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THE IMPACT OF VINEYARD AND CELLAR FACTORS ON THE COLOR AND ANTHOCYANIN PROFILE OF PINOT NOIR GRAPES AND TABLE WINES

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GERARD ANTHONY LOGAN

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G. Stanley Howell
Major Professor's Signature

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THE IMPACT OF VINEYARD AND CELLAR FACTORS ON THE COLOR AND ANTHOCYANIN PROFILE OF PINOT NOIR GRAPES AND TABLE WINES

By

GERARD ANTHONY LOGAN

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ABSTRACT

THE IMPACT OF VINEYARD AND CELLAR FACTORS ON THE COLOR AND ANTHOCYANIN PROFILE OF PINOT NOIR GRAPES AND TABLE WINES.

By

GERARD ANTHONY LOGAN

In the cool climate winegrowing regions of Michigan USA and Canterbury New Zealand, *Vitis vinifera* L Pinot noir is an economically important red winegrape cultivar. Each region has problems with the final color of young Pinot noir wines based on anthocyanin presence and concentration. The anthocyanin concentration of *V. vinifera* Pinot noir fruit and wine was investigated using four clones and two international growing locations. Utilizing HPLC techniques, the five main anthocyanins in the fruit (labeled 1-5) were identified based on a cyanidin-3-glucoside standard, and total anthocyanin concentration was compared. Spectrophotometric methods were used to analyze the samples absorbance to give an indication of color perception by the human eye. Wines were made from Michigan sample vines, and analyzed in the same manner. Both growing season and location of culture were significant effects on the absorbance and anthocyanin concentration of fruit and wine extract samples ($p \leq 0.01$, and $p \leq 0.002$ respectively). The clone of Pinot noir grown is shown to have no statistical effect on the above parameters. Climate of growing region and winemaking extraction techniques are compounding limiting factors in the boundaries of anthocyanin concentration and final color of Pinot noir wines.

TO MY GRANDPARENTS, FAMILY AND FRIENDS

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KEY TO SYMBOLS OR ABBREVIATIONS

ABBREVIATION	MEANING
HPLC	High Performance Liquid Chromatography
pH	Hydrogen Ion Concentration
RH	Relative Humidity
SWMREC	Southwest Michigan Research and Extension Center
TA	Titratable Acidity
VSP	Vertical Shoot Positioning
MAR	Mariafield (UCD23) Clone of Pinot noir
UCD (or UCD13)	UCD 13 Clone of Pinot noir
113	Dijon 113 Clone of Pinot noir
115	Dijon 115 Clone of Pinot noir
MEOH	Methanol
ETOH	Ethanol
C-3-G	Cyanidin-3-glucoside
Sample Type	Used in statistical analysis. S1=Day 0, S2=Day 30, S3=Day 70and WINE = Wine samples after fermentation.
kg	Kilogram(s)
nm	Nanometers
S1	Early Season (first) sample date (Day 0)
S2	Mid-season (second) sample date (Day 30)
S3	Late Season (third) sample date (Day 70)

CHAPTER 1

INTRODUCTION

Pinot noir has become a cultural icon recently after the release of the popular movie "Sideways," which elevated attention to Pinot noir among American wine drinkers above the usual Bordeaux-steadfast, Merlot. The popularity of these cultivars is high because they are well known for consistently producing excellent quality wines – when managed correctly (Barr 1992, Haeger 2004, Jackson 2000).

Pinot noir is most famously grown in the Burgundy region of France. However, Pinot noir has achieved prominence, especially in cool climate regions of New World wine regions such as North America, Australia, South Africa and in New Zealand where it is one of the main exports (Haeger 2004, http://www.nzwine.com, http://www.uncork.com.au).

Pinot noir is one of the hardest cultivars to grow and vinify well, constantly providing challenges to viticulturalists and winemakers alike. To produce a top quality Pinot noir requires scientific knowledge and artistic ability. Optimum understanding of any topic or effort comes from two sources: a) trial and error; b) research and development. Research on Pinot noir production has increased dramatically in the last 20 years, ranging from clonal parentage investigations to wine ageing trials, and topics in between, all attempting to understand the cultivar more thoroughly.

One area of Pinot noir research that does not seem to be exhausted is international clonal consistency under commercial conditions, especially regarding the activity of anthocyanins. This consistency can be based on many factors, but today image is highly valued and wine is no exception (Winkler et al 1974). The color of wine is one of the first features a consumer or wine judge notices about a wine, besides the packaging (Haeger 2004). This is more important in red cultivars, and even more so in those wines which do not possess as much color density as the heavy consumer driven color benchmarks, such as the Bordeaux reds. Too often consumers mistakenly demand their Pinot noirs to have the color density and hue of an Australian Shiraz, without the understanding of cultivar or clonal differences, anthocyanin accumulation. (Barr 1992, Jackson and Schuster 2001).

Pinot noir stands apart from most red wine cultivars in that it does not possess any acylated anthocyanins, a feature which helps to stabilize the anthocyanin molecule in solution (Boulton 2001). Therefore, the anthocyanins present in the skins of Pinot noir fruit, and wine, are not as stable as in other cultivars, and not able to produce or hold a dark red color. Instead, most cool or colder climate wines produced from Pinot noir normally possess a slightly lighter and sometimes almost rosé-style pinkly-red color (Waterhouse and Kennedy 2004).

Young Pinot noir wine color comes from free anthocyanin, and is another reason for its lack of stability (Boulton 2001). During ageing however, the anthocyanins do begin to interact with other phenols, including non-colored polymeric pigments and other

anthocyanins, to form co-pigments. These newly formed compounds are more stable and darker than either of the co-pigments constituents alone (Boulton 2001).

Too often winemakers attempt to produce darker red color by extending the skin contact time during maceration. This may improve color, but can extract an overabundance of bitter tannin and heavy phenolic compounds that diminish flavor and palatability (Jackson, 2000).

The issue of color improvement without over-extraction of undesirable compounds was studied by Parley, et al (2001) on the cold soak (or cold maceration) mechanism for pre-fermented Pinot noir must. This process aided in color extraction over short to medium periods of time at cool temperature (4-10°C) without the extraction of large quantities of harsh phenolics (Parley et al 2001).

The State of Michigan, USA, has minimal history with Pinot noir. More recently, Michigan has begun to realize a potential for *V. vinifera* wine grape cultivars, and is producing excellent examples of Pinot noir, Chardonnay, Riesling, and Cabernet Franc. Consequently, there is considerable interest and little historical data or proven methods to aid in the consistent production of these cultivars (Howell et al 1999). Michigan wine growers enjoy Pinot noir a great deal, as it is early ripening, challenging, fairly new to Michigan, yet well renowned internationally as a great wine (Howell et al 2000).

The goal of this research effort is to investigate the role of geographic location, vintage variation and clonal differences influencing color, and anthocyanin content, that all lead toward the final quality of the wine product desired.

To achieve the goal, during a two season period in Michigan, USA, and Canterbury, New Zealand, vines of common Pinot noir clones were treated uniformly, employing commercial viticultural methods with regard to canopy management, sprays and vineyard hygiene. A series of treatments; varying hemispheres (New Zealand versus Michigan), vintage (2004 versus 2005) and Pinot noir clone (UCD13, 113, 115) were employed to test the following hypotheses:

1) The hemisphere/location of culture will not affect the level of anthocyanin pigment in Pinot noir grapes at harvest.
2) The vintage/growing season will not affect the level of anthocyanin pigment in Pinot noir grapes at harvest.
3) Clone of Pinot noir will not affect the level of anthocyanin pigment in Pinot noir grapes at harvest.
4) There will be no differences among treatments in the level of anthocyanin pigment in the grapes at harvest and the final wine produced from that fruit.

CHAPTER 2

REVIEW OF LITERATURE

INTRODUCTION

The genus *Vitis* is one of the most successful in the plant kingdom. Species are growing in every continent excluding Antarctica and have been cultivated for wine production for an estimated 10,000 years (Meredith 2001). During this time the resulting quality of *Vitis* species fruit fermentation has been constantly improving, more especially in *V. vinifera* cultivars. One aspect of quality improvement in those vines producing red fruit has been the study of anthocyanins and their response to various factors involved with said quality.

There are currently five known anthocyanins in the skins of red *V. vinifera* winegrape cultivars, the most predominant being Malvidin-3-glucoside, composing almost 90% of total anthocyanin in Pinot noir fruit (Boulton 2001, Gao et al 1997, Jackson 2000), and the major proportion in others (Boss et al 1996, Keller et al 1998, Roggero et al 1986). In *V. vinifera* L. cv. Pinot noir, the anthocyanins are located entirely in the skins, and occur at a much lower concentration than in other red *V. vinifera* cultivars. This results in a need to fully understand the extraction techniques before, during and after fermentation to adequately extract anthocyanin to obtain the highest quality possible – a feature of necessity in the current winemaking protocol.

The study of anthocyanin biosynthesis and activity in fruits has been extensively documented (Boss et al 1996, Brouillard and Delaporte 1977, Chandra et al 2001, El-Kereamy et al 2003, Gao et al 1997, Gil et al 1997, Holton and Cornish 1995, Jayaprakasam et al 2005, Keller and Hrazdina 1998, Roggero et al 1986, Seeram and Nair 2002, Seeram et al 2002, Wang et al 1999). Such work suggests external factors influencing final composition and concentration of anthocyanins in various fruits that include temperature, light, plant genetics, water and even crop-loading. However such external factors as location of culture (Terroir) and clone of *V. vinifera* L. Pinot noir have not been so well investigated.

The following review includes a discussion of many factors involved in the culture of quality *V. vinifera* L. Pinot noir vines and fruit, phenolic compounds in fruit and wines, external factors influencing the anthocyanin presence in the skins of winegrapes, and the effect of winemaking on the anthocyanins for extraction into the wine.

Vitis vinifera L. PINOT NOIR

History and Initial Geography of Pinot noir

V. vinifera is a species of thorn-less, dark-stemmed, bark-shedding plants, most of which include some sort of vine with tendrils opposing leaves on herbaceous stems that bear fruit. The cultivars of *V. vinifera* that exist are primarily the product of crossings either naturally or by breeding throughout time. Pinot noir is no exception; however it is very unclear where exactly it originates (Barr 1992, Fall et al 2002, Haeger 2004). There

are several opinions on the origins of *V. vinifera*. The first is that cuttings were taken from domestic vines in ancient world Transcaucasia (located between the Black and Caspian Seas, which now is known as Turkey, Iraq and Iran), in carpetbags with travelers on trade routes from the 10th millennium B.C.E. and distributed around Western Asia and Europe (Haeger 2004).

Another theory is, that in the first millennium B.C.E., Greek and Roman settlements in the western Mediterranean littoral, had already established its domestication – compounded by evidence that ceramic wine jars found in that area had the interiors identified as *V. vinifera* wine residue by chemical means, suggesting that one had to culture the grapes before making the wine. In Egypt, there have been hieroglyphic drawings of grapevines growing on trellis structures found, dated at third millennium B.C.E. Simple spreading of wild *V. vinifera by* reproduction is another likely theory (Barr 1992, Haeger 2004, Regner et al 2000).

DNA analysis has been employed in efforts to identify the origins of Pinot noir and other *V. vinifera* species (Aradhya 2003, Bowers et al 1993, Haeger 2000, Meredith 2001, Regner et al 2000). The difficulty associated with attempting to reconstruct the Pinot noir 'family tree' through progressive genetic steps is compounded by absence of cultivars in this sequence, leaving no trace of their existence. Work by Carole Meredith, formerly of the University of California, Davis, has established that Pinot noir, along with Gouais is in the genetic parents of Chardonnay, Gamay, and 14 others which are fairly common modern cultivars (Meredith 2001). Meredith also noted that the progeny of

Pinot noir and Gouais were unable to produce any successful offspring of their own, which alluded to how strange the cross of Pinot noir and Gouais was to begin with. This work was unable to identify an exact parental origin of Pinot noir all those millennia ago, but it does suggest how very old it is (Haeger 2000, Meredith 2001)).

Pinot noir is among the oldest *V. vinifera* cultivars. Its name comes from the noble Pinot family of Burgundy, France due to its pinecone shaped clusters (Haeger 2000). Pinot noir arrived in Burgundy in the 1st century AD, and there are many legends surrounding its arrival; a) one states that it came as cuttings by the Aedui from their invasions of Lombardy and Italy; b) a second suggests it arriving via the Romans during their invasions (Haeger 2000, Regner et al 2000).

When the Barbarian invaders drove the Romans from the Burgundy region, the Catholic church took custody of the fine Pinots collection. The monks used the Pinot noir vines to make wine that was used in the sacraments of the church, and they began to enjoy the Pinot noir wines produced sufficiently to refine its cultivation via study and experimentation. Such refinements included canopy management, irrigation and pruning (Barr 1992, Haeger 2000).

By the 6th century, most of the Burgundy region was divided into Church-owned vineyards. Despite improved cultivation methods, increased use and further study, no documentation of Pinot noir was found earlier than 1345. The monks also brought Pinot noir vines to the Rheingau region where it has been cultivated since 1470. These Church-

owned vineyards were seized and distributed to families in Burgundy during the French revolution around 1789 which resulted in the small family ownership and operation of these vineyards that is the current situation (Regner et al 2000).

International Distribution of Pinot noir

Pinot noir remains a steadfast cultivar in Burgundy, France. Over the last century, the cultivar has been slowly yet successfully, infiltrating the winegrowing regions of North America, South America, South Africa, Australia and New Zealand (http://www.nzwine.com). The distribution of Pinot noir is most likely a result of finding further suitable places to grow based on modern viticultural recommendations, and population expansion (Barr 1992, Haeger 2004).

CHALLENGES IN PRODUCING PINOT NOIR GRAPES AND WINE

Clonal Importance

Pinot noir is one of the least resistant of all the *V. vinifera* cultivars to biological mutation, it adapts very easily to new climates and physical conditions, to ensure it may benefit from, and succeed at growing in that area, although has not yet mastered warmer climates. This mutation results in a vast number clones available today, and because each one has a specific set of different attributes, these are useful in management as well as winemaking. Table 2.1 outlines the clones of Pinot noir assessed in Michigan, and gives qualitative attributes for each. For Pinot noir, it is exceedingly important, and nonetheless extremely helpful to have this many clones. Blending different clones in the winery has the potential to enhance the color, flavor and aroma of the cultivars' wine,

without needing to use other cultivars such as Cabernet Sauvignon which would remove the ability to claim the wine as purely Pinot noir (Jackson 2000).

Clone	Yield	Fruit Quality		Relative
		Fine	Rot	Cold Hardiness
1A	***	No	*	**
2A	**	Yes	*	**
4	***	Yes	**	****
9	***	Yes	**	****
13	*	Yes	*	*
15	*	?	**	***
17	*	No	*	*
22	***	No	**	***
23	*	No	*	**
29	**	Yes	**	****
31	*	No	*	*
32	**	?	*	**
33	**	No	**	**
K. Frank	***	?	*	**

**** Excellent

*** Very Good

** Acceptable

*Questionable

Table 2.1 Viticultural Factors Relating to Pinot noir Clones Evaluated by Howell et al (2002) at Michigan State University Research and Extension Center, Benton Harbor, Michigan.

In terms of viticulture, Pinot noir can be difficult to grow despite all the clones available. It tends to have a recumbent habit regardless of the common use of upright trellising systems in commercial viticulture around the globe, it has small tightly packed

clusters which lend it to numerous fungal diseases, and it is less cold hardy than most other *V. vinifera* cultivars (Barr 1992, Graham 1998, Haeger 2004, Howell et al 2000).

Specific challenges of the cultivar relate to vineyard and cellar impacts on pigments, especially those for color, as Pinot noir is very low in anthocyanins, relative to Cabernet Sauvignon and Merlot for example, and these pigments are only found in the skin of the grape berry. Therefore fruit and wine color are key components in wine quality assessment (Jackson 2000).

VITICULTURE

Current Status of Pinot noir Viticulture

Burgundy – The Region of Modern Origin

Located at 47° 16' 0" North, 5° 5' 0" East, Burgundy (Dijon, France) is the home of Pinot noir, and all red wine produced there is such. The wines of Burgundy are mostly produced by family owned estates, where the methods of vine culture and winemaking have been passed down through the generations. This, along with good clone selection and weather, can combine to yield high quality wines consistently (Barr 1992, Haeger 2004).

The climate in Burgundy seems ideal for the production of high quality Pinot noir; the limestone subsoil created after the area was underwater for over 80 million years is one of the greatest assets according to French winemakers, who believe that the soil is more important than the remaining climatic elements (Barr 1992, Haeger 2004).

Generally the climate is considered continental, which leads to less moderation of the seasons (hotter summers and colder winters) and faster, shorter ripening seasons. Burgundy is considered the model for Pinot noir production (Jackson 2000).

Vineyards in Burgundy are under strict appellation control, in which all vineyards must adhere to specific rules such as trellising system, vines per hectare, crop load, and vine type. This ensures that all estates are keeping the same level of quality consumers expect, and to avoid wines being improved by blending with other regions fruit (Haeger 2004).

Terroir

Terroir is a concept used to assist in defining a wine's quality based on its origin. Used initially in France, it only included soil type associations, but now has been more broadly defined. It is newly defined as including "physical and chemical aspects of soil, configuration of the terrain, meso-climate, rootstock, cultivar, vine age, cultural practices, grape berry micro-flora, vinification practices, and transport of the fruit and finished wines" (Reynolds and de Savigny 2001). Each one of these aspects is an important part of the life cycle of the vine and production of wine, and this is why the new definition was required. The use of the word "terroir" has become fashionable in the last ten years among wine drinkers, but there is not a complete understanding of its impact on the production of the wines (Haeger 2004). However, Wilson, J. (1999), suggests that terrior includes more than simply the elements of the vineyard habitat – "there is an additional dimension – the spiritual aspect that recognizes the joys, the heartbreaks, the pride, the

sweat and the frustrations of its history" (Wilson 1999). Most notably across the literature the term is not standardized, nor is it fully understood to be anything more than a current marketing technique, although recent research has included and attempted to define the term more scientifically (Barham 2003, Douguet and O'Connor 2003, Reynolds and de Savigny 2001).

PINOT NOIR IN THE NEW WORLD

Regions

The regions associated with Pinot noir production in the New Wine World include California, Oregon, Australia, South Africa and New Zealand. Vines here are primarily cared for in larger commercial vineyards, where mechanization is very important to ensure vines are under control (Jackson and Schuster 2001, Jackson 2000). Mechanization is a deviation from the time-honored methods of Burgundy, and result in more single canopy systems optimized for ease of mechanical use. The New World regions mechanize most of the work that in Burgundy is done by hand, including canopy management, leaf removal, weed control and harvest (Barr 1992). Some of these may detract from the ability of the fruit to express its potential in the wine, the harvest causing the greatest damage to fruit. For highest wine quality, vintners in the New World are evaluating less mechanized approaches, in order to improve their Pinot noir to its peak potential (Kliewer and Dokoolian 2005, Peterlunger et al 2002).

Climates and Soils

The New World wine regions have quite different climate and soil profiles to that of Burgundy. Most of these are more coastal and posses maritime climates such as New Zealand, Oregon, and South Africa. These climates allow more moderation of temperature extremes, longer growing seasons and lower disease conflicts due to decreased humidity (Haeger 2004). The soils are generally younger than those of Burgundy. New Zealand, for example, produces its best Pinot noirs on shallow sandy silty loams, compared with the very deep limestone bedrock of Burgundy.

Clones of Pinot noir

Clones are either genetic mutations of known stock, which once identified are propagated vegetatively to maintain their characteristics. Alternatively, the differences are due to individual varieties being selected many times from a wild population – this seems to be the way in which the vast number of Pinot noir clones came about (Howell et al 2000).

There are over a hundred known clones of Pinot noir, only a few of them are in constant commercial production. When looking at the clones for color and fruit quality, it depends on the location of culture, but generally more modern clones such as French 113, 115, 667 and 777 (UC Davis 44, 73, 72 and 71 respectively) are those which in cooler climates, produce higher color and wine quality than others. Below is table 2.2 showing the known clones of Pinot noir as catalogued by the University of California at Davis' Department of Enology and Viticulture, and their origin (Haeger 2004, Regner et al

2000). The picture is complicated by the fact that several sets of names and numbers can be used for these clones, and when dealing with such a large array it is easy to misunderstand the literature.

