predominate over bitterness (Noble 1994; Peleg et al. 1999). Skin differs from seed tannin in having a higher mean degree of polymerization (mDP), the trihydroxylated flavan-3-ol (-)-epigallocatechin (EGC) and lower galloylation. The mDP for Pinot noir seeds was reported to be in the range of 6-9 while skins were found to be from 27-42 flavan-3-ol units (Pastor del Rio & Kennedy 2006). Studies have shown there are also differences in sensory properties related to the identity of the monomeric unit, the specific linkages, extent of galloylation, and formation of derivatives (Peleg et al. 1999; Vidal et al. 2003; Lesschaeve & Noble 2005). Although it had been suggested that skin tannin played an important role in wine tannin (Meyer & Hernandez 1970), many questions remain in how skin tannin modifies astringency.

In this study, the sensory descriptive analysis found astringency to be the most important sensory attribute in differentiating the vigor zone wines. Astringency was found to be highly correlated with skin and wine proanthocyanidin and total proanthocyanidin. In wine, astringency was also strongly correlated with pigmented polymers which are proanthocyanidin –anthocyanin adducts formed during fermentation and aging. The pigmented polymers were twice as high in the low vigor zone compared to the high vigor zone wines. This brings up an interesting question about the role pigmented polymers play in wine astringency. At this time, there have not been any sensory studies looking specifically at the influence of pigmented polymers in wine astringency perception.

Pigmented polymers are also the major contributors to wine color accounting for 50-70% of total color in a one year old wine ( ). In this study, there were minimal differences in native anthocyanins in the fruit; however, pigmented polymers were much higher in low vigor zones than in high vigor zones wines. As pigmented polymers consist of primarily proanthocyanidins with an anthocyanin bound in the terminal position, the proanthocyanidin concentration is important in wine. The proanthocyanidin concentration was found to be positively correlated with pigmented polymers and pigmented polymers were positively correlated with wine color density. The concentration of native anthocyanin in the fruit and wine were not found to be as strongly associated with pigmented polymer formation.

This showed that although anthocyanin concentration in the fruit is important, other factors driving pigmented polymer formation should be investigated. Simply measuring anthocyanins in fruit is not enough to predict wine color.

The differences observed in skin proanthocyanidin and pigmented polymers in the first year led to a fruit shading experiment in the second season. It was expected that the two major factors responsible for differences in flavonoid accumulation and composition were vine water status and sunlight exposure in the canopy. Variations in sunlight exposure were also expected to influence canopy temperatures. Light exclusion boxes were placed in the low vigor zone of Site A with the idea that a shady canopy could be created that would be similar to the shade found in the high vigor vines except for the available water and nutrients would still be the same as the low vigor zone.

Many of the response patterns in this shading 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. However, skin proanthocyanidin concentration and percent skin EGC were lower in the high vigor zone (characterized by a dense, shady canopy) 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 (+)-catechin relative to (-)-epicatechin. The pattern of lower anthocyanins and a reduction in the percent

delphinidin glucosides in the shaded treatment in the present study is similar to what was observed in high vigor vines. This suggests the responses in flavonoid accumulation found in this study were primarily due to changes in light exposure with limited influence from nutrient or water status.

Although proanthocyanidin concentration and astringency are only one aspect of wine quality, astringency appeared to me one of the major differences in the wines from the vine vigor zones. The sensory study results suggest that flavonoid compounds are vital to overall wine quality in providing the backbone for both astringency/mouth feel and color. Flavor and aroma analysis that has yet to be completed may detect other differences important to sensory perception.

Investigating vineyard spatial variation was beneficial in developing our understanding of the link between the site environment, vine growth, fruit composition/chemical analyses and wine chemistry.

In this study, it was possible to determine chemical compositional differences in proanthocyanidins from both the fruit and wine from two sites that were considered by the winemaker to produce wine of differing quality. These chemical differences were confirmed with the sensory analysis. This study provides evidence for the importance of variations in the site environment on fruit phenolic analysis and wine chemistry. Further studies on how viticulture practices influence skin proanthocyanidin accumulation, extraction differences in fruit, factors influencing the formation of pigmented polymers, and the sensory impact of skin proanthocyanidins and pigmented polymers on wine astringency are needed.

In addition, further research is necessary to develop practical applications in vineyards. Possible applications of vineyard spatial variation data would be to reduce vineyard variability through adjusting management practices, harvest vigor zones separately or at different times, target fruit for specific wines or in determining wine blends. Further investigation is needed to: 1) reduce the time needed to divide sites by vine vigor and wine composition, 2) develop rapid vineyard fruit sampling assessment techniques and 3) utilize these results to modify vineyard practices to produce fruit to specification.

In Summary, this research improves our understanding of the relationships between vineyards and wine chemistry. Several important new discoveries came from this research including the high degree of variation that can occur in flavonoid accumulation related to the site environment, the influence of sunlight exposure on the accumulation of skin proanthocyanidin in fruit, the differences in skin proanthocyanidin concentration in wine, the higher extraction of skin proanthocyanidin in low vigor wines and the large increases seen in pigmented polymer formation in low vigor zone wines. This provides justification for continued research towards understanding differences in plant response to environment in terms of fruit ripening biochemistry and wine fermentation processes.

Bibliography

Arnold, R. A.; Noble, A. C.; Singleton, V. L. Bitterness and astringency of phenolic fractions in wine. *J. Agric. Food Chem.* **1980,** 28, 675-678.

Asen, S.; Stewart, R. N.; Norris, K.H. Co-pigmentation of anthocyanins in plant tissues and its effect on color. *Phytochem.* **2003,** 11, 1139-1144.

Asenstorfer, R. E.; Markides, A. J.; Iland, P. G.; Jones, G. P. formation of vitsin A during red wine fermentation and maturation. *Aust. J. Grape Wine Res.* **2003,** 9, 40-46.

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

Bao Do, C.; Cormier, F. Accumulation of peonidin-3-glucoside enhanced by osmotic stress in grape (*Vitis vinifera* L.) cell suspension. *Plant Cell, Tissue and Organ Culture.* **1991,** 24, 49-54.