Clone Number	Origin	Clone Number	Origin	Clone Number	Origin
01A	Switzerland	51	France	84	France
02A	Switzerland	54	France	85	France
9	California	55	California	86	Italy
13	California	66	California	87	California
15	California	68	Italy	88	France
16	California	69	France	89	France
18	UC Davis	70	France	90	California
19	UC Davis	71	France	91	France
22	UC Davis	72	France	92	Italy
23	Switzerland	73	France	93	France
31	France	74	France	94	France
32	France	75	California	95	California
37	California	76	France	96	California
38	France	77	France	97	California
39	France	78	France	98	France
40	France	79	France	99	France
44	France	80	France	100	France
46	France	81	France	236	France
47	France	82	France	667	France
48	France	83	France		

Table 2.2 Pinot noir clonal list with country of clone origin. Modified from University of California, Davis' Website (http://fpms.ucdavis.edu/).

Rootstocks for Pinot noir

This area is of great significance as the point of contact and nutrient flow between the ever important soils and the cultivar scions above. Ever since phylloxera *Daktulosphaira vitifoliae* (Fitch) (Downie and Granett, 1998) was introduced to European vineyards, using a rootstock of a resistant *Vitis* species has been crucial to

ensuring vine growth, productivity and longevity. There have been many studies over the years on suitable rootstocks for different cultivars, and Pinot noir is no different. Courdec 3309 has long been used as a rootstock for Pinot noir, it is deep rooted and therefore more resistant to drought and physical damage, and it aids in production of good fruit through lower yields (Howell et al 1994). Other rootstocks also work well with Pinot noir, 101-14 produces a larger volume of structural wood than 3309, promotes low vigor, although it is shallow rooted and encourages slightly higher cropping (Haeger 2004). Riparia Gloire and Schwartzman are two further examples of suitable rootstocks, they provide low vigor and high quality fruit to their scion, and are fairly drought tolerant. In New Zealand the predominant rootstocks used for Pinot noir are; Schwartzman 01, Courdec 3309, Kober 5BB and 125AA, while Courdec 3309 predominates in Michigan (Howell et al 1987, Howell et al 2000, Jackson 2000).

Training Systems

Old World

In most old world winegrowing areas, especially Burgundy, there are rules dictating the training systems that may be used to support Pinot noir grapevines under certain conditions. Mostly, "V" type trellising is used, and this allows light to reach both sides of the fruit at once, as opposed to single canopy systems that only expose one side of each cluster regardless of canopy management approach (Haeger 2004). These "V" type systems require a split, cane pruned approach, when training a vine to suit the trellis chosen. This system does not allow as much accumulation of carbohydrate reserves as the cordon and spur pruned system, which keeps the main cordon, only pruning the new

growth back to short spurs (which depending on the system, range from two buds to eight). Some areas such as the Cote d' Or still use free standing vines in rows, and these are pruned in a bush type manner.

New World

This is where you will find much mechanization, and therefore mainly cane pruned single canopy grapevines. In New Zealand, the primary training system used for Pinot noir, is the two cane VSP system, trained to a single canopy vertical trellis. This ensures ease of mechanical operation, while still allowing easy access for manual tasks that are steadily overtaking mechanical in boutique wines (Jackson and Schuster 2001). Michigan wine industry, being familiar with successful juice grape cultivation, has also implemented VSP systems for Pinot noir, but also uses spur pruned cordon systems as well (Howell et al 1994, Howell et al 1987). Studies conducted by Peterlunger et al 2002, concluded that Pinot noir training systems affected yield, but showed little or no impact on grape or wine composition. The sensory analysis conducted in the trials also could not show relevant differences between training systems used for Pinot noir (Peterlunger et al 2002). However this does not agree with the work of Kobler et al (1994), who found that Pinot noir training systems did affect grape composition significantly. The conflict between the two studies appears to relate to vine growing locations. Peterlunger et al, (2002) used vines grown in Northeastern Italy, whereas Kobler et al (1994), used vines grown in Switzerland, which is further north and has less suitable growing conditions for Pinot noir.

Canopy Management

Canopy management is a critical, labor intensive part of producing fine Pinot noir grapes, and has received much research over the last quarter century (Candolfi-Vasconcelos et al 1994, Howell et al 1994, Howell 2001, Kliewer and Dokoolian 2005, Koblet et al 1994, Miller et al 1996, Peterlunger et al 2002, Spayd et al 2002). The canopy must be kept open to allow for air and light to penetrate lowering the risk of fungal infection, and increasing the ability of fruit to ripen, respectively. Fruit ripening is affected by canopy openness because the relationship between sunlight exposure and temperature of grape clusters is important to berry composition and metabolism (Spayd et al 2002). The canopy needs to be open enough to allow sufficient sunlight exposure required for anthocyanin synthesis without stripping the canopy of leaves to shade the fruit from over-exposure to the sun resulting in berry sun-burn and reduced quality (Spayd et al 2002). The techniques to achieve this include shoot positioning, leaf removal from the fruiting zone, canopy trimming and spraying (Haeger 2004, Howell et al 1994, Miller et al 1996).

Shoot positioning ensures a single canopy, and prevents vine shoots from becoming overlapped and too dense this requires slow manual work. Leaf removal occurs at veraison and again if required, where leaves are removed from shoots in a small area around the fruiting zone, typically about 30 cm to reduce the shading of fruit and increase exposure to the sun which increases ripening and accumulation of fruit pigments (Howell 2001, Howell et al 1994, Jackson 2000). Canopy trimming normally occurs mechanically, although it can be achieved manually in smaller vineyards. Vines have

excess shoots removed at a certain pre-set canopy height and width (Howell et al 1987, Jackson 2000). Generally lateral shoots will sprout from these topped primary shoots and the process will have to be repeated again before harvest. Trimming is also used to reduce canopy shading (Jackson and Schuster 2001). Spraying the canopy with various fungicides and insecticides, aids in the prevention or suppression of pests and diseases that destroy both vegetative and reproductive parts of the vine (Howell 2001). Commonly, sprays are timed to coincide with canopy and cluster development stages which optimizes their performance (Howell 2001, Howell et al 1987, Howell et al 2000, Jackson 2000).

Cropping

Pinot noir vines are generally low producing vines, possessing small and compact clusters (Haeger 2004). Many factors influence crop levels, and these start with anlagen initiation, and continue to include cluster initiation, flowering, fruit-set, vine vigor, health status, irrigation, and genetic predisposition of the vine (Barr 1992, Haeger 2004, Howell et al 2000).

Suggested crop levels for the fine Pinots are around 6.5-11 metric tons per hectare depending on conditions of culture such as soil type, vine health and climate. Pinot noir destined for sparkling wine can be cropped at near 20 tons per hectare, since fruit maturity at harvest is less, and is normally achieved with a high yielding clone such as UCD 23 (Barr 1992, Howell et al 2000, Jackson and Schuster 2001).

Fruit Thinning

To achieve recommended cropping levels, methods of crop estimation must be employed, and if required may be followed up with a technique called fruit thinning or crop adjustment. This occurs by hand, and can include the removal of primary set over-crop, but also the second-set provided by the lateral shoots which is very common in Pinot noir (Jackson 2000). Usually the fruit will be removed from the apical position first, and on every second or third shoot, depending on current and desired cropping level. The timing of fruit thinning is important to ensure the process impacts the fruit quality sufficiently to justify its use (Howell 2001). Several physiological times are used for fruit thinning. Green drop as the name suggests occurs at veraison while the least ripe fruit are still green (in the case of red cultivars) or pre-veraison. Fruit thinning may also occur post-veraison (Jackson 2000). The work of Smithyman, Howell et al (1998) suggests that post-set cluster thinning is a viable cultural practice to reduce harvest season cluster rot and improve yields in Seyval blanc grapevines. Performance of the final crop is greatly enhanced by the removal of excess set clusters which drain the vines resources and extend the required maturity time beyond reasonable limits (Creasy and Lombard 1993, Howell et al 1987, Howell el at 2000, Jackson 2000, Winkler et al 1974).

Harvest

Criteria

The criteria on which the decision to harvest is based are extremely important. Generally, harvest has been decided on numbers such as °brix (or Baume in France), titratable acidity, pH, and the upcoming weather conditions. More traditionally in Old

World areas, the harvest has been decided not by the numbers alone, but by components of berry sensory analysis, including color, flavor, aroma, and turgidity. These methods are now becoming more commonplace in New World regions also, but continue to include a consideration for the numbers. Optimum Pinot noir harvest criteria for still red wine include: a) berries that appear slightly shriveled; b) full color coverage across the cluster; c) the presence of deep red, ripe cherry, raspberry, cinnamon and plum flavors; and d) a fresh fruity and floral aroma. Suitable numbers commonly complementing the sensory criteria include 23 - 24°Brix, 8.5 - 11.0g/L TA and 3.15 – 3.35 pH, all of which will lead to a full bodied, well balanced and desirable wine which has the potential to be great.

Methods

Once the decision to harvest has occurred, there are two main methods, yet only one should really be considered for fine Pinot noir. The use of machine harvesters, while common and desirable for white wines is too rough and damaging to the delicate Pinot noir clusters. It is unsuitable because of the thin skins of Pinot noir berries, and the speed at which harmful oxidation takes place destroying the color pigments, along with aroma and flavor compounds (Jackson 2000).

Hand harvesting Pinot noir is the only way to physically ensure that the fruit arrives at the winery in best possible condition, with minimal berry splitting and oxidation (Jackson 2000). Hand harvest is more laborious and thus more expensive, but it provides a superior product from which winemakers can craft quality wines to justify

such means. Hand harvesting usually consists of people cutting clusters into small bins, and then collecting those into slightly larger bins for transport to the winery (Jackson 2000).

Climate Challenges

A cool climate is one in which grapes after harvest should not need chilling, nor heating during processing, or in preparation for the ferment (Jackson and Schuster 2001). Pinot noir is a cool climate grape that performs best when ripening slowly over a long season, as compared with rapid ripening in a warm, short season, or very slowly maturing to inadequate ripeness in a cold climate (Haeger 2004, Howell et al 2000, Jackson and Schuster 2001). Burgundy is the northern limit for producing fine red table wines from Pinot noir, as Champagne, only 150 miles north, seldom ripens a single cluster, and for this reason became very successful at producing fine Champagne sparkling wines. The opposite is also true of Pinot noir. Southern California produces high sugar (alcohol) wines that are beyond the winemakers control because of the intense heat and length of season that occurs there. For whatever reason, Pinot noir of any clone is adapted to growing and producing its best crop in a cool climate, when managed correctly (Jackson and Schuster 2001).

Challenges of the Michigan climate include severe winter temperatures, that sometimes reach -35°C (not including wind chill effect) but more regularly average about -25°C, and last for much longer than more southern areas. This extreme cold in winter causes extreme stress to most species of wildlife and plants, not to mention tender *V.*

vinifera cultivars, which in many cases can cause complete winter-kill of entire mature vines (Howell et al 1987). Although the Great Lakes provide some maritime climate stability, Michigan is still at the interior of a large continent, which can cause very high humidity, 80-85% RH. The high humidity becomes more noticeable during summer months when the air temperature regularly achieves 30 - 35°C, although is still present during winter months. High humidity can cause great problems for vineyards, with an increased risk of fungal activity and destruction of vines and fruit (Howell et al 1987). Michigan also has low sunlight interception, as complete cloud cover in the skies above occurs on more than half of the days in any year which results in lower ripening potentials from any crop, although does have low winds and few birds to destroy crops (Howell et al 1987).

Canterbury, New Zealand is a maritime climate, and is void of almost any of the types of problems experienced in Michigan. However it has many of its own. The area is known nationally for its high winds that during summer can regularly become severe. These gale force winds cause havoc in vineyards, can break trellising, and damage fruit. The small land area which leads to high concentration of vineyards has in turn produced a high density pest and disease problem. Vineyards in this area must be covered with netting during ripening to prevent birds stripping the entire crop, which can occur in a matter of hours rather than days. Fungal infections are very prolific, and due to their high density and the regions mild winters, tend to over-winter easily and return to the vineyard consistently without proper spray programs in place (Jackson and Schuster 2001). Rainfall in Canterbury is very low, especially during the ripening season, and almost

every vineyard needs an extensive irrigation system to ensure vines obtain optimum water requirements for growth and production.

RED WINE COLOR

Wine color is the very first thing that a consumer perceives about a wine, making this a very important topic to winemaking and sales. There are many chemical issues to consider when discussing this part of winemaking; fortunately, there is currently a lot of research and understanding in this area (Creasy and Logan 2003).

Human Perception of Color

The perception of color differs among individuals and can be altered by each individual's eye sight, retinal damage, color-blindness, DNA, experience (with what colors actually look like) or a plethora of other reasons. When studying wine color, it is important to take into account that not everyone will see the color in the same way, and since the color is the first sensory assessment that the consumer makes of the wine, this often influences the highly subjective quality assessment of the product (Creasy and Logan 2003, Neguereula et al 1995).

The Chemistry of Color in Grapes

The Grape Berry

Berries of winegrapes (*Vitis vinifera*) are composed of many compounds sequestered into 4 physical areas: the seed, juice, pulp and skin. Each part of the berry has a different composition of phenolic compounds, which are precursors of color, flavor

and aroma pigments (Waterhouse and Kennedy 2004). The knowledge of this within-berry partitioning is a crucial part of winemaking, as it determines the processing and fermentation methods, and the style of the resulting wine (Mazza et al 1999). The seeds possess the highest concentration of phenolics in the berries; however this is mainly composed of tannins, which on their own do not provide color to the wine. Anthocyanins (which provide color to the wine, either on their own or in complexes with other molecules such as tannins) are found mainly in the skins. This is the main reason for skin contact with the juice before, during and after the fermentation. The juice and pulp of wine grape berries together contain less than 5% of the total phenolics found in each berry (Lorenzo et al 2005).

WINE-GRAPE PHENOLIC COMPOUNDS

Phenolic Compounds

Phenolic compounds are very important to wine as they influence its sensory characteristics especially color, flavor, aroma, haze/sediment formation and anti-microbial properties (Jackson 2000). Red wines have a much higher concentration of phenolic compounds than white wines (German and Walzem 2000, Ibern-Gómez 2002, Monagas et al 2005). This concentration of phenolic compounds heavily influences the style of the resultant wine. Phenolic compounds found in winegrapes include flavonoids and non-flavonoids, the former being primarily important in red wine color. When flavonoids and non-flavonoids form polymers in wine either together, or separately, they are commonly known as tannins (Jackson 2000).

The most common flavonoids in wine include flavonols, catechins (flavan-3-ols), anthocyanins (in red wine) and small amounts of leucoanthocyanins (flavan-3, 4-ols) (Jackson 2000). The flavonoids may exist free, polymerized to other flavonoids, sugars or non-flavonoids, or a combination of the above free and polymerized entities. Synthesis of flavonoids occurs in the endoplasmic reticulum before being transported to, and stored in, the central vacuole of the producing cell (Jackson 2000). They form in response to ultraviolet light radiation exposure from sunlight and are easily extracted into the wine, and water soluble (Pérez-Magariño and González-San José 2004). The flavonols, which are very bitter, make very strong co-pigments and have low redox potential (Boulton 2001). Flavonoids comprise more than 85% of the total phenolic content of a red wine, but less than 20% of that present in white wines (Garcia-Alonzo et al 2005, German and Walzem 2005, Mazza et al 1999).

Tannins – Complex Phenolics

Tannins consist of catechin, epicatechin and epicatechin gallate subunits which occur mainly in the stems and seed coat of wine grape berries, and not in the skins (Jackson 2000). Those tannins present in the skins of the grape, are characterized by a higher degree of polymerization than those from the seeds and stems (figure 2.7 and 2.8). Some cultivars, such as Pinot noir, do not produce any skin tannins. This is one of many reasons and mechanisms whereby Pinot noir's final wine color is less stable or intense than Bordeaux cultivars, such as Cabernet Sauvignon or Merlot (Haeger 2004, Jackson 2000).

When the crushed fruit is fermented in contact with the seeds, as in most red wine ferments, the tannins are extracted from the seeds into the wine and begin forming stable compounds with other entities (Sacchi et al 2005). These other entities include anthocyanins, in reactions called co-pigmentation which lead to more stable color, flavor and aroma molecules. However, for the most part, tannins add complexity, mouthfeel, bitterness and astringency to a wine (Boulton 2001).

Pigmented Polymers

The most important colorless compounds involved in wine color are pigmented polymers (Boulton 2001). These arise from catechins, proanthocyanidins and other wine pigments reacting together during the ageing phase of wine to produce these larger, and more stable compounds (Boulton 2001). Recently, this polymerization of the wine pigments, including the anthocyanidins, has been thought to vastly increase the stability of the perceived color from less stable color pigments in wine. Normally during the wine ageing process, the bright red colors from anthocyanins in a young red wine, will form associations with other molecules, particularly oxygen, to alter color to the brick-red hue experienced in an older red wine. However, with pigmented polymers occurring in the wine, this color degradation during the ageing process becomes slower, as the anthocyanidins and anthocyanins are larger, more stable pigments, making them harder to alter via the traditionally accepted methods (Peng et al 2002, Waterhouse and Kennedy 2004).

Anthocyanins

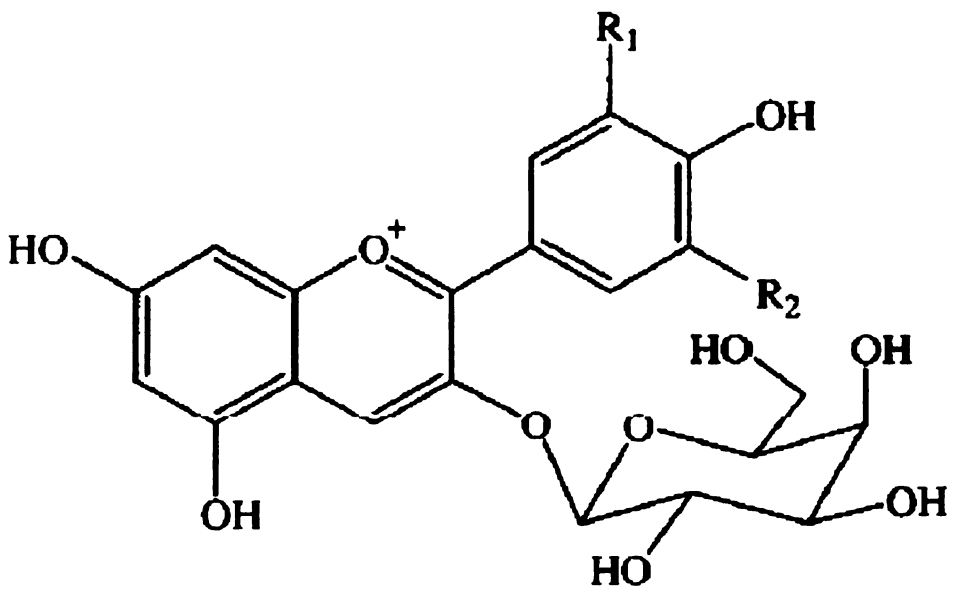
Anthocyanins are found in the skins of red grape varieties, and may combine with other compounds to become more stable in solution during such reactions as co-pigmentation and acylation. These reactions and subsequent color can be affected by many things that are common to wine: ethanol, pH, sulfur dioxide and oxidation, not to mention plant regulatory genes (Ali and Strommer 2003, Boulton 2001, Brouillard et al 2003, Creasy and Logan 2003, Garcia-Alonzo et al 2005, Holton and Cornish 1995, Jackson 2000).

The anthocyanins in grapes predominantly exist as glucosides that are formed through the conjugation of the flavonoid component (an anthocyanidin) with glucose (Sacchi et al 2005). Those present in grapes, are found in five different forms: Malvidin-, Peonidin-, Petunidin-, Delphinidin-, and Cyaninidin-3-glucoside (Boulton 2001, Lorenzo et al 2005, Monagas et al 2005). This glycosylation increases the chemical stability and water solubility of the anthocyanidin. This can now be further complexed by bonding with acetic-, coumaric-, or caffeic acid at the sugar component (Boulton 2001, Jackson 2000, Sacchi et al 2005, Wang and Sporns 1999, Wightman et al 1997).