Bergqvist, J.; Dokoozlian, N.; Ebisuda, N. Sunlight exposure and temperature effects on berry growth and composition of Cabernet Sauvignon and Grenache in the central San Joaquin Valley of California. *Am. J. Enol. Vitic.* **2001,** 52, 1-7.

Bieza K.; Lois, R. An Arabidopsis mutant tolerant to lethal ultraviolet-B levels shows constitutively elevated accumulation of flavonoids and other phenolics. *Plant Physiol.* **2001,** 126, 1105-1115.

Bishop, P. D.; Nagel, C. W. Characterization of the condensation product of Malvidin3,5-diglucoside and catechin. *J. Agric. Food Chem.* **1984,** 32, 1022-1026.

Boselli, E.; Boulton, R. B.; Thorngate, J. H.; Frega, N. G. chemical and sensory characterization of DOC red wines from Marxhe (Italy) related to vintage and grape cultivars. *J. Agric. Food Chem.* **2004,** 52, 3843-3854.

Bogs, J.; Downey, M. O.; Harvey, J. S.; Ashton, A. R.; Tanner, G. J.; Robinson, S. P. Proanthocyanidin synthesis and expression of genes encoding leucoanthocyanidin reductase and anthocyanidin reductase in developing grape berries and grapevine leaves. *Plant Physiol.* **2005,** 139, 652-663.

Bogs, J.; Ebadi, A.; McDavid, D.; Robinson, S. P. Identification of the flavonoid hydroxylases from grapevine and their regulation during fruit development. *Plant Physiol.* **2006,** 140, 279-291.

Bohm, B. A. Flavonoid functions in nature. *In.* Introduction to flavonoids. Chemistry and biochemistry of organic natural products. Vol. 2. Bohm, B. A. Eds. Harwood Academic Publishers, Amsterdam. **1988,** pp. 365-393.

Boss, P. K.; Davies, C.; Robinson, S. P. Analysis of the expression of anthocyanin pathway genes in developing Vitis vinifera L. cv Shiraz grape berries and the implications for pathway regulation. *Plant Physiol.* **1996a,** 111, 1059-1066.

Boss, P. K.; Davies, C.; Robinson, S. P. Expression of anthocyanin biosynthesis pathway genes in red and white grapes. *Plant Mol. Biol.* **1996b,** 32, 565-569.

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

Bramley, R.G.V.; Hamilton, R.P. Understanding variability in winegrape production systems 1. Within vineyard variation in yield over several vintages. *Aust. J. Grape Wine Res.* **2004,** 1, 32-45.

Bramley, R.G. V.; Proffit, A. P. B. Managing Variability in Viticultural Production. *The Australian Grapegrower and Winemaker* **1999,** 427:11-16.

Bramley, R.G. V.; Proffitt, T. Variation in grape yield and quality in a Coonawaara vineyard'. In: *Proceedings 5th International Symposium on Cool Climate Viticulture & Oenology*, Melbourne, Australia, **2000,** 16-20.

Bramley, R. V. G. Progress in the development of Precision Viticulture – variation in yield, quality, and soil properties in contrasting Australian Vineyards. In: Precision tools for improving land management. Occasional report No 14. Fertilizer and Lime Research Center, Palmerston North, New Zealand, **2001,** 25-43.

Bramley, R. V. G.; Proffitt, A. P. B.; Corner, R. J.; Evans, T. D.Variation in grape yield and soil depth in two contrasting Australian vineyards. In: Soil 2000: New Horizons for a New Century, Australian & New Zealand 2nd Joint Soils Conference, 2: Oral Papers. Eds J. A. Adams and A. K. Metherell, (NZSSS). **2000,** 29-30.

Bravdo, B.; Hepner, Y.; Loinger, C; Cohen, S; Tabacman, H. Effect of irrigation and crop level on growth, yield and wine quality of cv. Cabernet Sauvignon. *Am. J. Enol. Vitic.* **1985,** 36, 132-139.

Brossaud, F.; Cheynier, V.; Asselin, C.; Moutounet, M. flavonoid compositional differences of grapes among site test plantings of Cabernet franc. *Am. J. Enol. Vitic.* **1999,** 50, 277-284.

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

Burger, A. L.; Botha, F. C. Ripening-related gene expression during fruit ripening in *Vitis vinifera* L. cv. Cabernet sauvignon and Clairette blanche. *Vitis* **2004,** 43, 59-64.

Buttrose, M. S.; Hale, C. R.; Kliewer, W. M. Effect of temperature on the composition of Cabernet Sauvignon berries. *Am. J. Enol. Vitic.* **1971,** 22, 71-75.

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

Careri, M.; Corradini, C.; Elviri, L.; Nicoletti, I.; Zagnoni, I. direct LC analysis of quercetin and trans resveratrol in red wine, grape, and winemaking products. *J. Agric. Food Chem*. **2003,** 51, 5226-5231.

Castellarin, S. D.; Di Gaspero, G.; Marconi, R.; Nonis, A.; Peterlunger, E.; Paillard, S.; Adam-Blondon, A.F.; Testolin, R. Colour variation in red grapevines (Vitis vinifera L.): genomic organization, expression of flavonoid 3'5'hydroxylase, flavonoid 3'5'-hydroxylase genes and related metabolite profiling of red cyaniding-/blue delphinidin-based anthocyanins in berry skin. *BMC Genomics.***2006,** 7, in press.

Chalker-Scott, L. Environmental significance of anthocyanins in plant stress responses. *Photochem. Photobiol.* **1999,** 70, 1-9.

Chaves, N.; Escudero, J. C. Variation of flavonoid synthesis induced by ecological factors. In *Principles and Practices of Plant Ecology, Allelochemical Interactions.* (Eds Inderjit, K.M.M. Dakshini & C.L. Foy) CRC Press, Boca Raton, Florida, USA. **1999;** pp267-285.

Cheynier, V.; Rigaud, J. HPLC separation and characterization of flavonols in the skins of *Vitis vinifera* var Cinsault. *Am. J. Enol. Vitic.* **1986,** 37, 248-252.

Cheynier, V.; Fulcrand, H.; Brossaud, F.; Asselin, C.; Moutounet, M. Phenolic composition as related to red wine flavor. In *Chemistry of Wine Flavor*. A.L. Waterhouse and S.E. Ebeler Eds. Oxford University Publishing, New York, NY. **1998,** pp. 125-141.

Cheynier, V. Polyphenols in food are more complex than often thought. *Am. J. Clin. Nutr.* **2005,** 81, (suppl), 223S-229S.

Coombe, B. G. The development of fleshy fruits. *Ann. Rev. Plant Physiol.* **1976,** 27, 507-528.

Coombe, B. G.; Bovio, M.; Schneider, A. Solute accumulation by grape pericarp cells V. Relationship to berry size and the effect of defoliation. *J. Exp. Biol.* **1987,** 38, 1789-1798.

Coombe, B. G.; McCarthy, M. G. Dynamics of grape berry growth and physiology of ripening. *Aust. J. Grape Wine Res.* **2000,** 6, 131-135.

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

Cortell, J. M.; Kennedy, J. A. Effect of shading on accumulation of flavonoid compounds in *Vitis vinifera* L. Pinot noir fruit and extraction in a model system. *J. Agric. Food Chem.* **2006,** in press.

Crippen, D. D.; Morrison, J. C. The effects of sun exposure on the compositional development of Cabernet Sauvignon berries. *Am. J. Enol. Vitic.* **1986,** 38, 235-242.

Curtin, C.; Zhang, W.; Franco, C. Manipulating anthocyanin composition in *Vitis vinifera* suspension cultures by elicitation with jasmonic acid and light irradiation. *Biotechnology Letters,* **2003,** 25, 1131-1135.

Czochanska, Z.; Foo, L. Y.; Lawrence, J. P. Compositional changes in lower molecular weight flavans during grape maturation. *Phytochemistry* **1979,** 18, 1819-1822.

De Frietas, V.A.P.; Glories, Y.; Monique, A. Developmental changes of procyanidins in grapes of red *Vitis vinifera* varieties and their composition in respective wines. *Am. J. Enol. Vitic.* **2000**, *51*, 397-403.

del Carmen-Llaudy, M.; Canals, R.; González-Manzano, S.; Canals, J. M.; Santos-Bulega, C.; Zamora, F. Influence of micro-oxygenation treatment before oak aging on phenolic compounds composition, astringency, and color of red wine. *J. Agric. Food Chem.* **2006,** 54, 4246-4252.

Dixon, R. A.; xie, D. Y.; Sharma, S. B. Proanthocyanidins – a final frontier in flavonoid research? *New Phytologist.* **2005**, 165, 9-28.

Dokoozlian, N. K; Kliewer W. M. Influence of light on grape berry growth and composition varies during fruit development. *J. Am. Soc. Hort. Sci.* **1996,** 121, 869-874.

Downey, M. O.; Harvey, J. S.; Robinson, S. P. The effect of bunch shading on berry development and flavonoid accumulation in Shiraz grapes. *Aust. J. Grape Wine Res.* **2004,** 1, 55-73.

Downey, M. O.; Harvey, J. S.; Robinson, S. P. Synthesis of flavonols and expression of flavonol synthase genes in the developing berries of Shiraz and Chardonnay (*Vitis vinifera* L.) *Aust. J. Grape Wine Res.* **2003a,** 9, 110-121.

Downey, M. O.; Harvey, J. S.; Robinson, S. P. Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. *Aust. J. Grape Wine Res.* **2003b**, 9, 15-27.

Eglinton, J.; Griesser, M.; Henschke, P.; Kwiatkowski, M.; Parker, M.; Herderich, M. Yeast mediated formation of pigmented polymers in red wine. In *Red wine color: revealing the mysteries;* Waterhouse, A. L., Kennedy, J., Eds.; ACS Symposium series 886; American Chemical Society: Washington, DC, 2004; pp 125-142.

Ewart, A.; Kliewer, W. M. Effects of controlled day and night temperatures and nitrogen on fruit-set, ovule fertility, and fruit composition of several grape cultivars. *Am. J. Enol. Vitic.* **1977,** 28, 88-95.

Fischer, U.; Boulton, R. B.; Noble, A. C. Physiological factors contributing to the variability of sensory assessments: relationship between salivary flow rate and temporal perception of gustatory stimuli. *Food Qual. Pref.* **1994,** 5, 55-64.

Fischer, U.; Noble, A. C. The effect of ethanol, catechin concentration and pH on sourness and bitterness of wine. *Am. J. Enol. Vitic.* **1994,** 45, 6-10.

Flamini, R. Mass spectrometry in grape and wine chemistry. Part I: polyphenols. *Mass Spectrom. Rev.* **2003,** 22, 218-250.

Flint, S. D.; Jordan, P. W.; Caldwell, M. M. Plant protective response to enhanced UV-B radiation under field conditions: leaf optical properties and photosynthesis. *Photochem. Photobiol.* **1985,** 41, 95-99.

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

Francis, F. J. Food colorants: Anthocyanins. *Crit. Rev. Food Sci. Nutr*.**1989,** 28, 273-314.

Frank, I. E.; Kowalski, B. R. Prediction of wine quality and geographical origin from chemical measurements by partial least square regression modeling. *Anal. Chim. Acta* **1984,** 162, 241-251.

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

Fulcrand, H.; Atanasova, V.; Salas, E.; Cheynier, V. The fate of anthocyanins in wine: Are there determining factors?. In *Red wine color: revealing the mysteries;* Waterhouse, A. L., Kennedy, J., Eds.; ACS Symposium series 886; American Chemical Society: Washington, DC, 2004; pp 68-88.

Fujita, A., Soma, N., Goto-Yamamoto, N., Shindo, H., Kakuta, T., Koizumi, T.; Hashizume, K. *Am. J. Enol. Vitic.* **2005,** 56, 336-342.

Gao, Y.; Cahoon, G. A. Cluster shading effects on fruit quality, fruit skin color, and anthocyanin content and composition in Reliance (*Vitis* hybrid). *Vitis* **1994,** 33, 205-209.