Structure and Biosynthesis of Anthocyanins

The structure of winegrape anthocyanins is given below (figure 2.1). Anthocyanins are usually categorized by the number of sugar molecules per anthocyanidin. As such, most grape species produce anthocyanins with both mono-glucoside and diglucoside structures, the later being more stable yet more susceptible to

browning. However *V. vinifera* can only produce the monoglucoside form of anthocyanins, as it lacks the dominant allele that regulates the production of diglucoside anthocyanins (Jackson 2000).



Anthocyanin	R_1	R_2
Malvidin-3-glucoside	OCH_3	OCH_3
Petunidin-3-glucoside	OCH_3	OH
Peonidin-3-glucoside	OCH_3	H
Delphinidin-3-glucoside	OH	OH
Cyanidin-3-glucoside	OH	H

Figure 2.1 Anthocyanins present in *Vitis vinifera* L. Pinot noir fruit and young wines. Modified from Seeram et al 2002.

Recently, there has been a large increase in both variety and magnitude of studies related to anthocyanins in plants (Ali and Strommer 2003, Boulton 2001, Brouillard et al 2003, Chandra et al 2001, Creasy and Logan 2003, El-Kereamy et al 2003, Garcia-Alonzo et al 2005, Hyung et al 2003, Jayaprakasam et al 2005, Kennedy et al 2001, Kennedy et al 2002, Seeram et al 2002, Seeram et al 2003, Wada and Ou 2002, Zhang et al 2005). These all resonate similar techniques regarding the structure and synthesis of

anthocyanins, alterations to the biosynthesis occurring as a function of the plant medium and location.

Different plants are composed of different anthocyanins, for example in Cherries (*Prunus avium* L.) the major, and sometimes only anthocyanin found is cyanidin (Gao and Mazza 1995). The dominant anthocyanin by far in winegrapes and their resultant wines is that of malvidin-3-glucoside, which, in the case of Pinot Noir, accounts for 90% of total grape anthocyanins (Boulton 2001, Gao et al 1997, Jackson 2000) and a similarly high proportion in all other *V. vinifera* grapes and wine (Boss et al 1996, El-Kereamy et al 2003, Kennedy et al 2001, Roggero et al 1986).

The biosynthesis of anthocyanins has been extensively investigated over the last few decades (Boss et al 1996, Brouillard and Delaporte 1977, Chandra et al 2001, El-Kereamy et al 2003, Holton and Cornish 1995, Roggero et al 1986). Largely, the work has been conducted in three primary species: maize (*Zea mays*), snapdragon (*Antirrhinum majus*) and petunia (*Petunia hybrida*), although petunia has more recently become the most popular organism for the research (Holton and Cornish 1995). Figure 2.2 gives a generalized biosynthetic pathway as exists in the three aforementioned plants. It shows the production of pelagonidin-, cyanidin-, and delphinidin-3-glucoside via regulatory genes from precursors p-Coumaroyl- and Malonyl-coenzymes. This pathway produces different products in every species because of the effect of plant-specific genetic make-up. The work of Holton and Cornish (1995) discusses the specific anthocyanin products occurring in petunia flowers (*Petunia hybrida*), showing how only cyanidin-, and

delphinidin-3-glucoside are produced initially, and the reactions showing how cyanidin-3-glucoside produces peonidin, and how delphinidin-3-glucoside leads to the production of both petunidin, and malvidin via a separate biosynthetic pathway (figure 2.3).

Using *Vitis vinifera* L. cv. Shiraz berries, figure 2.4 shows a truncated version of the synthetic pathway for the five anthocyanins found in *V. vinifera* and this alludes that, when malvidin-3-glucoside is the primary anthocyanin in grapes, the delphinidin pathway is preferred (Boss et al 1996). The anthocyanin biosynthetic pathway found in *V. vinifera* L. Pinot noir is notarized to show the entire (shikimic acid) pathway from photosynthesis to the final production of the five grape anthocyanins (figure 2.5). This figure shows how important each step in the pathway is to the final concentration of each anthocyanin in the grapes, and how many genes have a direct affect on the production of the end products throughout the pathway. The last step in each branch of the pathway (cyanidin side and the separated delphinidin side) labels the conversion to peonidin, petunidin and malvidin from their precursors using the gene methyltransferase, which is a determining factor in the final concentrations of anthocyanins. This biosynthesis pathway, while starting with photosynthesis, does not initiate until veraison, although it continues from that point right through to harvest or late season senescence. Factors involved in determining the level of anthocyanins produced overall, can be influenced by variety, growing region and conditions of culture (Boss et al 1996).

Figure 2.2 Anthocyanin and flavanol biosynthetic pathway that yields Pelargonidin-, Cyanidin- and Delphinidin-3-glucoside. (Modified from Holton and Cornish 1995).

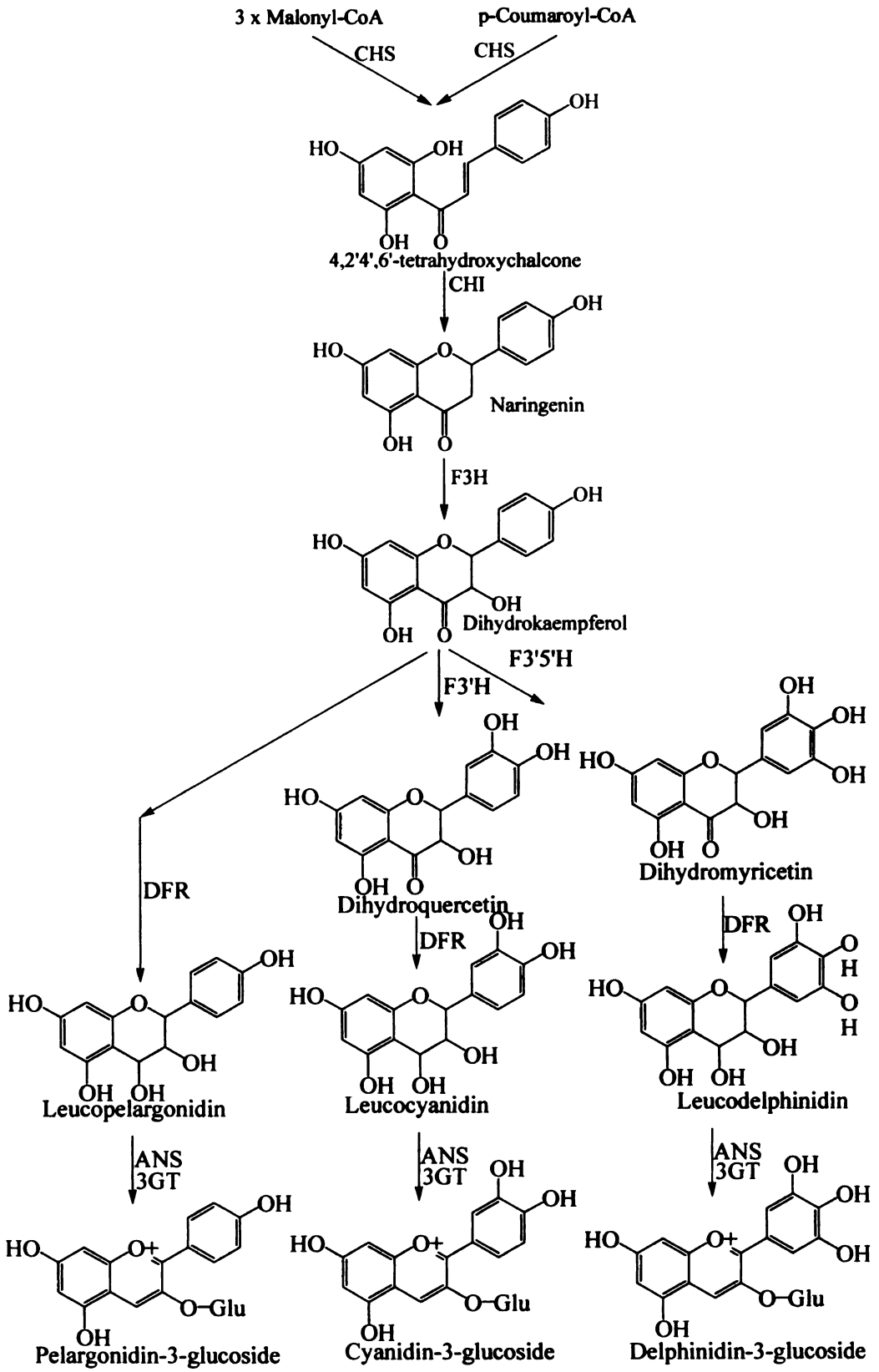


Figure 2.3 Genetic control of anthocyanin modifications as occur in petunia (*Petunia hybrida*) to show Petunidin and Malvidin synthesis. (Modified from Holton and Cornish 1995).

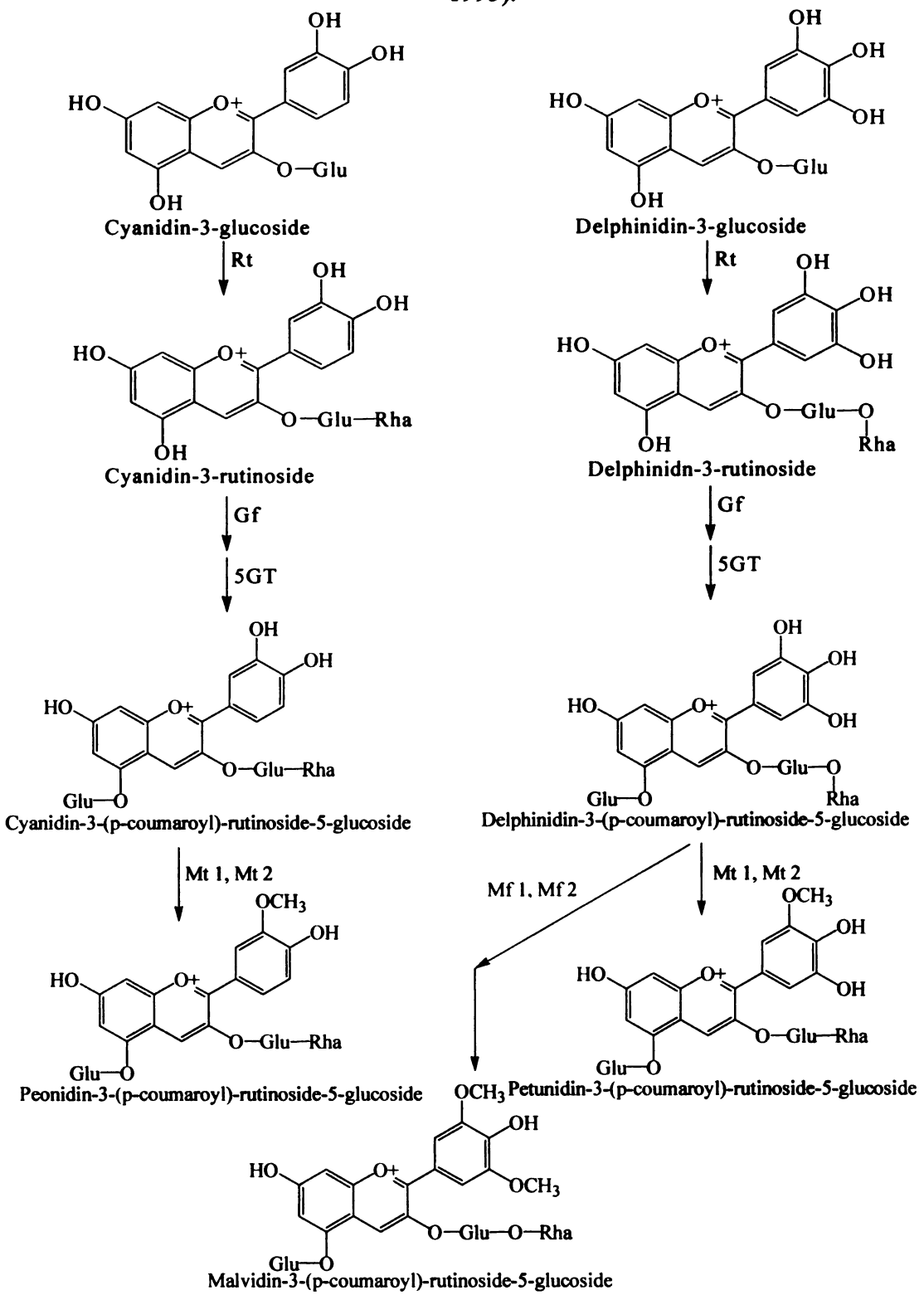


Figure 2.4 The simplified schematic of the anthocyanin biosynthetic pathway modified to account for the major products found in grapes. (Modified from Boss et al, 1996).

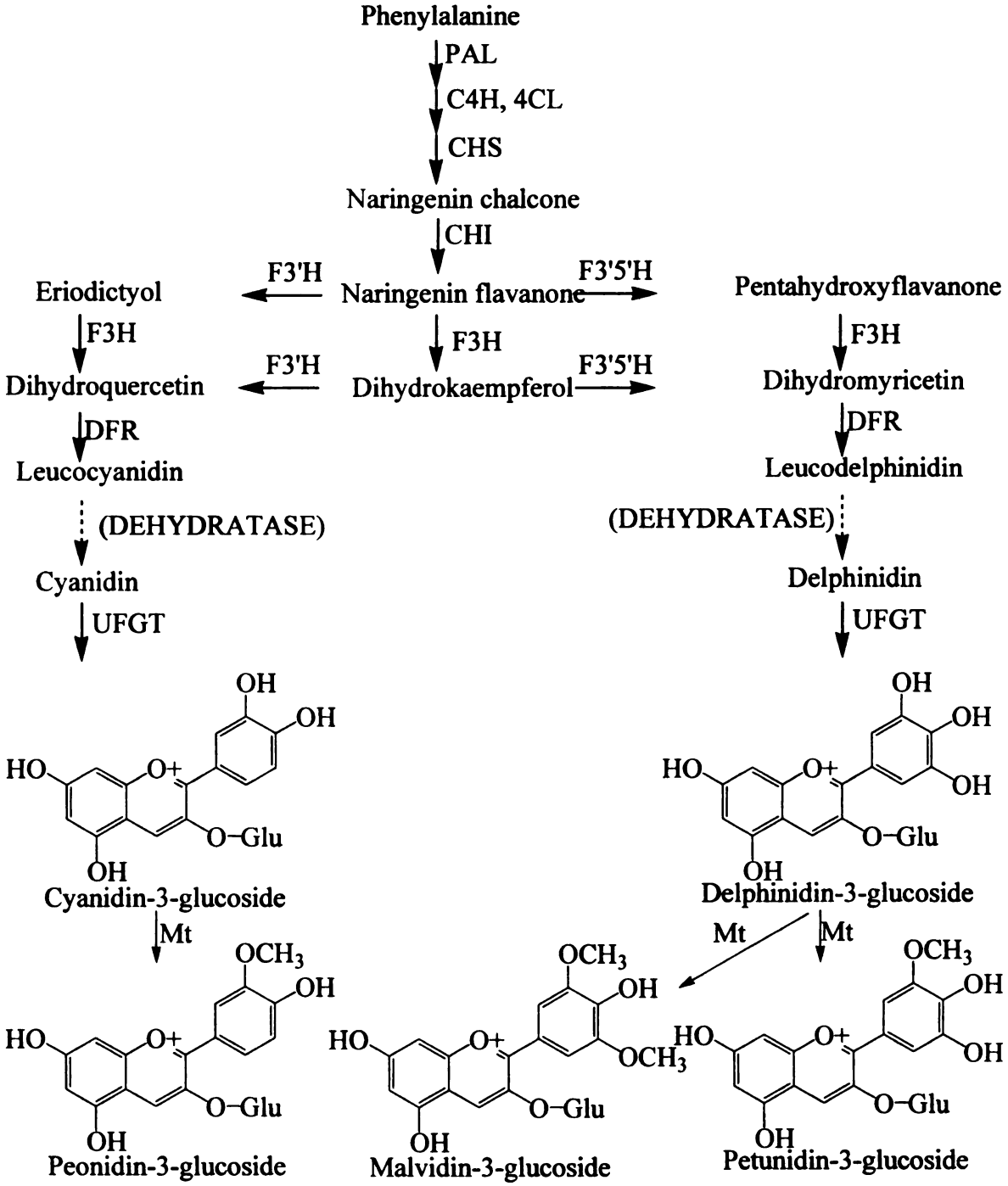
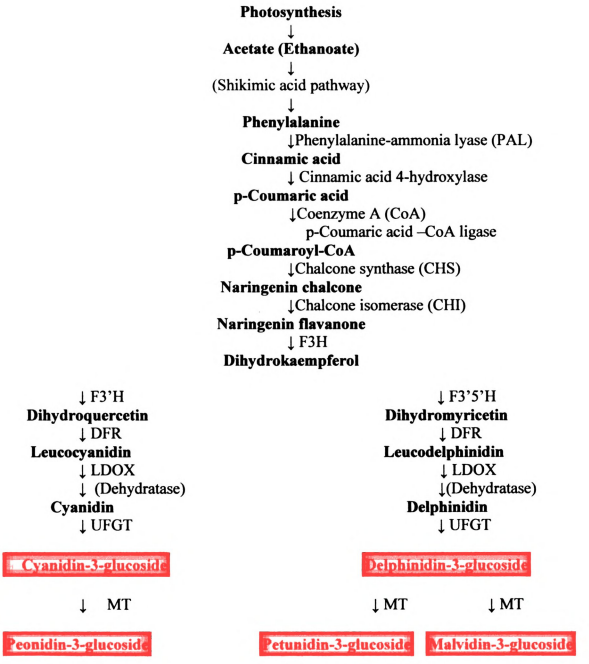


Figure 2.5 Anthocyanin biosynthetic pathway starting at photosynthesis, as occurs in *Vitis vinifera* L. Pinot noir Adapted from Boss et al 1996, Holton and Cornish 1995, and Sullivan 1998.



Compounds Influencing Pinot noir Color

The predominant anthocyanin in grapes, malvidin-3-glucoside, provides most of the color to a young red wine (Boulton 2001, Brouillard et al 2003). In the berry, the intensity of red colors adheres to the steric effect, whereby it increases with increasing methylation of the anthocyanin molecule. However, due to the nature of the anthocyanin chemistry, there is no correlation between the concentration of anthocyanins in a young red wine and its color (Boulton 2001). The process by which anthocyanins are removed from the grape skins to add color to the wine is called maceration, which extracts diverse phenolics from the berry skins and grape solids, especially hydroxycinnamic esters and flavonoids (mostly made up of anthocyanins, when speaking purely of color) (Jackson 2000). However, due to the reactivity of the anthocyanins, they disappear gradually from the wine, due to their involvement in different reactions, and as time passes, more stable pigmented polymers appear which are responsible for the color in aged wines (Ali and Strommer 2003, Boulton 2001, Brouillard et al 2003, Creasy and Logan 2003, Lorenzo et al 2005, Medina et al 2005, Monagas et al 2005, Sacchi et al 2005).

Factors that affect the expression of the wine color by the anthocyanins are pH, SO_2, polymerization and co-pigmentation (Jackson 2000).

pH

The effect that the pH has on the wine solution is huge, notwithstanding the impact on the color of the wine. The pH affects the anthocyanins that exist in the flavylium state – a low pH leads to an increase in the concentration of the flavylium state,

which in turn improves the red color of the wine. As the pH rises, the proportion of anthocyanins in the flavylium state decreases rapidly (Jackson 2000). Therefore, in a wine solution, as the pH is brought down artificially with hydrochloric or a similar acid, the solution becomes less blue, and more red, and the reverse occurs at pH levels above the normal wine pH where a dark blue is obtained. During the early stages of winemaking, it is imperative to ensure that the pH level in the grape must and ferment is controlled, in order to keep the color in a suitable range (Rankine 2002). In primarily cooler climates such as Michigan, deacidification with calcium carbonate can be undertaken in order to raise the pH above 3.20, and in warmer climates such as California, it may be necessary to perform acidification, normally carried out with the addition of tartaric acid, to attain the pH below 3.80 (Iland et al 2000, Jackson 2000, Rankine 2002).

Sulfur Dioxide

Free sulfur dioxide, while protecting the wine against oxidation, may also affect the color of the wine. The amount of free sulfur dioxide in wine must therefore be monitored closely throughout the entire winemaking process to ensure that it is high enough to prevent oxidation, but not so high as to cause widespread anthocyanin damage. This is reversible in solution, and as the free sulfur dioxide is consumed by oxidative reactions occurring in wine, its ability to bleach the anthocyanins is vastly reduced (Jackson 2000).