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

Gawel, R. Red wine astringency. *Aust. J. Grape Wine Res.* **1998,** 4, 74-95.

Ginestar, C.; Eastham, J.; Gray, S.; Iland, P. Use of sap-flow sensors to schedule vineyard irrigation. II. Effects of post-véraison water deficits on composition of Shiraz grapes. *Am. J. Enol. Vitic.* **1998,** 49, 421-428.

Gladstones, J.S. *Viticulture and Environment* Winetitles, Adelaide, Soth Australia, 1992.

Glories, Y.; Saucier, C. Tannin evolution from grape to wine. Effects on wine taste. *Proc. Amer. Soc. Vit. Enol. 50th Anniversary Annual* Meeting, Seattle, Washington. **2000,** 353-355.

Gómez-Cordovés, C. New pigments in red wines via different enological processes. In *Red wine color: revealing the mysteries;* Waterhouse, A. L., Kennedy, J., Eds.; ACS Symposium series 886; American Chemical Society: Washington, DC, 2004; pp 89-124.

Hall, A.; Lamb, D.W.; Holzapfel, B.; and Louis, J. Optical remote sensing applications for viticulture – a review. *Aust. J. Grape Wine Res.* **2002,** 8, 36-47.

Harbertson, J. F.; Kennedy, J. A.; Adams, D. O. Tannin in skins and seeds of Cabernet Sauvignon, Syrah, and Pinot noir berries during ripening. *Am. J. Enol. Vitic.* **2002,** 53, 54-59.

Harbertson, J. F.; Picciotto, E. A.; Adams, D. O. Measurement of polymeric pigments in grape berry extracts and wine using a protein precipitation assay combined with bilsulfite bleaching. *Am. J. Enol. Vitic.* **2003,** 54, 301-306.

Harbourne J. Phytochemistry of fruits and vegetables: an ecological overview. In *Phytochemistry of Fruits and Vegetables;* Tomas-Barberan, F., Eds; Oxford University Press; New York, **1997**; pp 353-367.

Hardie, W. J.; Considine, J. A. Response of grapes to water-deficit stress in particular stages of development. *Am. J. Enol. Vitic.* **1976,** 27, 55-61.

Hardie, W. J.; Johnson, J. O.; Weaver, R. J. The influence of vine water regime on ethephon enhanced ripening of zinfandel. *Am. J. Enol. Vitic.* **1981**, 32, 115-121.

Haselgrove, L.; Botting, D.; van Heeswijck, R.; HØj, P. B.; Dry, P.R.; Ford, C.; Iland, P. G. Canopy microclimate and berry composition: The effect of bunch exposure on the phenolic composition of *Vitis vinifera* L. cv Shiraz grape berries. *Aust. J. Grape Wine Res.* **2000,** 6, 141-149.

Haslam, E. Review: Symmetry and promiscuity in procyanidin biochemistry. *Phytochem.* **1977,** 16, 1625-1640.

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

Heymann, H.; Noble, A. C. Descriptive analysis of commercial Cabernet Sauvignon wines from California. *Am. J. Enol. Vitic.* **1987,** 38, 41-44.

Hilbert, G.; Soyer, J. P.; Molot, C.; Giraudon, J.; Milin, G. S.; Gaudillere, J. P. Effects of nitrogen supply on must quality and anthocyanin accumulation in berries of cv. Merlot. *Vitis* **2003,** 42, 69-76.

Holton, T.A.; Cornish, E. C. Genetics and Biochemistry of anthocyanin biosynthesis. *Plant Cell* **1995,** 7,1071-1083.

Holzapfel, B. S.; Rogiers, K.; Degaris, G.; Small, G. 1999. Ripening grapes to specification: effect of yield on colour development of Shiraz grapes in the Riverina. *The Australian Grapegrower and Winemaker*. **1999,** 428, 24-28.

Holzapfel, B. S.; Rogiers, K.; G. Degaris, G.; Small, G. Identifying factors effecting grape berry ripening and berry colour development. In Poceedings 5th International Symposium on Cool Climate Viticulture and Oenology, Melbourne, Australia. **2000.**

Hrazdina, G.; Parsons, G. F.; Mattick, L. R. Physiological and biochemical events during development and maturation of grape berries. *Am. J. Enol. Vitic.* **1984,** 35, 220-227.

Ishikawa, T.; Noble, A. C. Temporal perception of astringency and sweetness in red wine. *Food Qual. Pref.* **1995,** 6, 27-33.

Jaakola, L.; Määttä-Riihinen, K.; Kärenlampi, S.; Hohtola, A. Activation of flavonoid synthesis by solar radiation in bilberry (*Vaccinium myrtyillus* L.) leaves. *Planta* **2004,** 218, 721-728.

Jackson, D. I.; Lombard, P. B. Environmental and management practices affecting grape composition and wine quality – A review. *Am. J. Enol. Vitic.* **1993,** 44, 409-430.

Jorgensen, E. M.; Marin, A. B.; Kennedy, J. A. Analysis of the oxidative degradation of proanthocyanidins under basic conditions. *J. Agric. Food Chem.* **2004,** 52, 2292-2296.

Kallithraka, S.; Bakker, J.; Clifford, M. N. Evaluation of bitterness and astringency of (+)-catechin and (-)-epicatechin in red wine and in model solution. *J. Sensory Stud.* **1997,** 12, 25-37.

Kallithraka, S.; Arvanitoyannis, I. S.; Kefalas, P.; El-Zajouli, A.; Soufleros, E.; Psarra, E.; Instrumental and sensory analysis of Greek wines: implementation of principle component analysis (PCA) for classification according to geographical origin. *Food Chem.* **2001,** 73, 501-514.

Kantz, K.; Singleton, V. L. Isolation and determination of polymeric polyphenols in wine using sephadex LH-20. *Am. J. Enol. Vitic.* **1991,** 42, 309-315.

Keller, M. K.; Hrazdina, G. Interaction of nitrogen availability during bloom and light intensity during véraison. II. Effects on anthocyanin and phenolic development during grape ripening. *Am. J. Enol. Vitic.* **1998,** 49, 341-349.