Co-pigmentation

Accounting for up to 50% of the color in young red wines, co-pigmentation is a very important phenomenon in wine and anthocyanin chemistry discussions. Traditionally it has been dismissed, although more recently it has become an area for intense research and much advancement. Co-pigmentation is a molecular association occurring between the colored forms of the anthocyanins and other compounds (normally colorless), whether phenolics or not, to form non-covalent complexes vertically stacked (Boulton 2001, Brouillard et al 2003, Lorenzo et al 2005). These associations are maintained by hydrophobic interactions between aromatic nuclei. The most important feature of co-pigmentation is not only the fact that it accounts for up to 50% of the young red wine color, but that this color comes from primarily colorless molecules (Boulton 2001). A range of chemical substances can act as co-pigments with anthocyanins. Examples of these chemical substances include flavonoids (including other anthocyanins), organic acids, amino acids, polysaccharides, and many others (Boulton 2001, Brouillard et al 2003, Sacchi et al 2005). Factors that affect the co-pigmentation of anthocyanins in wine or grapes include pH, ethanol concentration, temperature, and the amount and type of other compounds such as phenols and flavonoids available to act as co-pigments. Both types of co-pigmentation complexes, that is anthocyanin-anthocyanin complexes and anthocyanin-other phenolic complexes, increase the light absorbtion and the color density of both grape skins and resultant wines (Boulton 2001, Brouillard et al 2003, Lorenzo et al 2005, Parley et al 2001, Sacchi et al 2005).

Co-pigments need not be colored, but they must have a flat and polarizable part in their structure that will allow them to associate with the anthocyanin (Boulton 2001). These co-pigmentation interactions and reactions explain the variety of color hues and intensities found in flowers and fruits, at pH values where anthocyanins are normally colorless. Figure 4.3 shows anthocyanins interacting by the process of self-association, in which flavylium and quinoidal colored anthocyanins can form associations in a left hand spiral arrangement that is held together by electrostatic and hydrophobic interactions (Boulton 2001, Waterhouse and Kennedy 2004).

Co-pigmentation reactions can increase the observed color by up to ten times that of the non-co-pigmented anthocyanin. Considering that this ten-fold increase is caused by colorless entities can be somewhat difficult to believe, but it has been shown numerous times (Lorenzo et al 2005, Pozo-Bayón et al 2004, Waterhouse and Kennedy 2004).

Recent unpublished work of Boulton, R, gives the protocols for estimating the content of co-pigmented anthocyanins in the wine solution: Measurments include; A^{acet}: 20μL of 10% acetaldehyde solution is added to 2 mL of wine sample in a 10 mm plastic cuvette, after 45 minutes, the sample is placed in a 2 mm cuvette and the absorbance is measured at 520nm, the reading is corrected the shorter path length by multiplying by 5 (1 mm cuvettes may need to be used for highly colored samples). A^{20}: 100μL of wine sample is placed into 1900μL of the buffer in a 10 mm cuvette, after a few minutes, the absorbance is measured at 520nm, the reading is corrected for the dilution by multiplying by 20 (for highly pigmented young wines (A^{acet} >10 AU) a dilution of 40 or 50 is

recommended, then multiplied by the corresponding correction factor). $\mathbf{A^{SO2}}$: 160μL of 5% SO2 solution is added to 2 mL of wine sample in a 10 mm cuvette, the absorbance is measured at 520 nm. $\mathbf{A^{280}}$: Depending on the full scale range of the spectrophotometer, the A^{acet} or A^{20} samples may be used to measure A^{280}, provided they are quartz cuvettes, otherwise a dilution of 1/100 is required (100 mL into 10 mL of water and the reading corrected by multiplying by 101). $\mathbf{A^{365}}$: As for the A^{280} measurement but at 365 nm. The buffer solution is: 24 mL pure ethanol added to 176 mL distilled water, dissolve 0.5 g of potassium bitartrate into the solution, the pH of the solution should be adjusted to 3.6 with HCl or NaOH as needed. Calculations include:1) Color due to Co-pigmented Anthocyanin: [C] = (A^{acet} - A^{20}) absorbance units (at pH 3.6). 2) Total Anthocyanin: [TA] = (A^{20} - A^{SO2}) absorbance units (at pH 3.6). 3) Color due to Polymeric Pigment: Ep[P] = A^{SO2} absorbance units (at pH 3.6). 4) Estimate of Flavone Cofactor Content: [FC] = A^{365} absorbance units. 5) Estimate of Total Phenols (Monomers plus Tannins): [TP] = A^{280} absorbance units. 6) Fraction of color due to Co-pigmentation: (A^{acet} - A^{20})/A^{acet}. 7) Fraction of color due to Free Anthocyanins: (A^{20} - A^{SO2})/A^{acet}. 8) Fraction of color due to polymeric pigment: A^{SO2}/A^{acet}.

MEASUREMENTS OF ANTHOCYANINS IN WINE

The literature has a plethora of references (Ali and Strommer 2003, Boulton 2001, Brouillard et al 2003, Byers 1999, Creasy and Logan 2003, Garcia-Alonzo et al 2005, Ibern-Gómez et al 2002, Jayaprakasam et al 2005, Mazza et al 1999, Peng et al 2002, Waterhouse and Kennedy 2004, Wightman et al 1997) to, and discussions about the measurements of said compounds and even more about the mechanisms of the machines

themselves, offering suggestions to more suitable or more reliable methodologies. The material presented below goes into some depth to describe each type of device used to measure anthocyanins and why each is so important to the study of wine color.

High Pressure Liquid Chromatography

HPLC is the most common form of chromatography in use today for viticulture and enology. It is such as it provides the user with a qualitative and accurate quantitative assessment of grape and wine extracts, and in particular phenolics and anthocyanins (Ali and Strommer 2003, Boulton 2001, Ibern-Gómez et al 2002, Monagas et al 2005). Anthocyanins are detected at 520nm, due to the colored flavylium forms having a maximum absorption at about this wavelength (Iland et al 2000).

For the HPLC of anthocyanins, the analytical process uses reverse phase chromatography in which a non – polar stationary phase and a polar mobile phase are used (Waterhouse and Kennedy 2004). The stationary phase is usually a C_{18} polymer bonded to a silica support, although polymer columns are now becoming more common and they are stable over a wider pH range (Zoecklein et al 1995). This stationary phase listed here is that which can separate monomeric, oligomeric and polymeric anthocyanin fractions from all other phenolic compounds. The packing for these columns is usually of a very small particle size of 4-5μm which ensures a high number of theoretical plates hence, optimum resolution and efficiency. The mobile phase for HPLC is usually methanol or acetonitrile that are both common organic modifiers and solvents (Weston and Brown 1997).

Acidification of the anthocyanins prior to injection is necessary to prevent the ionization of the acid groups in the phenolics, which should take the pH to around 1.5 for anthocyanins to get sharp peak resolution, although some authors recommend pH 3.0 or 3.6 (Boulton 2001). When the anthocyanin solutions are injected for analysis at higher pH values, then there is broadening of peaks, due to the interference of anthocyanins in forms other than the flavylium form (Iland et al 2000, Lough and Wainer 1996, Waterhouse and Kennedy 2004, Weston and Brown 1997, Zoecklein et al 1995).

Spectrophotometry

The measurement of wine absorbance by spectrophotometry is widespread. It can be used to determine concentration of both phenolic compounds and anthocyanins present in wine, aside from color hue and density measurements. These spectral techniques are all extremely useful and allow for meaningful measurement of wine color (Byers 1999, Gore 2000, McLaren 1980).

The concentration of anthocyanins in young wines can be calculated from absorbance readings taken at a wavelength of 520nm. As red wines age, this absorbance at A_{520nm} decreases while the absorbance at A_{420nm} increases, due to the shift between monomeric and polymeric anthocyanins. Recently, there has been a recommendation by several authors to also record the absorbance of wine at 620nm (Waterhouse and Kennedy 2004). The red zone of young wines has not been fully dealt with in previous calculations, and as a result included a new calculation with the other two – called the

colorant intensity or CI. This is mainly for use in young red wines, but may also be of some consequence to the grape skin extracts. This new method shows good correlation with the intensity measures of the CIE tri-stimulus method (Boulton 2001, Neguereula et al 1995, Waterhouse and Kennedy 2004).

The use of spectrophotometry for the analysis of anthocyanins is quite common, although more so for the analysis of anthocyanins in wine as opposed to diluted grape skin extracts (Zoecklein et al 1995). The difference being that the wine has already been engaged in a process of maceration which is a time when all possible phenols are extracted from the must, as opposed to skin extracts which are simply taken, then diluted, hence a different amount of interacting phenols all in the wine as opposed to the extract solution (Zoecklein et al 1995).

Spectral methods have an advantage over other methods as they are both fast and simple. They provide a useful link between objective methods and the subjective appreciation of wine. Spectral methods tend to serve as supplementary values to other methods of color analysis as the wavelengths and intensities of different phenolic compounds are very different (Gore 2000).

IMPACT OF WINE PRODUCTION METHODS ON COLOR

Vineyard Impacts

The color of a red wine comes from the skins of the grapes used to make the wine (Jackson 2000). Therefore, it is rather evident that wine color issues begin far in advance

of wine or the actual fruit processing point. During the ripening phase of the fruit in the vineyard, there are many factors that affect the berry's ability to accumulate color-causing anthocyanin molecules and other related chemical entities.

Sunlight is one of the main components responsible for increasing the formation and accumulation of anthocyanins in the grape berry skin. Work by Creasy and Logan, 2003, (Creasy and Logan 2003) shows that anthocyanins still accumulate in complete shade in the skins of *V. vinifera* L. Cabernet Sauvignon, although the rate of this is highly impeded by the lack of ultra-violet light. Another discovery by the aforementioned, show how the colors behave throughout the ripening phase of winegrapes, and how similar this is to the behavior of leaves of deciduous trees, when looking at RGB values (Creasy and Logan 2003). During the ripening phase of the fruit, the colors are steady up until the point of veraison, which begins with the berries softening, accumulating sugars and presenting an increasing amount of red coloration. At this time, the skins of the berries do not become more red, as we perceive that they do. Instead they simply become less green and blue – the red value of the skins, when measured by computer software never changes, although to the human eye, it becomes more expressed because there is less interference by the green and blue colored entities (Creasy and Logan 2003).

There are a myriad of other reasons that provide a lower or higher potential for color formation, expression, stability or enhancement in wine resulting from viticultural impacts. Examples are: vineyard site, sun exposure, water availability, soil nutrient status, heat unit summation, canopy management, vegetative : reproductive tissue ratio, pest and

disease status, crop load, trellising system and pruning methods used (Iland et al 2000, Kliewer and Dokoolian 2005, Rankine 2002). The harvest is the point at which the quality of the fruit is (hopefully) at its peak, and the main objective now, is to ensure that the quality degradation is slowed as much as possible, after all, wine color, among many other wine parameters, is a very qualitative entity (Creasy and Logan 2003, Jackson and Schuster 2001, Jackson 2000, Winkler et al 1974).

Winery Impacts

Now that the fruit has been harvested, by hand or machine (hand harvesting is preferred for quality fruit), it is brought to the winery and, in order to ensure the highest color stability possible, the fruit should be cooled and processed at once. Red wines require skin contact, to extract the color into the wine, and so crushing and de-stemming the fruit is the preferred processing technique (you can make white wine or sparkling from red fruit by simply pressing the fruit gently to extract only the colorless juice, which as discussed above contains very little (<1%) phenolics and no anthocyanins) (Jackson 2000, Rankine 2002).

Fruit must (a combination of skins, pulp, juice and seeds) is pumped through 4 inch stainless steel (316) pipes to the fermentation tanks, and, on its way is cooled by passing ethylene glycol over the outside of such piping in a cross flow fashion inside a sealed pipe of its own. The tanks for fermentation of red fruit have open tops to ensure the ability to access and manage the cap of the ferment, where the fruit rises to the top and could dry if not managed correctly. These open top tanks allow for the staff to keep

the skins in contact with, and moving through the ferment which ensures optimum phenolic extraction. During this phase, the acid, temperature and sulfur dioxide among many others will be monitored to ensure they are in the correct range to avoid color bleaching (Jackson 2000, Parley 1997, Rankine 2002).

The extraction of phenolic compounds from a grape berry (skin, seeds and pulp) is achieved by a process mentioned briefly before, called maceration (Brouillard et al 2003, Zoecklein et al 1995). Wines that are to be aged for a long period are usually macerated on the seeds and skins for up to three weeks, which results in a decline in free anthocyanin content, however this lengthened maceration period may enhance color stability in the resultant wine after aging (Rankine 2002). The degree of this extraction (maceration) influences the amounts and stability of the color, the astringency and tannin structure of the wine, the potential of the wine to age, and the balance between fruitiness and heavier more complex flavors and aromas in the wine (Jackson 2000). However, only about a third of the total available phenolic compounds in grapes are ever extracted into the wine, and these can come from any of the following skins, seeds, pulp, and stems. The extraction of anthocyanins from grape skins, seeds and pulp is very quick initially, and then slows down with time. This extraction relationship follows an exponential curve to begin with, and then a parabolic downward sloping curve afterwards, when the graph of anthocyanin extraction versus time is analyzed (Jackson 2000, Parley 1997, Rankine 2002).

Techniques used to extract color from the skins of wine grape berries vary as much as winemaking practices have over the last few millennia since winemaking has been positively identified. There are however, several mainstream methods in current use that facilitate such extraction, they include cold soaking (Parley et al 2001), fermenting on the skins, post ferment maceration, and additives such as enzymes to assist the extraction (Sacchi et al 2005). Cold soak is simply taking the must after crushing, and covering it with carbon dioxide and sulfur dioxide to prevent browning, and chilling it to around 5°C for up to ten days (Parley et al 2001). Must fermentation involves fermenting the juice with the skins, seeds and pulp in contact, and can range in temperature from 28-40°C, during this time, the rising ethanol content is manly responsible for the color extraction from the skins (Jackson 2000, Sacchi et al 2005, Zoecklein et al 1995). Post ferment maceration is a period of remaining in contact with the fermented must for up to 10 additional days at room temperature, where again the ethanol concentration plays the largest role in extraction of color (Jackson 2000, Zoecklein et al 1995). Lastly, additions to the must such as enzymes and different yeast selections can affect the extraction of color, and even the expression of color based on many of the chemical concepts discussed above (Jackson and Schuster 2001, Jackson 2000, Parley 1997, Parley et al 2001, Pozo-Bayón et al 2004, Rankine 2002, Waterhouse and Kennedy 2004).

Now, wine production influences are limited in number for wine color and color chemistry. The wine is drained off the skins using gravity, and the remaining lot is pressed off, yet this contains higher concentrations of bitter tannins. The wine then undergoes many inputs to clarify, stabilize or improve its longevity, during which time

the wine undergoes many millions of chemical reactions, and over time, transformations (Iland et al 2000, Jackson 2000, Parley 1997, Zoecklein et al 1995). It may be filled into oak barrels and set aside for several years to allow the wine to extract tannins and lignin from the wood to aid in its stability and improve flavor, before being bottled, stored again, then transported and sold. During the entire process above, the color chemistry in wine changes dramatically, optically moving from bright red, to brick/yellow red, and chemically, the color predominantly comes from the free and co-pigmented anthocyanins to begin with, and as it ages, this changes to procyanidin polymers that may include tannins and anthocyanins (Iland et al 2000, Jackson and Schuster 2001, Jackson 2000, Parley 1997, Rankine 2002, Zoecklein et al 1995).

THE PROJECT

This color concern has led to the research effort reported here to assess the impact of climate (region of vineyard culture), genotype (clone of choice) and cellar methods as they influence the type, quantity and stability of wine color. The investigation will include the use of current extraction techniques such as the cold soak, commercially utilized viticultural inputs, and common methods of crop control to assess the impact on anthocyanin concentration and final color of grape extracts in New Zealand and Michigan, and the wine produced from that fruit in Michigan.

CHAPTER 3

THE IMPACT OF VINEYARD FACTORS ON THE COLOR AND ANTHOCYANIN PROFILE OF PINOT NOIR GRAPES FROM VERAISON TO HARVEST IN CANTERBURY, NEW ZEALAND AND MICHIGAN, USA.

In the cool climate winegrowing regions of Michigan USA and Canterbury New Zealand, *Vitis vinifera* L Pinot noir is an economically important red winegrape cultivar. Each region has problems with the final color of young Pinot noir wines based on anthocyanin presence and concentration. The anthocyanin concentration of *V. vinifera* Pinot noir fruit was investigated using four clones and two international growing locations. Utilizing HPLC techniques, the five main anthocyanins in the fruit (labeled 1-5) were identified based on a cyanidin-3-glucoside standard and total anthocyanin concentration was compared. Spectrophotometric methods were used to analyze the samples absorbance to give an indication of color perception by the human eye. Both growing season and location of culture were significant effects on the absorbance and cyanidin-3-glucoside concentration of fruit extract samples ($p \leq 0.01$, and $p \leq 0.002$ respectively). The clone of Pinot noir grown was shown to have no statistical effect on the above parameters ($p \leq 0.4670$), however differences did exist in clone behavior. Climate of growing region was a compounding limiting factor in the boundaries of anthocyanin concentration and final color of Pinot noir fruit.

INTRODUCTION

Vitis vinifera L. Pinot noir is important commercially throughout the world, especially in the South Island of New Zealand and Michigan, USA. Color is an important indicator of fruit maturity, and an imperative factor in wine quality as perceived by the consumer (Creasy and Logan 2003). The development of red color in grape berries occurs with an increase of a class of phenolic compounds known as anthocyanins. Anthocyanins are extremely important compounds in nature, as they determine the color of many flowers and fruits (Zhang et al 2005). They have been extensively studied in various plant types including red *V. vinifera* cultivars, where they are responsible for the color of berries, although most of the research has focused on maize (*Zea mays*), snapdragon (*Antirrhinum majus*) and petunia (*Petunia hybrida*) where their biosynthesis has been well established (Holton and Cornish 1995).

There are many factors involved in the final concentration of anthocyanins in the skins of winegrapes. The concentration is primarily controlled by vine genes and photosynthesis, although vineyard environment, vintage, and cultural techniques play an important role (Boss et al 1996). The biosynthesis of anthocyanins begins at veraison, and continues until harvest or vine senescence, whichever occur first (Esteban et al 2001). Environmental factors that affect anthocyanin synthesis include sunlight, temperature and soil type - all are functions of vineyard growing location and vintage. Cultural techniques affecting anthocyanin development include canopy management, yield, pruning method and nutrient availability (Spayd et al 2002). Indirect effects of vine habitat on concentration of anthocyanins in berries includes precipitation and

evaporation, which affect vine physiology and berry dilution which alter the berry composition (Esteban et al 2001).

To determine the behavior of color and anthocyanin concentration in *V. vinifera* L. Pinot noir fruit in response to different vineyard environments and vintages, an approach was applied to measure the fruit from veraison to harvest on the basis of anthocyanin concentration and perceived color. To that end, the ripening fruit was sampled during that developmental period and compared on the basis of different clones during two seasons in separate hemispheres.

MATERIALS AND METHODS

Vineyard Sites

The vineyard sites for this experiment were situated in two geographic locations, Benton Harbor, Michigan, USA, and Lincoln, Canterbury, New Zealand. The two sites were used as comparisons for the identical clones and samples throughout the experiment. The two sites were also chosen to evaluate very different growing conditions on the expression of color in Pinot noir.

Michigan, United States of America

The vineyard located in Benton Harbor, Michigan, USA, is part of the Michigan State University's Southwest Michigan Research and Extension Center (SWMREC). The vines for the experiment were located on north-south oriented rows of the SWMREC block. The vines were located on the lower West coast of the State of Michigan, planted

on Kalamazoo-silt-loam soils with grass inter-rows, a few kilometers inland, eastward from the shores of Lake Michigan at N 42.0841° W 86.3570°, 220 meters above mean sea level. The site has an average growing season length of 165 days (with 165 days being the optimum for winegrapes), yielding 1200 growing degree days (base 10°C), 950mm annual rainfall, with a mean temperature all year of 11.30°C (Glen Creasy 2005, personal communication).

Canterbury, New Zealand

The sister vineyard site for this experiment, was located on the campus of Lincoln University in Canterbury, New Zealand, and is part of the Centre of Viticulture and Oenology there. The vines were located on the west side of the original vineyard, on north-south oriented rows. This site, on the East coast of the South Island, has alluvial silt loam soils with grass inter-rows and is several kilometers inland, westward of the Pacific Ocean near S 43.2922° E 172.3204° (Christchurch International Airport Control Tower). This vineyard site has an average growing season length of 200 days, with 939 growing degree days (base 10°C), 635mm of annual rainfall with an average temperature over the whole year of 12.9°C (Glen Creasy 2005, personal communication).

Plant Material

Vines used in this experiment were *Vitis vinifera* L. Pinot noir, clones 113, 115, UCD13 and UCD23 (Mariafield Type). However clone UCD23 was not used in Michigan during the experiments. Each vine was pruned to a two-cane vertical shoot positioning system (VSP), in a commercially viable manner. The vines were selected

based on their similarity across the experiment, which includes a visual inspection for pest damage, diseases or other factors that could limit its ability to produce, ripen or mature fruit with minimal damage. During the budburst-veraison phase, the vines were treated similarly with regard to canopy management, spray application and viticulture. One vine from each clone was chosen as the whole cluster sample vine, and this remained the sample vine for the entire experiment. The remaining vines were harvested for winemaking purposes. Vines were uniformly thinned at veraison, with fruit removed to a level of 30 clusters per vine, equally of apical and basal clusters on fruiting shoots, and all the second-set fruit was removed. Sampling began at this time, considered day 0, for fruit analysis, then continued with another sample at day 30, and lastly a sample at day 70/harvest, at the same time as remaining fruit was harvested for winemaking (chapter 4).

Canopy management included: 1) Leaf removal from both sides of the vines fruiting zone, that was conducted by hand to around 60% exposure of fruit as occurs in commercial vineyards. This was repeated once when lateral leaves grew in place of primary leaves. 2) Shoot tucking to keep desired shoots behind foliage wires and prevent damage from machinery. 3) Dead plant material removal from the canopy to prevent disease inoculum build-up. 4) Normal canopy sprays including insecticides and fungicides applied at suitable periods similar to commercial vineyard management.

Field Design

The Michigan vines for this study were arranged by clone in a randomized block design, set out in three replicates of ten vines each of one clone per block at the

SWMREC vineyards at Benton Harbor, Michigan, USA. One vine from each clone was selected as the fruit sampling vine for the entire experiment and flagged. Treatments were applied to all vines similarly, and fruit harvested for the winemaking process was taken from all vines separated only by clone. The vines in New Zealand were arranged in two groups of four vines for each clone, in one row. Sample vines and treatments applied in Michigan were identical in all New Zealand based vines.

Field data to be obtained during experiments included: 1) cluster number per vine, 2) vine yields (kg/vine), 3) °Brix, and for winemaking purposes 4) titratable acidity (g/L), and 5) pH. During the experiments general weather data from each geographic location was being logged by outside sources (Michigan Automated Weather Network in Michigan, USA, and Lincoln University in Canterbury, New Zealand). This weather data would include; 1) growing degree days (GDD), 2) maximum and minimum air and soil temperatures (°C), 3) daily precipitation (mm), 4) wind speed (m/s^{-1}), 5) light intensity.

Sampling

The sampling protocols were sub-divided into several groups for ease of use. Each set specified a different area of the work and protocols used therein. The protocols are given as follows;

Sample Preparation

- Weigh each of the two-cluster samples separately (3 samples per date)
- Removed rachis (carefully removed berries)

- Removed seeds (crushed berries gently, without crushing seed)
- Weighed the skin/juice/flesh for each sample (two clusters (apical and basal))
- Add 3 N hydrochloric acid 'drop-wise' until pH 3 is reached (used a Fisher Scientific accumet® model 10 pH meter)
- Purée in a blender for two minutes (used a Virtis Research Equipment, model 30 blender)
- Pour out onto freeze drying tray, rinsing blender with a 20 mL of distilled water each time, washing thoroughly between samples.
- Record fresh weights
- Freeze dry
- Weigh powder
- Place into sealed plastic Glad© "zip-lock" bags after tare weight is established
- Weigh bag and powder
- Store immediately at -20°C until required for shipping and analysis by HPLC and spectrophotometry.

Experimental Conditions

The experiments undertaken were done to provide a basis for practical, commercial application in the future. Thus, the trial blocks had the treatments imposed with as many of the normal viticultural inputs in place so that the results would be reproducible in a regular commercial vineyard.

HPLC Analysis

HPLC Standards

The standards for the analysis of the anthocyanins in the Pinot noir fruit extract and wine samples were difficult to obtain and very expensive. For this reason, a pure extract of cyanidin-3-glucoside was obtained, and all peaks and results were compared on the basis of cyanidin-3-glucoside concentration in the fruit/wine sample (Chandra et al 2001, Wada and Ou 2002). The standard, (obtained from M. G. Nair at the National Food Safety and Toxicology Center, Michigan State University, East Lansing, Michigan) was pure, and re-hydrated with pure HPLC-Grade water spiked with HCl to pH 3.00 – to maintain continuity among samples. The standard produced as follows: a) 1.00mg cyanidin-3-glucoside powder was re-hydrated with 1.00mL of pH 3.00 water, and b) a serial dilution was prepared form the stock solution to yield: 0.500, 0.250, 0.125, 0.0625 and 0.03125mg/1.00 mL concentrations respectively. Each standard was injected in triplicate and used in the construction of the standard curve.

Sample Preparation for HPLC.

The preparation for the HPLC phase of sample analysis began with either freeze dried grape extract samples, in accordance with storage and shipping protocols. Each sample was compared on a per milliliter basis for concentrations to avoid errors between fruit extract and wine samples. This technique was more suitable for a representation of the fruit after crushing and processing to pre-ferment status at the winery, which was then compared directly on this bases of per milliliter (or per 1000 liters for a winery) to the wine produced from that fruit.

Solvents Used in HPLC Analysis

Solvents required for HPLC analysis of the anthocyanins in Pinot noir fruit and wine samples were as follows:

Solvent A → 0.10% triflouroacetic Acid

99.90% HPLC-Grade Water

Solvent B → 0.10% triflouroacetic Acid

1.00% Acetic Acid

48.50% Acetonitrile

50.40% HPLC-Grade Water

Solvent Gradient →

Time	Flow	%A	%B	%C	%D
0.01	1	80	20	0	0
26	1	40	60	0	0
35	1	80	20	0	0

Table 3.1 Solvent Gradient used in the HPLC analysis of all fruit and wine extract.

HPLC Configuration

Analysis of anthocyanins occurred using HPLC (Waters Corp., Milford, MA) with a PDA detector (Waters Corp., Milford, MA) and the techniques of published procedures (Seeram et al, 2002). The column used in the experiment is a Waters Chromatography Xterra C^{18} 150mm, 3.50μm particle size column. The solvents were used by percentages (v/v) under gradient throughout the entire 40 minute sample run (table 3.1). During this time the column was slightly heated and maintained at 40.0°C for the entire sample set. The mobile phase flow rate of 0.80 mL/min was used for all standards and samples, using 20μL injection aliquots for each. Peaks were detected at

520nm (Zhang et al, 2004). Blank samples of pure HPLC-Grade methanol were run every 10 samples to ensure the column remained clean and clear of debris build-up. Samples were injected in triplicate and the average peak areas were used in the data.

Spectrophotometric Analysis

Spectrophotometry was used to compare the results obtained with HPLC. The analysis used samples prepared at the same time as the HPLC samples – these were re-hydrated by weight with pH 3.00 HPLC-Grade water. The samples were then filtered through a 0.45μm syringe filter before being loaded into 10.00mm path-length quartz crystal cuvettes for analysis. Each sample was read at 520nm for anthocyanins and 700nm for sample purity / contamination. Dilution factor of 3.00 was required as most red wines were too dark when using 10.00mm path-length cuvettes. The dilution factors were corrected by simple multiplication, and recorded. The values obtained at 700nm should be very small, which would show little to no interference. The cuvettes were rinsed with distilled water and dried between samples to avoid contamination. Reference cuvette was filled with HPLC grade water to zero absorbance between samples. All measurements were taken on the same spectrophotometer to reduce experiment error where possible.

Statistical Analysis

Statistical analysis was conducted using SAS version 9.1 (SAS Institute Inc.). Methods utilized include the General Linear Model, Mixed Model and Analysis of Variance for total anthocyanin concentration per milliliter of fruit/wine extract where

appropriate. LSD Means separation was calculated for all of the comparisons on the basis of total anthocyanin concentration per milliliter of fruit/wine extract by SAS 9.1 also. Regression for standard curve and relationships between yield and soluble solids, year and location, clone and wine, for total anthocyanin concentration was calculated using Microsoft Excel. Finally 5% error bars and regression of anthocyanin concentration versus yield calculations were completed using Microsoft Excel.

RESULTS AND DISCUSSION

Images in this thesis are presented in color

Vineyard Production Data

The data of cluster sampling and lab preparatory work conducted for each of the sampling dates is displayed in tables A-1 to A-4. Harvest data for the Michigan trial vines is displayed in table 3.18 representing 2004 and 2005 respectively. Despite constant cluster numbers throughout the experimental units, the yield per vine during 2005 is more than twice that which occurs in 2004 (table 3.2). Although marginally reduced in 2005, brix remained largely unaffected despite the yield having doubled on the vines in 2005. The pH was elevated considerably in 2005 fruit in each clone when compared to 2004 pH data.

The weather at SWMREC may have had an influence on these parameters; table 3.19 shows daily averages for 1st May through 25th September, and figures 3.1 to 3.16 show data for daily actual precipitation and light intensity showing the period from 75 days before veraison to the harvest date. These data show there was about half as much rain, warmer air and soil temperatures, and lower wind speeds in 2005 than in 2004.

Additionally, the accumulated growing degree days (base 10°C) in 2005 at harvest were over 250 higher than those accumulated by harvest day in 2004. This table for accumulated growing degree days, being a combination of units to describe the climate suitability for growth, based on average daily temperatures above 10°C, and taking into account time above that temperature, gives a direct comparative basis to show how 2005 was a more suitable year in which to photosynthesize and ripen fruit, than in 2004, by almost 16% (Jackson 2000, Reynolds et al 1995).

Further, the effect of light intensity during the growing season has been shown to effect both the concentration and accumulation of anthocyanins in the skins of winegrapes. Studies undertaken by Spayd et al (2002), reported an increase in monomeric anthocyanins with light intensity, although when UV light was filtered out, they found no significant differences suggesting a possible need for the whole light spectrum. This effect is noticeable in these experiments, as figures 3.1 to 3.8 give the daily and accumulated daily light intensity readings taken at the two vineyard locations for the duration of the study. Most notably is the data in figures 3.2 and 3.4 – the comparison of the two locations accumulated light intensities. The data for Michigan shows greater light intensity in both years, which is accumulating at a higher rate than the light intensity for New Zealand. During both seasons, the concentration of anthocyanins – mainly comprised of malvidin-3-glucoside as previously reported in Pinot noir (Boulton 2001, Gao et al 1997, Jackson 2000) was higher at harvest in New Zealand than in Michigan. This relationship between increasing malvidin-3-glucoside concentration and lower light intensities, has been previously reported (Keller and Hrazdina 1998). During this work, it

was also reported that cyanidin-3-glucoside was significantly reduced in shaded environments, however, lower light intensity in New Zealand still produced higher concentrations of cyanidin-3-glucoside than in Michigan despite the higher light intensity recorded in both years. This suggested that other factors were involved in with the production, final concentration, or both of cyanidin-3-glucoside, a statement that is resounded throughout this work. Figures 3.23 and 3.24 show the light intensity separating vintage at each growing location. During 2004, both locations reported lower light intensities than found in the 2005 growing season. Most of the data shows higher concentrations of anthocyanins at both locations in 2005 than in 2004, suggesting again the involvement of other factors in this relationship.

The New Zealand harvest data is shown in table 3.4, for both 2004 and 2005. Yield did not change much between years, except for the noted differences experienced with clone 113 in both years. The data for brix was higher in 2005 than in 2004 for each clone, possibly related to the higher GDD experienced during 2005 (table 3.3).

The weather data for the New Zealand vineyard location is shown in table 3.5. The temperature is fairly consistent between years, however the average high wind speeds for the period was much higher in 2005, and growing degree days were down in 2005 also. Rainfall average in mm/day was also down during the 2005 growing season. The data for growing degree days in New Zealand vineyard location are much lower than the data for Michigan Vineyard GDD. This could contribute to the differences experienced between total anthocyanin concentration data reported in these experiments.

A: 2004

Clone	Fruit Weight (kg)	Vines	Yeild per Vine (kg)	Tonnes per Hectare	Average Cluster Weight (g)	Brix	TA g/L	pH
UCD13	45.69	31.00	1.47	2.64	49.00	23.90	10.50	3.15
113	24.15	16.00	1.51	2.71	50.00	24.80	8.50	3.35
115	51.48	35.00	1.47	2.64	49.00	24.10	9.60	3.21

B: 2005

Clone	Fruit Weight (kg)	Vines	Yeild per Vine (kg)	Tonnes per Hectare	Average Cluster Weight (g)	Brix	TA g/L	pH
UCD13	94.91	31.00	3.06	5.49	102.00	22.40	6.34	3.50
113	63.61	16.00	3.98	7.13	133.00	24.40	4.81	3.42
115	118.16	35.00	3.38	6.06	113.00	24.00	5.00	3.51

Table 3.2 Michigan harvest data including crop-load, total fruit weight, vine number and cluster weights with fruit harvest parameters for both 2004 (A) and 2005 (B) harvest seasons. Data is compiled for the entire experimental area on the basis of clone.

Year	Max. Air	Min. Air	Total	Precipitation	Precipitation	Light Intensity	Light Intensity	Max. Soil	Min. Soil	Max Wind	GDD
	Temp.	Temp.	Precipitation	Veraison to	Day 30 to	Total	Veraison to	Temp.	Temp.	Speed (km/h)	Base
	(ºC)	(ºC)	(mm)	Harvest	Harvest	(MJ/m^2)	Harvest	(ºC)	(ºC)		10ºC
2004	24.3	13.3	450	176.3	117.8	3267	1532	25.4	18.5	8.5	1320
2005	26.3	14.7	270	183.8	143.7	3465	1526	27.4	19.7	7.9	1573

Table 3.3 Weather summary for 2004 and 2005 at SWMREC vineyard location in Benton Harbor, Michigan. The data are calculated in the form of daily averages from 1st May – 25th September each year. Growing degree data for the same range are shown.

2004

Clone	Fruit Weight (kg)	Vines	Yield per Vine (kg)	Tonnes per Hectare	Average Cluster Weight (g)	Brix	TA	pH
MAR	5.64	3	1.88	3.37	62.7	22.1	NA	NA
UCD13	4.56	3	1.52	2.72	50.7	21.90	NA	NA
113	1.32	3	0.44	0.78	14.7	22.20	NA	NA
115	5.28	3	1.76	3.15	58.7	21.70	NA	NA
F-TEST	n.s.		n.s.	n.s.	n.s.	n.s.		

2005

Clone	Fruit Weight (kg)	Vines	Yield per Vine (kg)	Tonnes per Hectare	Average Cluster Weight (g)	Brix	TA	pH
MAR	5.51	3	1.8	3.29	61.2	24.2	NA	NA
UCD13	5.49	3	1.8	3.28	61.0	24.5	NA	NA
113	3.58	3	1.2	2.14	39.8	24.1	NA	NA
115	5.11	3	1.7	3.05	56.8	23.9	NA	NA
F-TEST	n.s.		n.s.	n.s.	n.s.	n.s.		

Table 3.4 New Zealand harvest data including crop-load, total fruit weight, vine number and cluster weights with fruit harvest parameters(TA and pH were not recorded as wine was not being produced).

Year	Max. Air	Min. Air	Total	Precipitation	Precipitation	Light Intensity	Light Intensity	Max. Soil	Min. Soil	Max Wind	GDD
	Temp.	Temp.	Precipitation	Version to	Day 30 to	Total	Version to	Temp.	Temp.	Speed (km/h)	Base
	(°C)	(°C)	(mm)	Harvest	Harvest	(MJ/m^2)	Harvest	(°C)	(°C)		10°C
2004	19.92	9.6	1.4	131.8	64.1	2019.9	640.8	NA	7	35.77	812
2005	20.37	10.2	1.22	134.2	101.8	2053.8	612.3	NA	7.77	65.27	795

NB: Precipitation data includes irrigation

Table 3.5 New Zealand weather data recorded from 1st January to 25th April each year, including air and soil temperature, precipitation, wind speed and Growing degree-days.

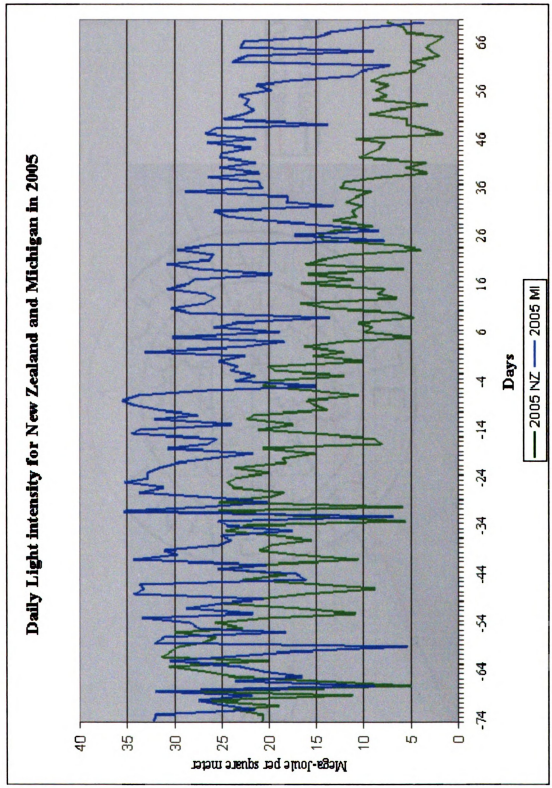


Figure 3.1 Daily light intensity for New Zealand and Michigan in 2005. Days measured are before and after veraison (Day 0).

Figure 3.2 Accumulated daily light intensity for New Zealand and Michigan in 2005. Days measured are before and after veraison (Day 0).

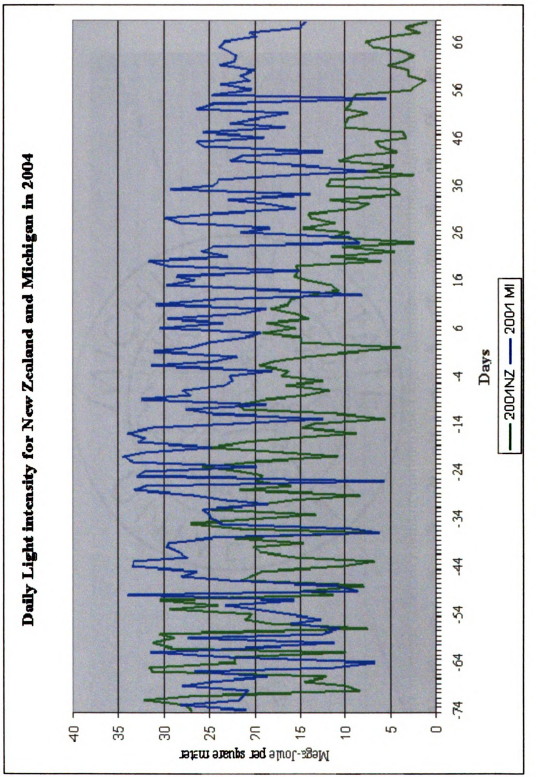


Figure 3.3 Daily light intensity for New Zealand and Michigan in 2004. Days measured are before and after veraison (Day 0).