Kennedy, J. A., Hayasaka, Y., Vidal, S., Waters, E. J. & Jones, G. P. Composition of grape skin proanthocyanidins at different stages of berry development. *J. Agric. Food Chem.* **2001,** 49, 5348-5355.

Kennedy, J. A.; Jones, G. P. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J. Agric. Food Chem.* **2001,** 49, 1740-1746.

Kennedy, J. A.; Matthews, M. A.; Waterhouse, A. L. Changes in grape seed polyphenols during fruit ripening. *Phytochemistry* **2000a,** 55, 77-85.

Kennedy, J. A.; Matthews, M. A.; Waterhouse, A. L. Effect of maturity and vine water status on grape skin and wine flavonoids. *Am. J. Enol. Vitic.* **2002,** 53, 268-274.

Kennedy, J. A.; Troup, G. J.; Pilbrow, J. R.; Hutton, D. R.; Hewitt, D.; Hunter, C. R.; Ristic, R.; Iland, P. G.; Jones, G. P. Development of seed polyphenolics in berries from *Vitis vinifera* L. cv Shiraz. *Aust. J. Grape Wine Res.* **2000b,** 6, 244-254.

Kennedy, J.A.; Taylor, A.W. Analysis of proanthocyanidins by high-performance gel permeation chromatography. *J. Chromatogr. A.* **2003,** 995, 99-107.

Kliewer, W. M.; Lider, L. A.; Schultz, H. B. Influence of artificial shading of vineyards on the concentration of sugar and organic acids in grapes. *Am. J. Enol. Vitic.* **1967,** 18, 78-86.

Kliewer, W. M.; Lider, L. A. Influence of cluster exposure to the sun on the composition of 'Thompson seedless' fruit. *Am. J. Enol. Vitic.* **1968,** 19, 175-184.

Kovac, V.; Alonso, E.; Revilla, E. The effect of adding supplementary quantities of seeds during fermentation on the phenolic composition of wines. *Am. J. Enol. Vitic.* **1995,** 46, 363-367.

Labarbe, B.; Cheynier, V.; Brossaud, F.; Souquet, J. M.; Moutounet, M. Quantitative fractionation of grape proanthocyanidins according to their degree of polymerization. *J. Agric. Food Chem.* **1999,** 47, 2719-2723.

Lamb, D. Vineyard Monitoring and Management Beyond 2000, Precision Viticulture: A workshop investigating the latest technologies for monitoring and managing variability in vineyard productivity. **2000,** (Eds; Lamb, D.) Available on the Internet or CRC for Viticulture, PO Box 154 Glen Osmond, South Australia 5064.

Lamb, D.W.; Weedon, M.M.; Bramley, R.G.V. Using remote sensing to predict grape phenolics and colour at harvest in a Cabernet Sauvignon vineyard: Timing observations against vine phenology and optimizing image resolution. *Aust. J. Grape Wine Res.* **2004,** 10, 46-54.

Lamuela-Raventos, R.M.; Waterhouse, A.L. A direct HPLC separation of wine phenolics. *Am. J. Enol. Vitic.* **1994,** 45, 1-5.

Lee, C. B.; Lawless, H. T. Time-course of astringent sensations. *Chem Senses* **1991,** 16, 225-238.

Lee, D. F.; Ewald, E. S.; Asenstorfer, R. E.; Jones, G. P. Factors affecting the formation of red wine pigments. In *Red wine color: revealing the mysteries;* Waterhouse, A. L., Kennedy, J., Eds.; ACS Symposium series 886; American Chemical Society: Washington, DC, 2004; pp 125-143.

Lesschaeve, I.; Noble, A. C.; Polyphenols: factors influencing their sensory properties and their effects on food and beverage preferences. *A. J. Clin. Nutr.* **2005,** 81 (suppl):330s-335s.

Li, J.; Ou-Lee, T. M.; Raba, R.; Amundson R. G.; Last, R. L. Arabidopsis mutants lacking phenolic sunscreens exhibit enhanced ultraviolet-B injury and oxidative damage. *Plant Cell* **1993,** 5,171-179.

Liao, H.; Cai, Y.; Haslam, E. Polyphenolic interactions. Anthocyanins: copigmentation and colour changes in red wines. *J. Sci. Food Agric.* **1992,** 59, 299-305.

Lui, S.; Pilone, Q. An overview of formation and role of acetaldehyde in winemaking with emphasis on microbiological implications. *Int. J. Food Sci. Tech.* **2000,** 35, 49-61.

Lorenzo, C.; Pardo, F.; Zalacain, A.; Alonso, L. G.; Salinas, M. R.; Effect of red grapes co-winemaking in polyphenols and color of wines. *J. Agric. Food Chem.* **2005,** 53, 7609-7616.

Mabrouk, H; Sinoquet, H. Indices of light microclimate and canopy structure of grapevines determined by 3D digitizing and image analysis, and their relationship to grape quality. *Aust. J. Grape Wine Res.* **1998,** 4, 2-13.

Makris, D. P.; Stamatina, K.; Kefelas, P. Critical Review: Flavonols in grape products and wines: Burden, profile and influential parameters. *J. of Food Comp. and Anal.* **2005,** in press.

Markham, K. R.; Tanner, G. J.; Caasi-Lit, M.; Whitecross, M. I.; Nayudu, M.; Mitchell, K. A. Possible protective role for 3', 4'-dihydroxyflavones induced by enhanced UV-B in a tolerant rice cultivar. *Phytochem.* **1998,** 49, 1913-1919.
Matthews, M. A.; Anderson, M. M. Fruit ripening in *Vitis vinifera* L.: Response to seasonal water deficits. *Am. J. Enol. Vitic.* **1988,** 39, 313-320.

Mazza, G.; Miniata, E. Grapes. In *Anthocyanins in Fruits, Vegetables, and Grain;* G., Miniata, E., Eds.; CRC Press: Boca Raton, 1993; pp 149-199.

Mazza, G. Anthocyanins in grapes and grape products. *Crit. Rev. Food Sci. Nutr.* **1995,** 35, 341-371.