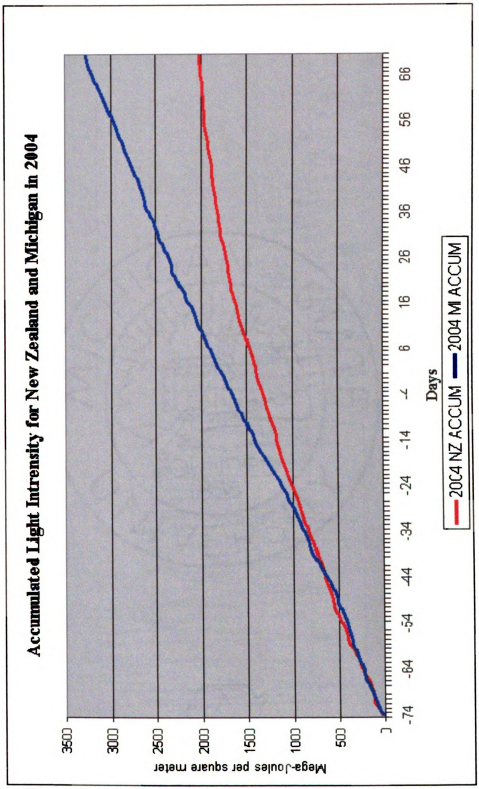


Figure 3.4 Accumulated daily light intensity for New Zealand and Michigan in 2004. Days measured are before and after veraison (Day 0).

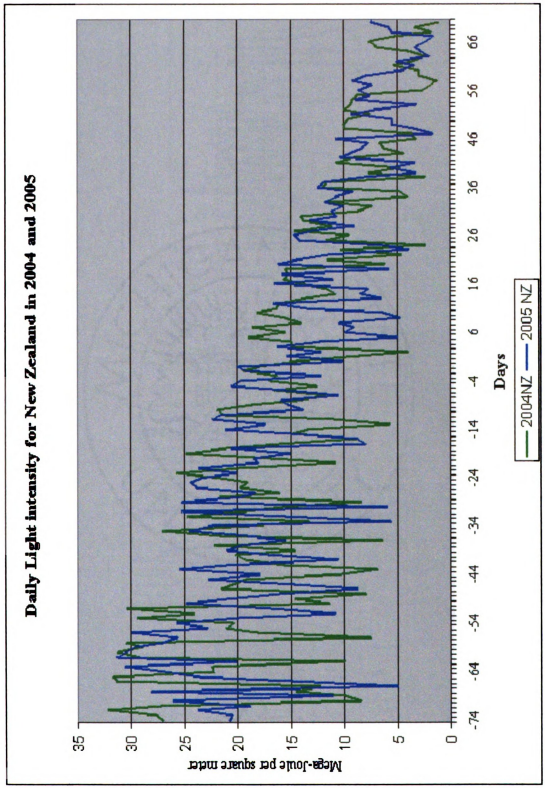


Figure 3.5 Daily light intensity for New Zealand in 2004 and 2005. Days measured are before and after veraison (Day 0).

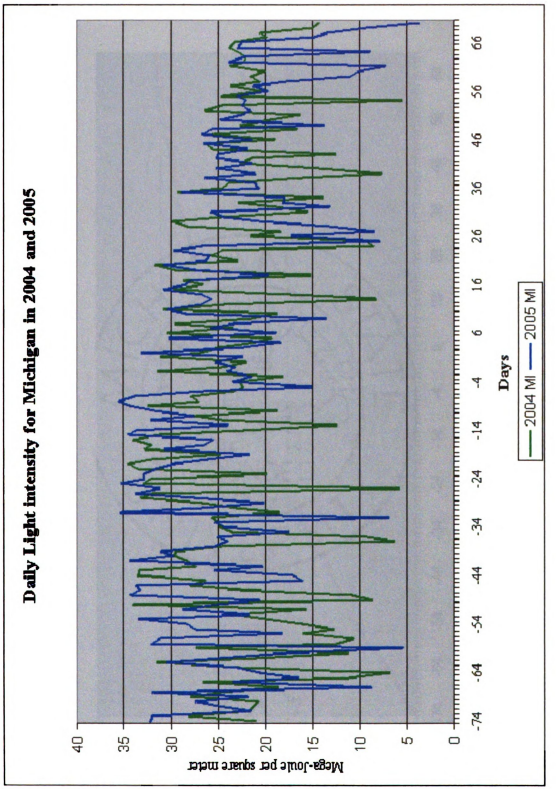


Figure 3.6 Daily light intensity for Michigan in 2004 and 2005. Days measured are before and after veraison (Day 0).

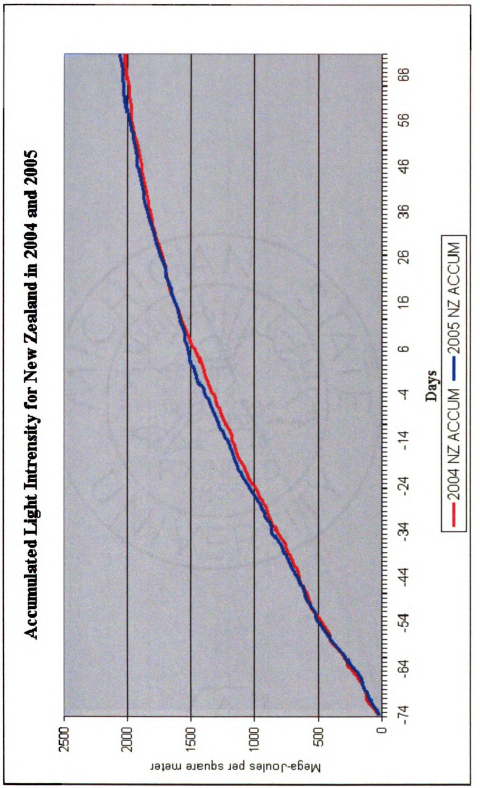


Figure 3.7 Accumulated daily light intensity for New Zealand in 2004 and 2005. Days measured are before and after veraison (Day 0).

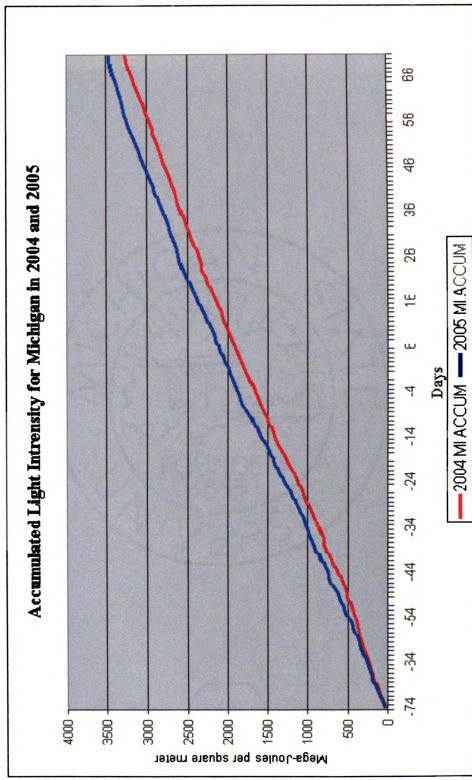


Figure 3.8 Accumulated daily light intensity for Michigan in 2004 and 2005. Days measured are before and after veraison (Day 0).

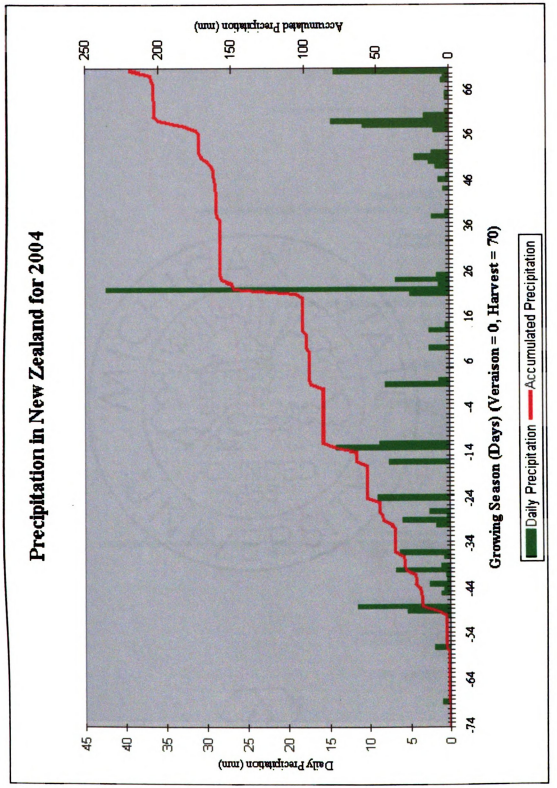


Figure 3.9 Daily and accumulated precipitation in New Zealand during 2004. Growing season measured in days, before and after veraison (Day 0).

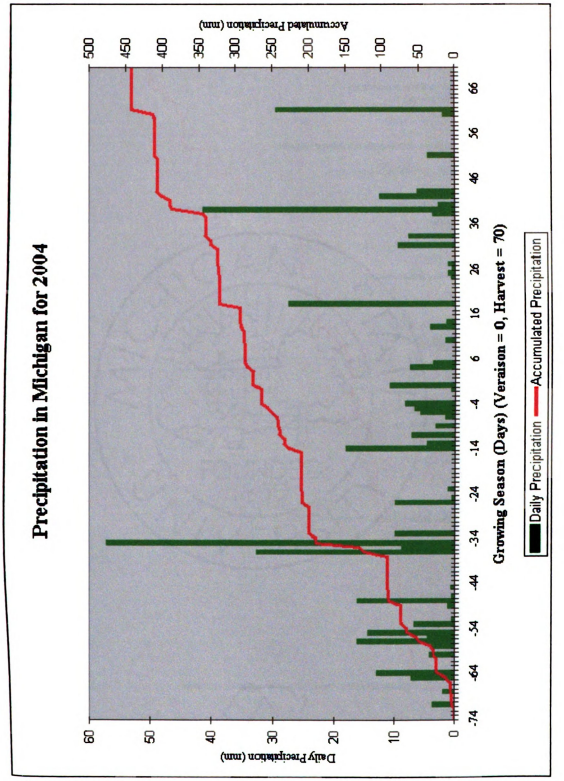


Figure 3.10 Daily and accumulated precipitation in Michigan during 2004. Growing season measured in days, before and after veraison (Day 0).

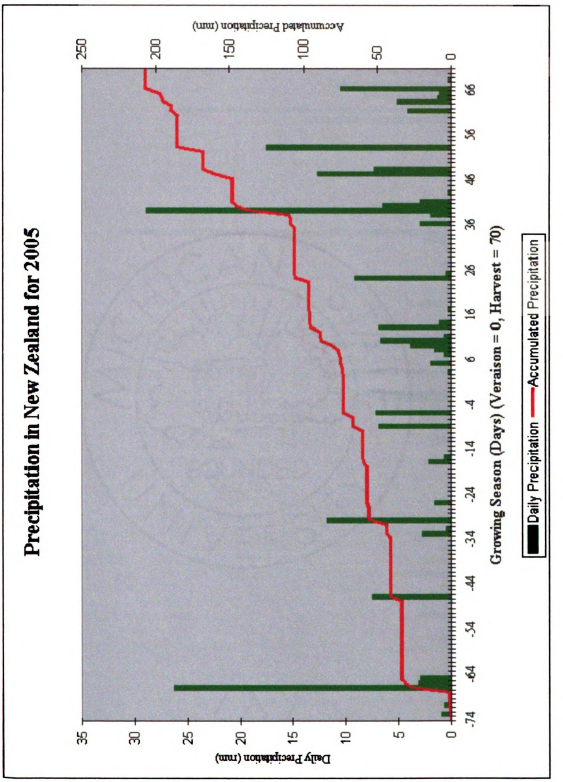


Figure 3.11 Daily and accumulated precipitation in New Zealand during 2005. Growing season measured in days, before and after veraison (Day 0).

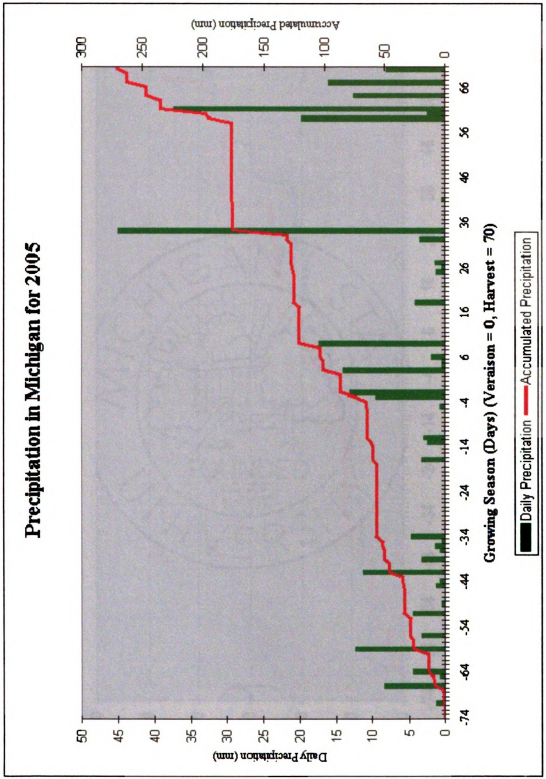


Figure 3.12 Daily and accumulated precipitation in Michigan during 2005. Growing season measured in days, before and after veraison (Day 0).

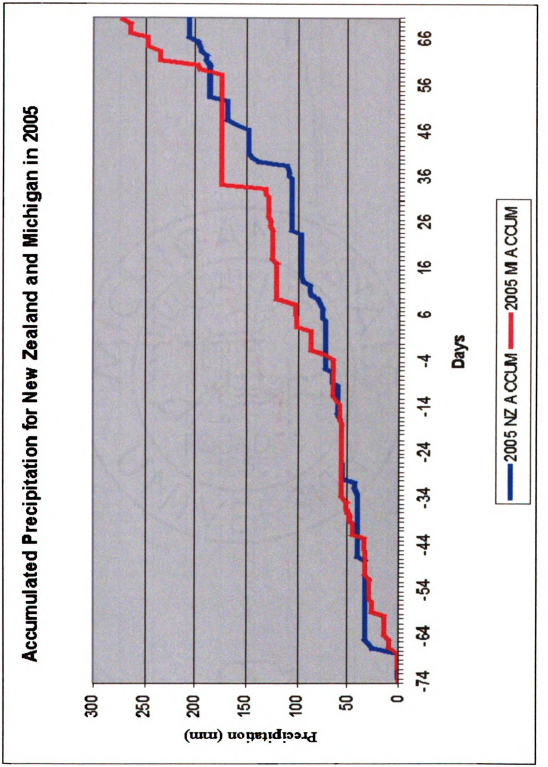


Figure 3.13 Accumulated precipitation for Michigan and New Zealand during 2005 growing season. Days measured are before and after veraison (Day 0).

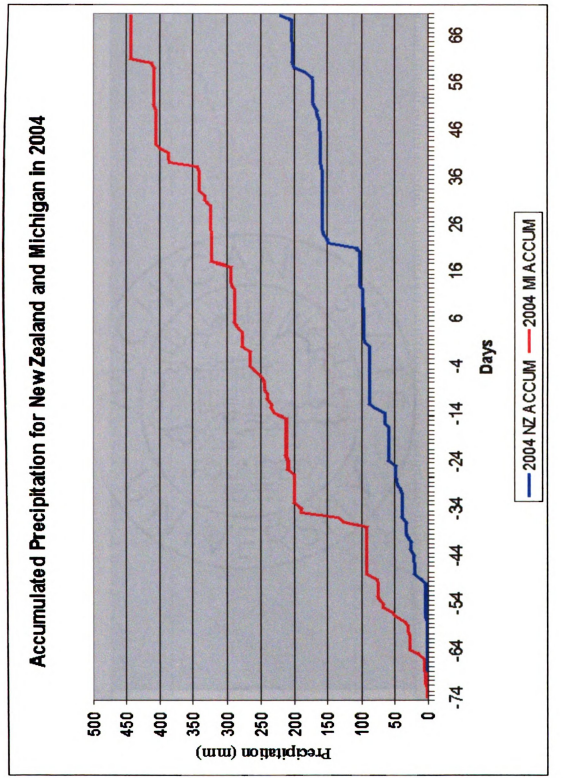


Figure 3.14 Accumulated precipitation for Michigan and New Zealand during 2004 growing season. Days measured are before and after veraison (Day 0).

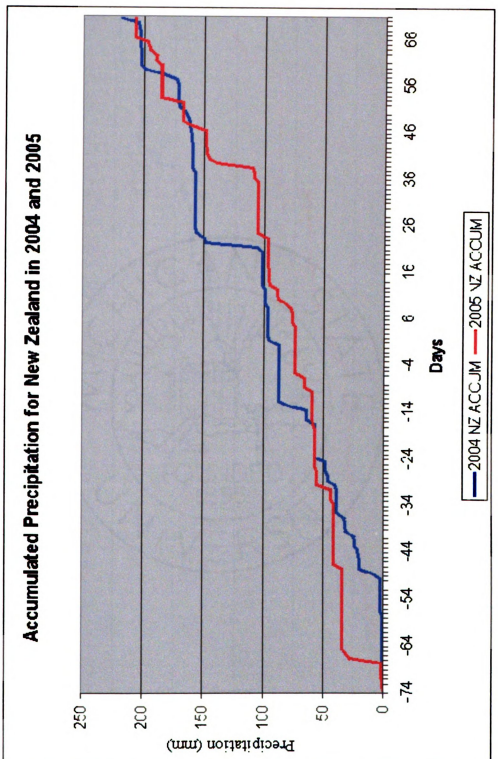


Figure 3.15 Accumulated precipitation for New Zealand during 2004 and 2005 growing season. Days measured are before and after veraison (Day 0).

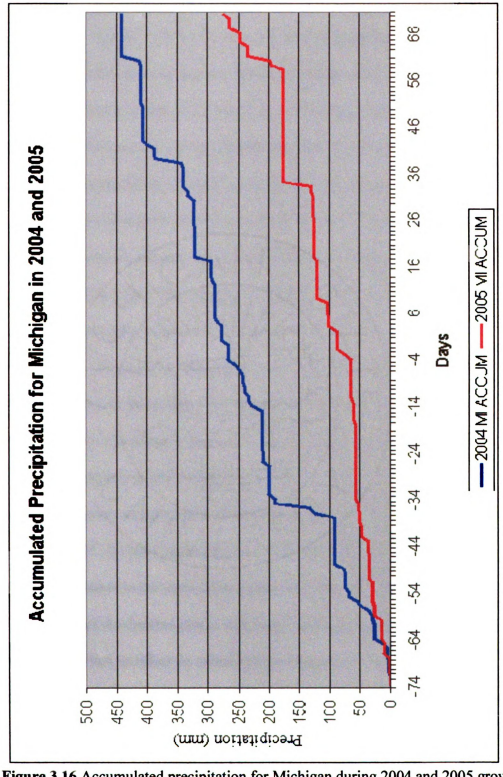


Figure 3.16 Accumulated precipitation for Michigan during 2004 and 2005 growing season. Days measured are before and after veraison (Day 0).

Additionally, the effect of precipitation on anthocyanin concentration has also been previously reported (Esteban et al 2001, Kennedy et al 2002, Matthews and Anderson 1989). Figures 3.13 to 3.16 show the data of precipitation across vintage and location. During both growing seasons, Michigan data reports large amounts of precipitation occurring in the ten days prior to harvest (figures 3.10 and 3.12), a factor, which has been reported in work by Esteban et al (2001), and Kennedy et al (2002) to result in lower concentrations of most berry constituents, namely anthocyanins by simply increasing the ratio of water to anthocyanin in the berry. The effect of precipitation noticed in Michigan during both seasons, suggests another reason for the reported lower anthocyanin concentration, when compared with New Zealand grown fruit. Further, studies of Matthews and Anderson (1989) reported the effect of water on vine physiology. They showed how reproductive development in *V. vinifera* was very sensitive to vine water status both in the current season, and the effects of the following season, affected by reproductive primordial. The work also showed the relationship between yield and precipitation – higher precipitation resulting in higher yields (reduced concentration of berry components). This factor was evident in the work reported here, where during 2005, the total precipitation was much lower in Michigan, and slightly lower in New Zealand in the maturation period before harvest (figures 3.15 and 3.16), and the concentration of anthocyanins was higher in 2005 in both locations, suggesting that precipitation had an effect on anthocyanin concentration, albeit indirectly. Additionally, figures 3.13 and 3.14, give the accumulated precipitation for each vintage, comparing location. Again, the New Zealand vineyard location, which produced higher concentrations of anthocyanins, experienced much lower precipitation than found in

Michigan, especially in 2004. This suggested how important precipitation was on berry composition, including that of anthocyanins, and is again similar to the results reported by Matthews and Anderson (1989).

Spectrophotometric Absorbance

The average results of the spectrophotometric analysis of fluid samples of grape extract at 520nm are collated in tables A-5 and A-6. Overall the data collected from New Zealand fruit samples yielded mostly shallow increases in absorbance throughout the season. The Michigan samples with some exceptions generally increased in absorbance at first, but then experienced dramatic reductions prior to intended harvest date.

The comparisons shown in figures 3.17 and 3.18, illustrate differences observed between the absorbance of each clone and sampling date from fruit grown in either New Zealand (green bars) or Michigan (blue bars) vineyards. During 2004 (figure 3.17), the Michigan-grown fruit expressed higher absorbance values in all measurements except the clone UCD13 late-season sample. The 2005 samples (figure 3.18) were not so clearly divided. The New Zealand-grown fruit was observed to show almost curve-linear increase in absorbance as the fruit ripened. Michigan fruit absorbance was not at all linear, although similarities between the 2004 and 2005 seasons could be observed on this basis. Overall, Michigan absorbance values in 2004, were noticeably higher than those resulting from 2005 season. The New Zealand fruit was different, yielding higher values in 2005, than 2004. This relationship is magnified when the locations are graphed

separately showing both growing seasons on the same figure as in figures 3.19 and 3.20. The effect of location was significant at $p \leq 0.01$.

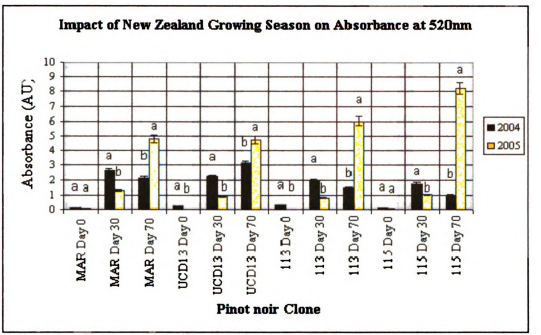


Figure 3.17 Impact of New Zealand growing season on absorbance at 520nm for each clone and at each sampling date (day 0, 30 and 70), comparing 2004 and 2005 (p=0.05).

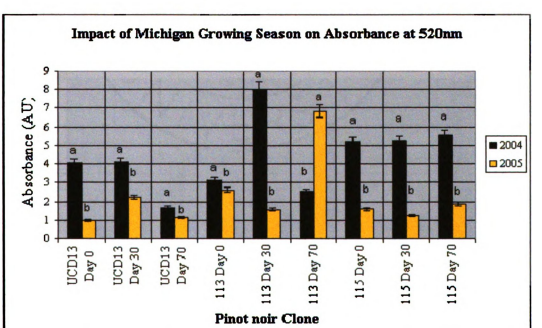


Figure 3.18 Impact of Michigan growing season on absorbance at 520nm for each clone and at each sampling date (day 0, 30 and 70), comparing 2004 and 2005 (p=0.05).

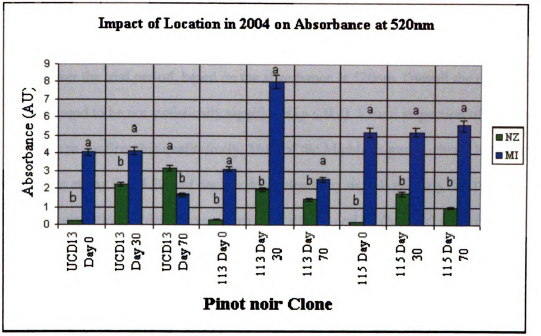


Figure 3.19 Impact of growing location in 2004 on absorbance at 520nm between each clone at each sampling date, comparing New Zealand and Michigan growing locations (p=0.05).

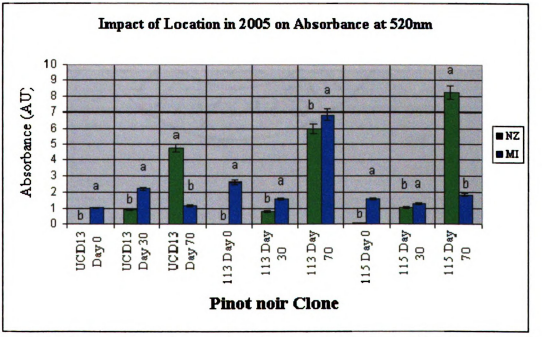


Figure 3.20 Impact of growing location in 2005 on absorbance at 520nm between each clone at each sampling date, comparing New Zealand and Michigan growing locations (p=0.05).

Only three of the four clones studied were available at both locations. Figures 3.21 to 3.26 compare data from clones UCD13, 113 and 115 vines in New Zealand and Michigan over the trial period, based on the absorbance at 520nm. Overall the effect of clone was found to be not statistically significant ($p \leq 0.4$). Visual inspection of figures 3.5 to 3.10 can confirm this qualitatively. Most of the Michigan-grown clones have high absorbencies at day 0 and 30, but decline dramatically by day 70 possibly due to the effect of precipitation, which increased between day 30 and 70 (harvest) (figures 3.9 to 3.16). The identical clones grown in New Zealand whilst increasing throughout the growing season, produce notably lower absorbencies for each clone, except in the case of clone 115 in the 2005 growing season.

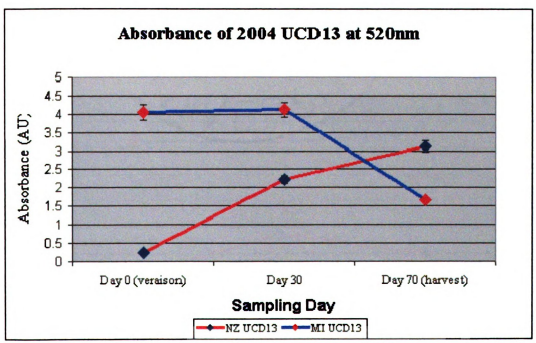


Figure 3.21 Absorbance (@520nm) of 2004 season clone UCD13 in New Zealand and Michigan growing locations from veraison to harvest (p=0.05).

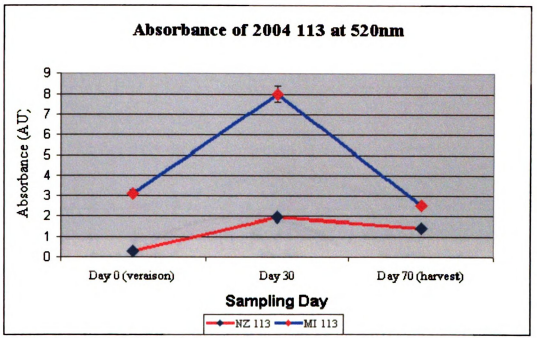


Figure 3.22 Absorbance (@520nm) of 2004 season clone 113 in New Zealand and Michigan growing locations from veraison to harvest (p=0.05).

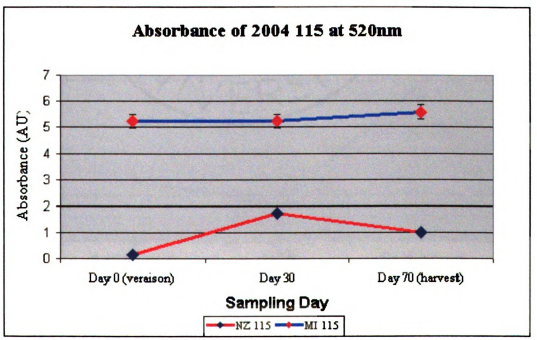


Figure 3.23 Absorbance (@520nm) of 2004 season clone 115 in New Zealand and Michigan growing locations from veraison to harvest (p=0.05).

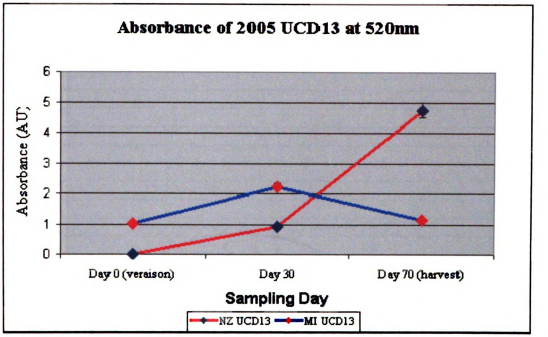


Figure 3.24 Absorbance (@520nm) of 2005 season clone UCD13 in New Zealand and Michigan growing locations from veraison to harvest (p=0.05).

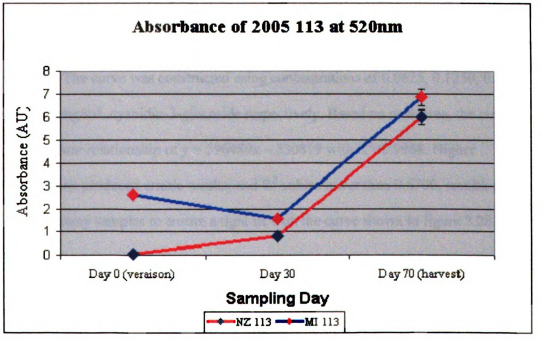


Figure 3.25 Absorbance (@520nm) of 2005 season clone 113 in New Zealand and Michigan growing locations from veraison to harvest (p=0.05).

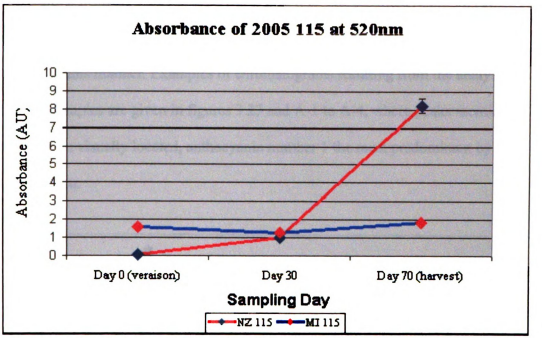


Figure 3.26 Absorbance (@520nm) of 2005 season clone 115 in New Zealand and Michigan growing locations from veraison to harvest (p=0.05).

Preparation of the Standard Curve

The standard curve established for cyanidin-3-glucoside (C-3-G) is displayed in figure 3.28. The curve was constructed using concentrations of 0.0625, 0.1250, 0.2500 and 0.5000 mg/mL cyanidin-3-glucoside respectively. Based on peak area, the curve yielded a linear relationship of $y = 596489x - 530819$ with R^2=0.9968. Higher concentrations produced erratic results, and R^2 values lower than 0.6700, resulting in a dilution of many samples to ensure a tight fit with the curve shown in figure 3.28.

Based on this curve of C-3-G, the five anthocyanins present in *V. vinifera* Pinot noir (including C-3-G) could be visually identified from fruit and wine sample chromatograms. However each anthocyanin can only be marked by magnitude, receiving

available. Table A-7 indicates anthocyanin labels and peak information used in the identification process. This iteration using C-3-G as the quantitative marker, allowed for calculation of total anthocyanin concentrations found in the fruit and wine samples analyzed in this manner. Examples of chromatograms resulting from the analysis of fruit and wine samples are given in figures 3.27 and A-1 to A-4, where peaks mentioned above can be visually located, anthocyanin number 1 the most predominant by concentration.

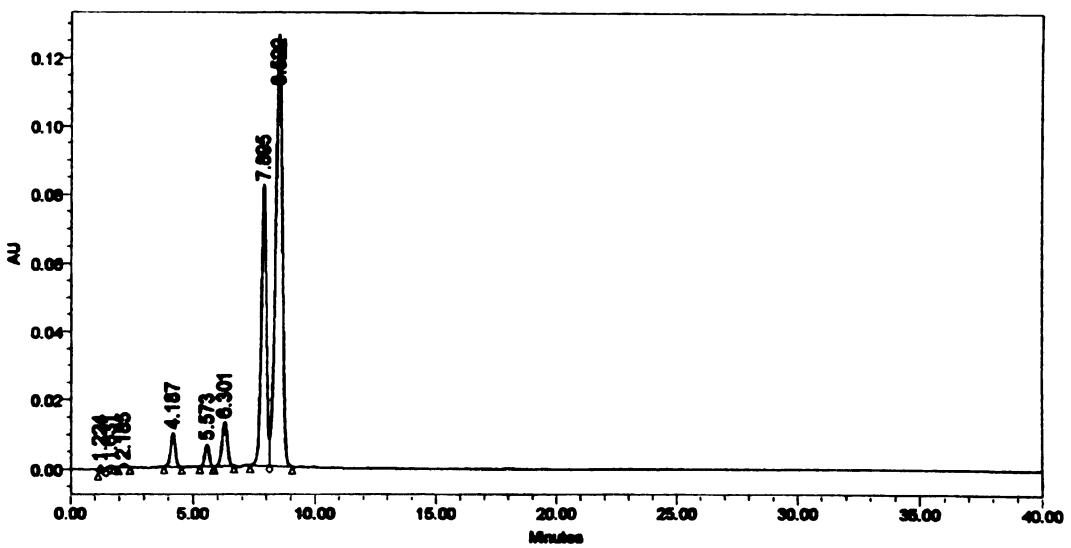


Figure 3.27 Chromatogram of 2004 Michigan clone 115 at day 0.

Calculation of C-3-G concentration is achieved by using the average response factor method (Singleton and Trousdale 1992) under the knowledge that the standard curve is linear to R^2=0.9968. The response factor (table A-8) for each calibration point, or injection incidence for each concentration of C-3-G, is summed and averaged across the curve. This average response factor is then multiplied by the area for each required peak, yielding a concentration of C-3-G in mg/mL. Tables A-9 to A-12 provide results of C-3-G concentration calculations for each of the samples analyzed.

Calculation of total anthocyanin (table A-13 and A-14) in a given sample simply requires the response factor for C-3-G to be multiplied by the peak area value of the identified peaks, and then completed by adding the five concentration values together. This produces a total anthocyanin concentration based on C-3-G concentration in the sample (Chandra et al 2001).

Figure 3.28 Standard curve of cyanidin-3-glucoside concentration (mg/mL) is prepared using HPLC standards of 0.0625, 0.125, 0.25 and 0.50 mg/mL respectively, and prepared on the basis of peak area.

Anthocyanin Concentration

The comparisons shown in figures 3.29 and 3.30 illustrate that the concentration of C-3-G present in the fruit during 2004 is generally higher in the Michigan samples. During 2005 however, New Zealand samples concentration of C-3-G far exceeds that of Michigan samples that year. Using the general linear model procedure, this effect of location on the concentration of C-3-G in samples was highly significant ($p \leq 0.002$). However, the impact of location on the total anthocyanin profile was not statistically significant ($p \leq 0.2263$). Differences between the significance of the total anthocyanin profile compared with the individual C-3-G concentration indicate that while C-3-G is statistically different at the two locations, the total anthocyanin profile does not change enough as a result of the location to be significant. This location effect on the C-3-G is likely based on climatic differences (light intensity, growing degree days and precipitation) between the two locations as discussed earlier in the text. When the data was separated by year (figures 3.31 and 3.32), the effects of location differences were magnified in the C-3-G figures, which further describes how clones performed erratically based on location of growth with regard to C-3-G concentration. The total anthocyanin profile however did not change enough to draw statistically significant differences from the two locations, leading to questions regarding the importance of C-3-G activity in the fruit and wines.

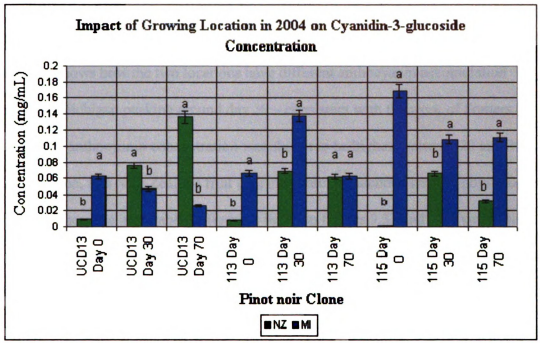


Figure 3.29 Impact of growing location in 2004 on cyanidin-3-glucoside concentration in clones UCD13, 113 and 115 at each sampling date (day 0, 30 and 70) between Michigan and New Zealand samples (p=0.05).

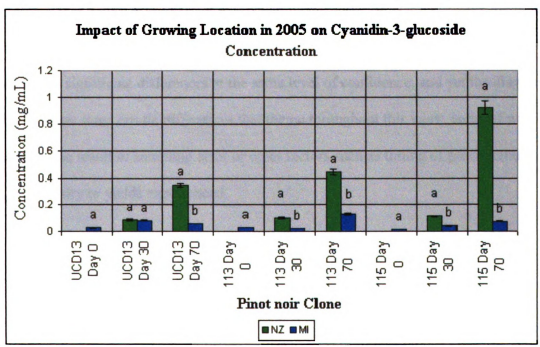


Figure 3.30 Impact of growing location in 2005 on cyanidin-3-glucoside concentration in clones UCD13, 113 and 115 at each sampling date (day 0, 30 and 70) between Michigan and New Zealand samples (p=0.05).

Overall this hints at a possible reason why New Zealand wines can obtain higher color intensity/anthocyanin concentration than Michigan wines – they have higher concentrations of anthocyanins, and as noted earlier, higher absorbance's near harvest. This also shows how the two locations have different anthocyanin accumulation behavior, Michigan peaking around day 30 – consistent with the work of Somers (1976), where fruit anthocyanin concentration was shown to be highest between 20 and 30 days post-veraison, and New Zealand fruit peaking nearer to intended harvest/physiological ripeness. There are noticeable differences between figures 3.33 and 3.34 which visually compare all clones and years for each location. The year 2005 yields higher anthocyanin concentrations in New Zealand, yet the Michigan location provides some data-points that far exceed the magnitude of all other data on either figure. While the effect of location on year is not significant ($p \leq 0.09$) some differences do exist in this work. Another way of showing the differences between Michigan and New Zealand locations is demonstrated in figures 3.35 – 3.40 where the total anthocyanin concentration is tracked over the time between veraison and harvest, and separated by location for each year. This result also yielded no significant differences at the same level of confidence, and yet the diagrams clearly show some erratic differences that theme throughout this work, some of which could be the result of sampling error or other factors such as timing of precipitation, daily light intensity or yields experienced.

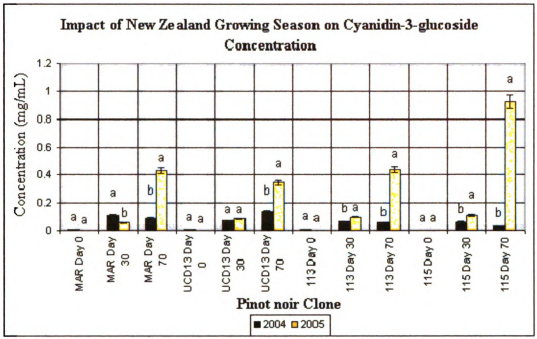


Figure 3.31 Impact of New Zealand growing season on cyanidin-3-glucoside concentration for each clone and at each sampling date (day 0, 30 and 70), comparing 2004 and 2005 (p=0.05).

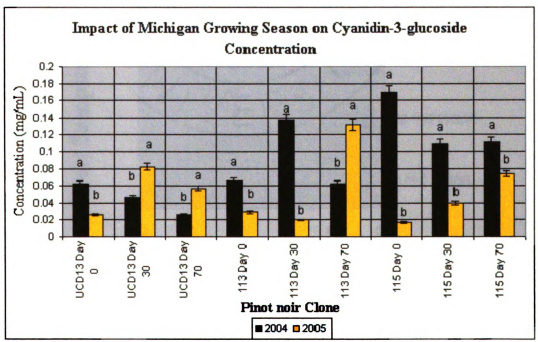


Figure 3.32 Impact of Michigan growing season on cyanidin-3-glucoside concentration for each clone and at each sampling date (day 0, 30 and 70), comparing 2004 and 2005 (p=0.05).