Mazza, G.; Fukumoto, L.; Delaquis, P.; Girard, B.; Ewert, B. Anthocyanins, phenolics, and color of Cabernet franc, Merlot, and Pinot noir wines from British Columbia. *J. Agric. Food Chem.* **1999,** 47, 4009-4017.

McCloskey, L.P.; Yengoyan, L.S. Analysis of anthocyanins in *Vitis vinifera* wines and red color versus aging by HPLC and spectrophotometry. *Am. J. Enol. Vitic.* **1981,** 32, 257-261.

Mercier, J. Role of phytoalexins and other antimicrobial compounds from fruits and vegetables in post harvest disease resistance. In *Phytochemistry of Fruits and Vegetables.* Tomas-Barberan, F., Eds; Oxford University Press; New York, **1987**; pp 221-241.

Meyer, J.; Hernandez, R. seed tannin extraction in Cabernet Sauvignon. *Am. J. Enol. Vitic.* **1970,** 21, 184-188.

Monagas, M.; Gómez-Cordovés, C.; Bartolomé, B.; Laureano, O.; Ricardo Da Silva, J. Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from Vitis vinifera L. Cv. Graciano, Tempranillo, and Cabernet Sauvignon. *J. Agric. Food Chem.* **2003,** 51, 6475-6481.

Morel-Salmi, C.; Souquet, JM.; Magali, B.; Cheynier, V. Effect of flash release treatment on phenolic extraction and wine composition. *J. Agric. Food Chem.* **2006,** 54, 4270-4276.

Morag, S. M.; Hughes, M.; Burns, J.; Lean, M. E. J.; Matthews, D.; Crozier, A. survey of the free and conjugated myricetin and quercetin content of red wines of different geographical origins. *J. Agric. Food Chem*. **1998,** 46, 368-375.

Morata, A.; Gómez-Cordovés, M. C.; Suberviola, J.; Bartolomé, B.; Colomo, B.; Suárez, J. A. Adsorption of anthocyanins by yeast cell walls during the fermentation of red wines. *J. Agric. Food Chem.* **2003,** 51, 4084-4088.

Mori, K.; Saito, H.; Goto-Yamamoto, N.; Kitayama, M.; Kobayashi, S.; Sugaya, S.; Gemma, H.; Hashizume, K. Effects of abscisic acid treatment and night temperature on anthocyanin composition in Pinot noir grapes. *Vitis,* **2005,** 4, 161-165.

Morrison, J. C.; Noble, A. C. The effects of leaf and cluster shading on the composition of Cabernet Sauvignon grapes and on fruit and wine sensory properties. *Am. J. Enol. Vitic.* **1990,** 41, 193-200.

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

Nagata, T.; Todoriki, S.; Masumizu, T.; Suda, I.; Furuta, S.; Du, Z.; Kikuchi, S. Levels of active oxygen species are controlled by ascorbic acid and anthocyanin in *Arabidopsis*. *J. Agric. Food Chem.* **2003,** 51, 2992-2999.

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

Noble, A. C.; Bitterness and astringency in wine. In *Developments in Food Science 25. Bitterness in Foods and Beverages*; Rouseff, R. L., Ed.; Elsevier: New York, NY, **1990,** pp 145-158.

Noble, A. C. Bitterness in wine. *Physiol. Behav.* **1994,** 6, 1251-1255.

Ojeda, H.; Andary, C.; Kraeva, E.; Carbonneau, A.; Deloire, A. Influence of pre- and post-veraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. *Am. J. Enol. Vitic.* **2002,** 53, 261-267.

Oretega-Regules, A.; Romero-Cascales, I.; Ros-García, J. M.; López-Roca, J. M.; Gómez-Plaza, E. A first approach towards the relationship between grape skin cell-wall composition and anthocyanin extractability. *Anal. Chimica Acta.* **2006,** 563, 26-32.

Ough, C. S.; Amerine, M. A. Methods for Analysis of Musts and Wines (2nd ed.) **1988,** John wile and sons, 377 pp.

Parley, A.; Vanhanen, L.; Heatherbell, D.; Effects of pre-fermentation enzyme maceration on extraction and colour stability in Pinot noir wine. *Aust. J. Grape Wine Res.* **2001,** 7, 146-152.

Pastor del Rio, J.; Kennedy, J. A. Development of proanthocyanidins in *Vitis vinifera* L. cv. Pinot noir grapes and extraction into wine. *Am. J. Enol. Vitic.* **2006,** 57, 125-131.

Peleg, H.; Bodine, K. K.; Noble, A. C. The influence of acid on astringency of alum and phenolic compounds. *Chem. Senses* 1998, 23, 371-378.

Peleg, H.; Gacon, K.; Schlich, P.; Noble, A. C. Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. *J. Sci. Food Agric*. **1999,** 79, 1123-1128.

Peng, Z.; Iland, P.G.; Oberholzer, A.; Sefton, M. A.; Walters, E. J. Analysis of pigmented polymers in red wine by reverse phase HPLC. *Aust. J. Grape Wine Res.* **2002,** 8, 70-75.

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

Peyrot des Gachons, C.; Kennedy, J.A. Direct Method for Determining seed and skin proanthocyanidin extraction in red wine. *J. Agric. Food Chem.* **2003,** 51, 5877-5881.

Pirie, A.J.G.; Mullins, M.G. Concentration of phenolics in skin of grape berries during fruit development and ripening. *Am. J. Enol. Vitic.* **1980,** 31, 34-36.

Price, S. F.; Breen, P. J.; Valladao, M.; Watson, B. T. Cluster exposure and quercetin in Pinot noir grapes and wine. *Am. J. Enol. Vitic.* **1995,** 46, 187-194.

Prieur, C.; Rigaud, J.; Cheynier, V.; Moutounet, M. Oligomeric and polymeric proanthocyanidins from grape seeds. *Phytochemistry* **1994,** 36, 781-784.

Ponder, F. Jr.; Fertilizer combinations benefit diameter growth of plantation black walnut. *J. of Plant Nutrition.* **1998.** 21, 1329-1337.

Proffitt, T.; Robinson, R.; Arney, M.; Hodder, S. P.; Iland, P. Canopy, fruit and wine characterstics of Cabernet sauvignon grown on contrasting soils in Coonawarra. In 'Proceedings 5th International Symposium on Cool Climate Viticulture & Oenology', Melbourne, Australia, **2000,** 16-20.

Rapp, A. Natural flavours of wine: correlation between instrumental analysis and sensory perception. *Fres. J. Analyt. Chem.* **1990,** 337, 777-785.

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

Reynolds, A. G.; Pool, R. M.; Mattick, L. R. Influence of cluster exposure on fruit composition and wine quality of Seyval blanc grapes. *Vitis* **1986,** 25, 85-95.

Reynolds, A. G.; Yerle, S.; Watson, B.; Price, S. F.; Wardle, D. A. Fruit environment and crop level effects on Pinot noir. III. Composition and descriptive analysis of Oregon and British Columbia wines. *Am. J. Enol. Vitic.* **1996,** 47, 329-339.

Reynolds, A. G.; Molek, T.; De Savigny, C. Timing of shoot thinning in *Vitis vinifera*: Impacts on yield and fruit composition Variables. *Am. J. Enol. Vitic.* **2005,** 56, 343-356.

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

Ribéreau-Gayon, P. Évolution des composés phénoliques au cours de la maturation du raisin. II. Discussion des resultants obtenus en 1969, 1970 et 1971. *Connaiss. Vigne Vin.***1972,** 6, 161-175.

Ribéreau-Gayon, P. The chemistry of red wine color. In *Chemistry of Winemaking*. (Eds Webb, A. D.) American Chemical Society, Washington, D.C. 1974; pp.81.

Ribéreau-Gayon, P.; Glories, Y.; Maujean, A.; Dubourdieu, D. Phenolic Compounds. *In* Handbook of Enology, The Chemistry of Wine Stabilization and Treatments. **2000,** John Wiley and Sons LTD, West Sussex, England, pp 129-185.

Ricardo da Silva, J. M.; Rigaud, J.; Cheynier, V.; Cheminat, A.; Moutounet, M. Procyanidin dimers and trimers from grape seeds. *Phytochemistry.* **1991,** 4, 1259-1264.

Robichaud, J. L.; Noble, A. C. Astringency and bitterness of selected phenolics in wine. *J. Sci. Food Agric.* **1990, 53,** 343-353.

Robinson, S.P.; Davies, C. Molecular biology of grape berry ripening. *Aust. J. Grape Wine Res.* **2000,** 6, 168-174.

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

Roby, G.; Matthews, M. A. Relative proportions of seed, skin and flesh, in ripe berries from Cabernet Sauvignon grapevines grown in a vineyard either well irrigated or under water deficit. *Aust. J. Grape Wine Res.***2004,** 10, 74-82.

Roggero, J. P.; Coen, S.; Ragonnet, R. High performance liquid chromatography survey on changes in pigment content in ripening grapes of Syrah. An approach to anthocyanin metabolism. *Am. J. Enol. Vitic.* **1986,** 37, 77-83.

Romero, C.; Bakker, J. Interactions between grape anthocyanins and pyruvic acid with effect of pH and acid concentration on anthocyanin composition and color in model solutions.

Romero, P.; Botia, P.; Francisco, G. Effects of regulated deficit irrigation under subsurface drip irrigation conditions on vegetative development and yield of mature almond trees. *Plant and Soil.* **2004.** 260, 169-181.

Romeyer, F.M.; Macheix, J.J.; Sapis, J.C. Changes and importance of oligomeric proanthocyanidins during maturation of grape seeds. *Phytochemistry* **1986,** 25, 219-221.

Romeyer, F.M., Macheix, J.J., Goiffon, J.P., Reminiac, C.C., Sapis, J.C. (1983) "The browning capacity of grapes. 3. Changes and importance of hydroxycinnamic acid-tartaric acid esters during development and maturation of the fruit," Jour. of Agricultural & Food Chemistry 31: 346-349.

Ryan, J. M.; Revilla, E. Anthocyanin composition of Cabernet Sauvignon and Tempranillo grapes at different stages of ripening. *J. Agric. Food Chem.* **2003,** 51, 3372-3378.

Ryan, K. G.; Swinny, E. E.; Markham, K. R.; Winefeld, C. Flavonoid gene expression and UV photoprotection in transgenic and mutant Petunia leaves. *Phytochemistry* 59, **2002,** 23-32.

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

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

Salas, E.; Le Guernevé, C.; Fulcrand, H.; Poncet-Legrand, C.; Cheynier, V. Structure determination and color properties of a newly synthesized direct-linked flavanol-anthocyanin dimer. *Tetrahedron Lett.* **2004,** 45, 8725-8729.

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

Salon, J. L.; Chirivella, C.; Castel, J. R. Response of cv. Bobal to timing of deficit irrigation in Requena, Spain: water relations, yield and wine quality. *Am. J. Enol. Vitic.* **2005,** 56, 1-8.

Santos-Buelga, C.; Scalbert, A. Review: Proanthocyanidins and tannin-like compounds – nature, occurrence, dietary intake and effects on nutritional health. *J. Sci. Food Agric.* **2000,** 80, 1094-1117.

Saucier, C.; Little, D.; Glories, Y. First evidence of acetaldehyde-flavanol condensation products in red wine. *Am. J. Enol. Vitic.* **1997,** 48, 370-373.

Scheffeldt, P.; Hrazdina, G. Co-pigmentation of anthocyanins under physiological conditions. *J. Food Sci.* **1978,** 43, 517-520.

Schwarz, M.; Picazo-Bacete; J. J.; Winterhalter, P.; Hermosín-Gutiérrez, I. Effects of copigments and grape cultivar on the color of red wine fermented after the addition of copigments. *J. Agric. Food Chem.* **2005,** 53, 8372-8381.

Schwarz, M.; Picazo-Bacete; J. J.; Winterhalter, P.; Hermosín-Gutiérrez, I. Effects of copigments and grape cultivar on the color of red wine fermented after the addition of copigments. *J. Agric. Food Chem.* **2005,** 53, 8372-8381.

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

Singleton, V. L.; Draper, D. The transfer of polyphenolic compounds from grape seeds into wine. *Am. J. Enol. Vitic.* **1964,** 15, 34-40.

Sipiora, M. J.; Gutierrez-Granda, M. J. Effects of pre-véraison irrigation cutoff and skin contact time on composition, color and phenolic content of young Cabernet Sauvignon wines in Spain. *Am. J. Enol. Vitic.* **1998,** 49, 152-162.

Skrede, G.; Wrolstad, R. E.; Durst, R. W. Changes in anthocyanins and polyphenolics during juice processing of highbush blueberries (*Vaccinium corymbosum* L.). *J. Food Sci.* **2000,** 65, 357-364.
Smart, R. E. Principles of grapevine canopy microclimate manipulation with implications for yield and quality. A review. *Am. J. Enol. Vitic.* **1985,** 36, 230-239.

Smart, R. E.; Smith, S. M.; Winchester, R. V. Light quality and quantity effects on fruit ripening for Cabernet sauvignon. *Am. J. Enol. Vitic.* **1988,** 39, 250-258.

Smart, R. E. Influence of light on composition and quality of grapes. *Acta Horticulture.* **1997,** 206, 37-47.

Smith, A. K.; June, H.; Noble, A. C. effects of viscosity on the bitterness and astringency of grape seed tannin. *Food Qual. Pref.* **1996,** 7, 161-166.

Solovchenko, A.; Schmitz-Eiberger, M. Significance of skin flavonoids for UV-B protection in apple fruits. *J. Exp. Biol.* **2003,** 54, 1977-1984.

Smith, G. J.; Markham, K.R. Tautomerism of flavonol glucosides: relevance to plant UV protection and flower colour. *J. Phytochem. Photobiol.* **1998,** 118, 99-105.

Somers, T. C. The polymeric nature of wine pigments. *Phytochemistry* **1971,** 10, 2175-2186.

Somers, T.C.; Evans, M.E. Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO_2, "chemical age". *J. Sci. Food Agric.* **1977**, 28, 279-286.

Souquet, J. M.; Cheynier, V.; Brossaud, F.; Moutounet, M. Polymeric proanthocyanidins from grape skins. *Phytochemistry* **1996,** 43, 509-512.

Spayd, S. E.; Tarara, J. M.; Mee, D. L.; Ferguson, J.C. Separation of sunlight and temperature effects on the composition of *Vitis vinifera* cv. Merlot berries. *Am. J. Enol. Vitic.* **2002,** 53, 171-182.

Stecher, G.; Huck, c. W.; Popp, M.; Bonn, G. K. Determination of flavonoids and stilbenes in red wine and related biological products by HPLC and HPLC-ESI-MS-MS. *Fresenius J. Anal. Chem.* **2001,** 371, 73-80

Sun, B.S.; Pinto, T.; Leandro, M.C.; Ricardo-Da-Silva, J.M.; Spranger, M.I. Transfer of catechins and proanthocyanidins from the solid parts of the grape cluster into wine. *Am. J. Enol. Vitic.* **1999**, *50*, 179-184.

Thorngate, J. H.; Noble, A. C. Sensory evaluation of bitterness and astringency of 3*R*(-)-epicatechin and 3*S*(+)-catechin. *J. Sci. Food Agric.* **1995,** 67, 531-535.

Thorngate, J. H. The physiology of human sensory response to wine: A review. *Am. J. Enol. Vitic.* **1997,** 48, 271-279.

Timberlake, C. F.; Bridle, P.; Interactions between anthocyanins, phenolic compounds, and acetaldehyde and their significance in red wines. *Am. J. Enol. Vitic.* **1976,** 27, 97-105.

Tomasi, D.; Pitacco, A.; Pascarella, G. Bunch and berry temperature and anthocyanin synthesis and profile in Cabernet sauvignon. *Rivista di Viticoltura e di Enologia,* **2003,** 56, 3-15.

Valentová, H.; Skrováková, S.; Panovská, Z.; Pokorny, J. Time intensity studies of astringent taste. *Food Chem.* **2002,** 78, 29-37.

Vidal, S.; Cheynier, V.; Waters, E.; Noble, A. c. effect of tannin composition and wine carbohydrates on astringency and bitterness. In: Meiselman H., Ed. 5th Pangborn Sensory Science Symposium. Boston, MA., Elsevier, **2003.**

Vidal, S.; Meudec, E.; Cheynier, V.; Skouroumounis, G.; Hayasaka, Y. Mass spectrometric evidence for the existance of oligomeric anthocyanins in grape skins. *J. Agric. Food Chem.* **2004,** 52, 7144-7151.

Vuorinen, H.; Maattta, K.; Torronen, R. Content of the flavonols myricetin, quercetin and kaempferol in Finnish berry wines. *J. Agric Food Chem.* **2000,** 50, 2675-2680.

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

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

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

Winkel-Shirley, B. It takes a Garden. How work on diverse plant species has contributed to an understanding of flavonoid metabolism. *Plant Physiol.* **2001,** 127, 1399-1404.

Winkel-Shirley. Biosynthesis of flavonoids and effects of stress. Current Opinions in Plant Biology. **2002,** 5:3:218-223.

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

Yamasaki, H.; Uefuji, H.; Sakihama, Y. Bleaching of the red anthocyanin induced by spueroxide radical. *Archives of Biochem Biophys.* **1996,** 332, 183-186.

Yokotsuka, K.; Nagao, A.; Nakazawa, K.; Sato, M. Changes in anthocyanins in berry skins of Merlot and Cabernet sauvignon grapes grown in two soils modified with limestone or oyster shell versus a native soil over two years. *Am. J. Enol. Vitic.* **1999,** 50, 1-12.

Zimman, A.; Joslin, W. S.; Lyon, M. L.; Meier, J.; Waterhouse, A. L. Maceration variables affecting phenolic composition in commercial-scale Cabernet sauvignon winemaking trials. *Am. J. Enol. Vitic.* **2002,** 53, 93-98.