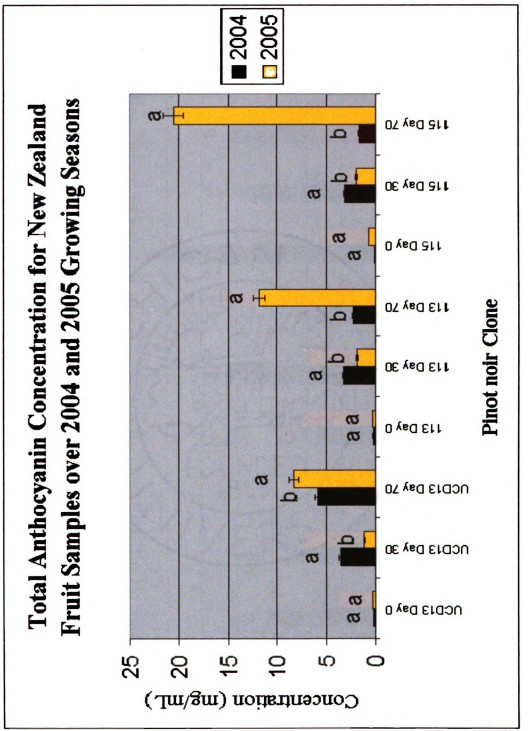


Figure 3.33: Total anthocyanin concentration for New Zealand fruit samples over 2004 and 2005 growing seasons (S1 = day 0, S2 = day 30, S3 = day 70 (harvest), from veraison) (p=0.05).

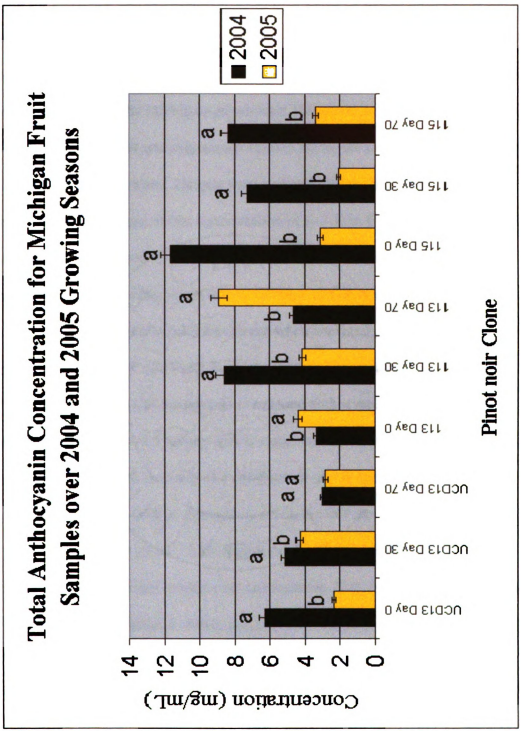


Figure 3.34: Total anthocyanin concentration for Michigan fruit samples over 2004 and 2005 growing seasons (S1 = day 0, S2 = day 30, S3 = day 70 (harvest), from veraison) (p=0.05).

The clone comparisons are displayed in figures 3.41 to 3.46. These show that except for 2004 113 and 115 samples, the concentration of C-3-G increases in a shallow positive linear fashion throughout the ripening period. The figures also showed that the New Zealand samples commonly had higher concentrations of C-3-G later in the season when compared with the Michigan-grown fruit. However, the samples taken from clones 113 and 115 in the 2004 growing season express far higher concentrations of C-3-G in Michigan than New Zealand. Despite these generalizations, there was no statistically significant effect of clone on the concentration of C-3-G in fruit samples ($p \leq 0.4670$). The concept, when based on total anthocyanin profiles was just as insignificant. The concentration of total anthocyanin across the three clones in both Michigan and New Zealand, was still not statistically significant when analyzed using the mixed procedure which yielded a p-value of 0.7145. It seems there was no statistical difference between the clones based on C-3-G concentration and total anthocyanin concentration. Considering the nature of a "clone" this is a positive result, suggesting that a) all clones are very similar even on the basis of a small group of molecules, and b) those molecules performed in a similar fashion throughout the clones of Pinot noir used in this experiment. The figures 3.47 – 3.50 diagrammatically showed the insignificance of the differences between clones in each year and location. They further amplify the results of C-3-G concentration between clones, and provide a basis for demonstrating the erratic nature of each year used in the experiments. However, the figures 3.470 – 3.50 do provide a clone performance indication of suitability in a given location and year during the experiments. Figure 3.47 shows that clone UCD13 performed better in New Zealand during 2004 regarding total anthocyanin

Figure 3.35: Total anthocyanin concentration in 2004 Pinot noir clone UCD13 fruit from veraison to harvest in both New Zealand and Michigan growing locations. Displaying 5% error bars.

Figure 3.36: Total anthocyanin concentration in 2004 Pinot noir clone 113 fruit from veraison to harvest in both New Zealand and Michigan growing locations. Displaying 5% error bars.

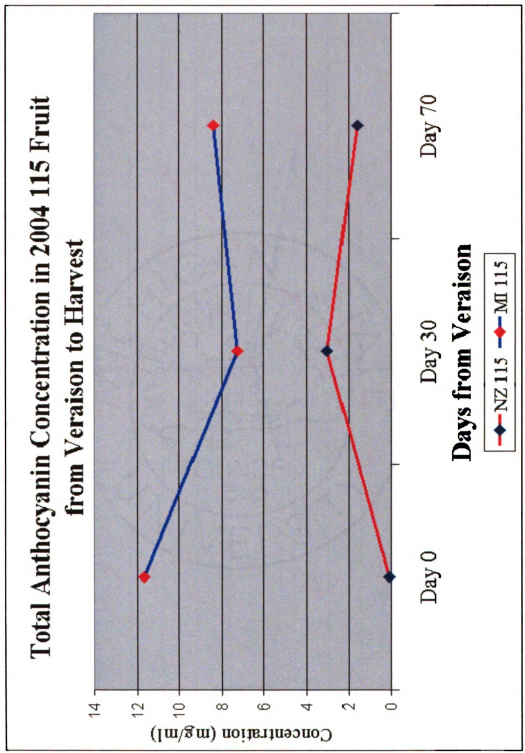


Figure 3.37: Total anthocyanin concentration in 2004 Pinot noir clone 115 fruit from veraison to harvest in both New Zealand and Michigan growing locations. Displaying 5% error bars.

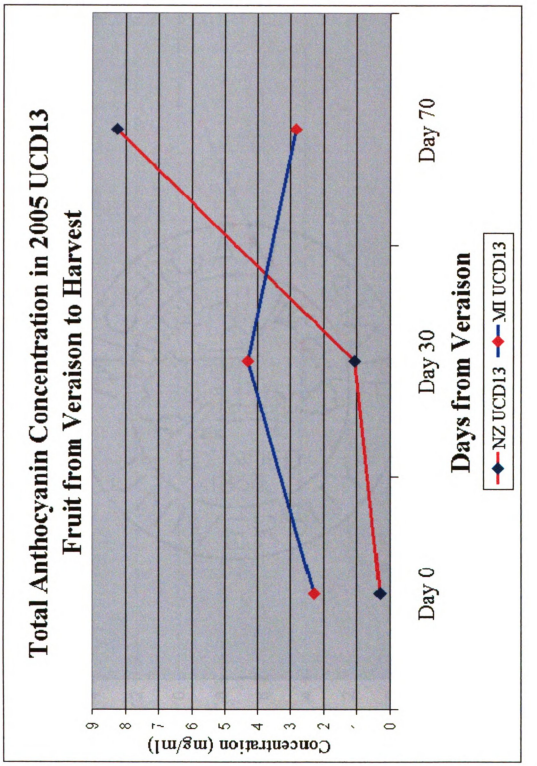


Figure 3.38: Total anthocyanin concentration in 2005 Pinot noir clone UCD13 fruit from veraison to harvest in both New Zealand and Michigan growing locations. Displaying 5% error bars.

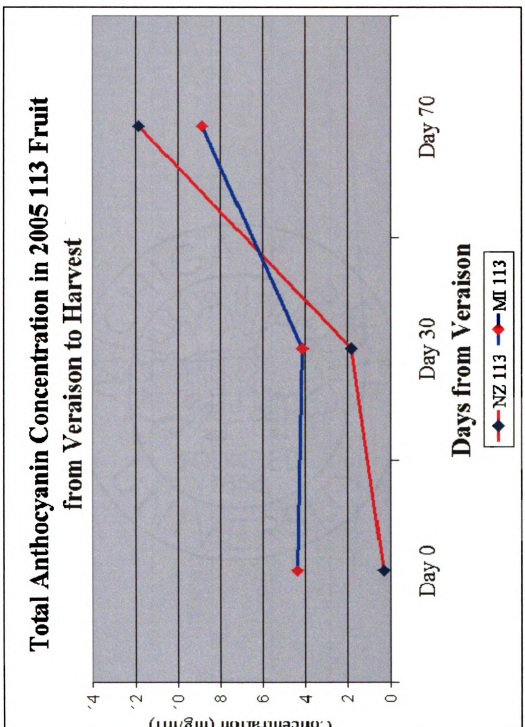


Figure 3.39: Total anthocyanin concentration in 2005 Pinot noir clone 113 fruit from veraison to harvest in both New Zealand and Michigan growing locations. Displaying 5% error bars.

Figure 3.40: Total anthocyanin concentration in 2005 Pinot noir clone 115 fruit from veraison to harvest in both New Zealand and Michigan growing locations. Displaying 5% error bars.

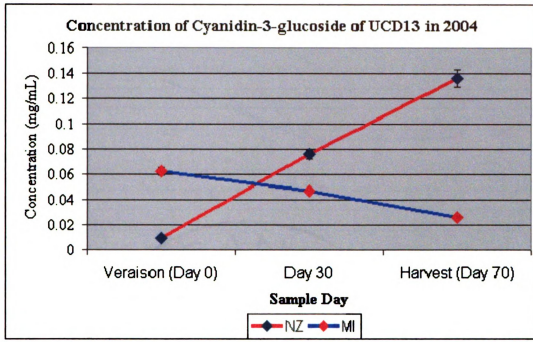


Figure 3.41 Cyanidin-3-glucoside concentration of 2004 season clone UCD13 fruit from veraison to harvest in both New Zealand and Michigan growing locations. Displaying 5% error bars.

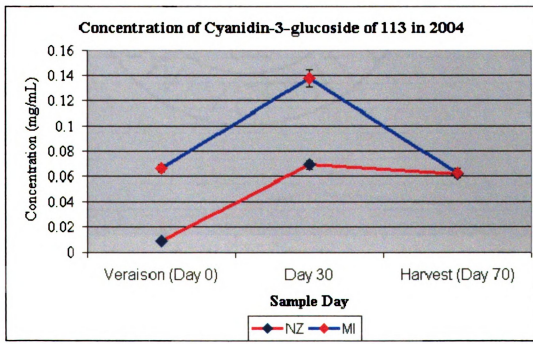


Figure 3.42 Cyanidin-3-glucoside concentration of 2004 season clone 113 fruit from veraison to harvest in both New Zealand and Michigan growing locations. Displaying 5% error bars.

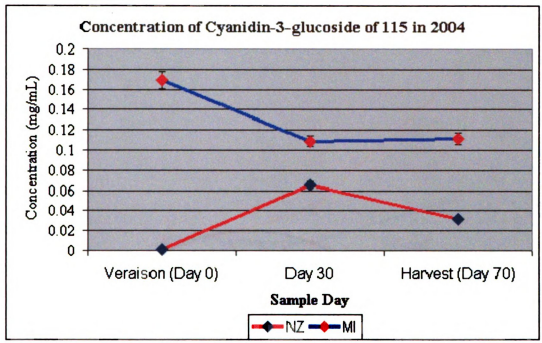


Figure 3.43 Cyanidin-3-glucoside concentration of 2004 season clone 115 fruit from veraison to harvest in both New Zealand and Michigan growing locations. Displaying 5% error bars.

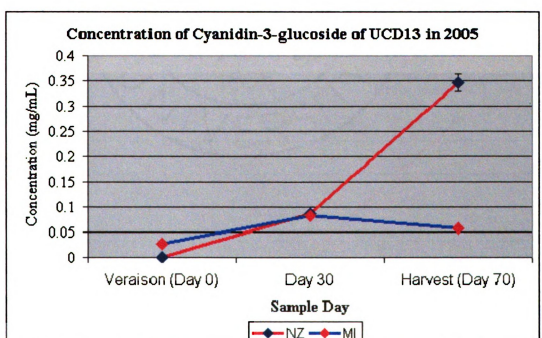


Figure 3.44 Cyanidin-3-glucoside concentration of 2005 season clone UCD13 fruit from veraison to harvest in both New Zealand and Michigan growing locations. Displaying 5% error bars.

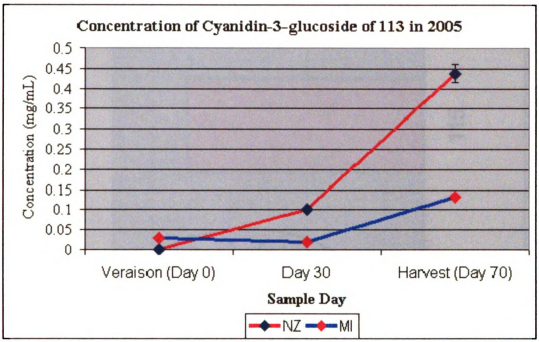


Figure 3.45 Cyanidin-3-glucoside concentration of 2005 season clone 113 fruit from veraison to harvest in both New Zealand and Michigan growing locations. Displaying 5% error bars.

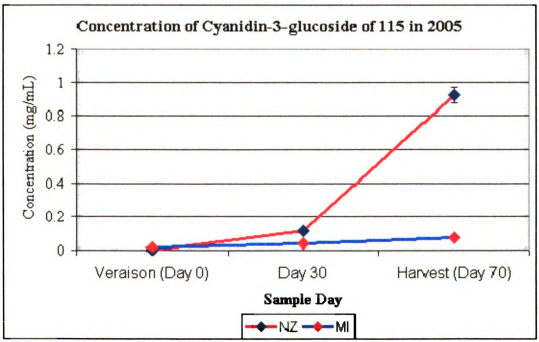


Figure 3.46 Cyanidin-3-glucoside concentration of 2005 season clone 115 fruit from veraison to harvest in both New Zealand and Michigan growing locations. Displaying 5% error bars.

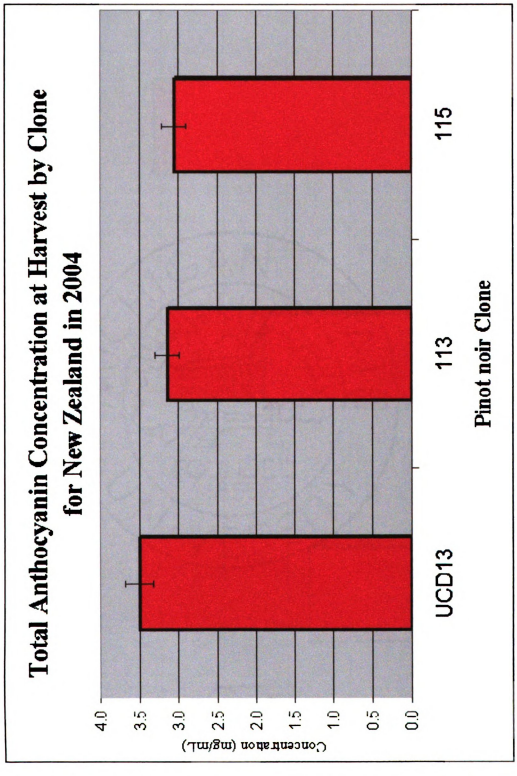


Figure 3.47: Total anthocyanin concentration at harvest by clone for New Zealand growing location in 2004. Displaying 5% error bars.

Figure 3.48: Total anthocyanin concentration at harvest by clone for New Zealand growing location in 2005. Displaying 5% error bars.

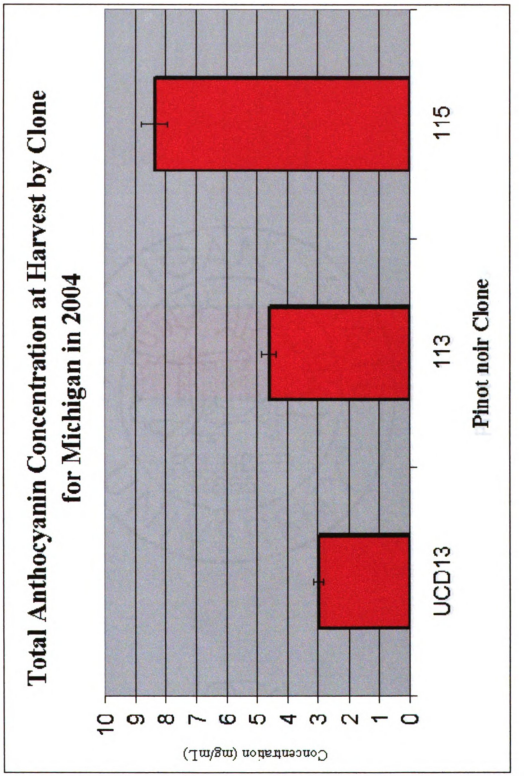


Figure 3.49: Total anthocyanin concentration at harvest by clone for Michigan growing location in 2004. Displaying 5% error bars.

Figure 3.50: Total anthocyanin concentration at harvest by clone for Michigan growing location in 2005. Displaying 5% error bars.

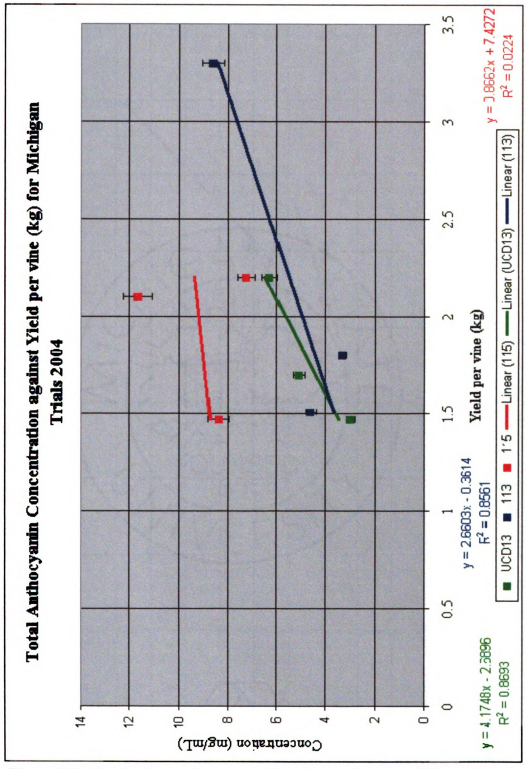


Figure 3.51: Total anthocyanin concentration regressed against yield per vine for Michigan data in 2004, separated by clone (p-values: UCD13=0.4076, 113=0.1041, 115=0.3035).

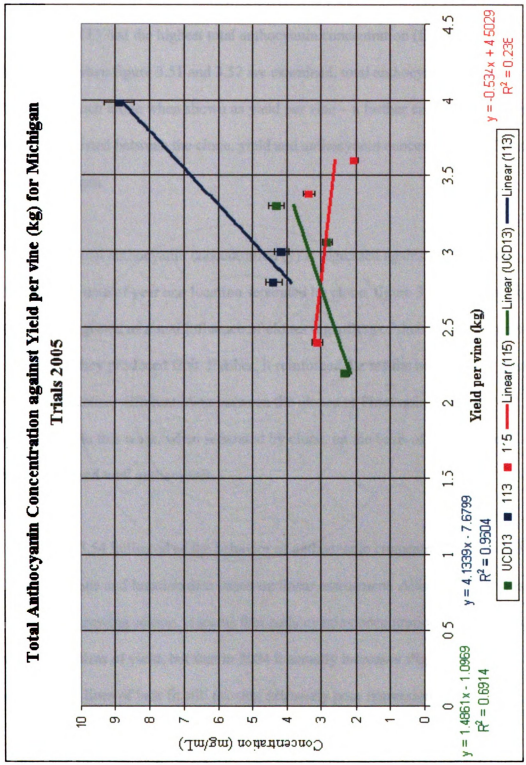


Figure 3.52: Total anthocyanin concentration against yield per vine for Michigan experimental data in 2005, separated by clone (p-values: UCD13=0.1612, 113=0.3345, 115=0.2759).

concentration, while 115 took better advantage of 2005 (figure 3.48). Figure 3.49 indicates how clone 115 outperformed both 113 and UCD13 during 2004 in Michigan, while in 2005 113 had the highest total anthocyanin concentration (figure 3.50). Additionally, when figure 3.51 and 3.52 are examined, total anthocyanin behaves differently in each clone when shown as yield per vine – a further example of how no relationship existed between the clone, yield and anthocyanin concentration of Pinot noir vines in Michigan.

When total anthocyanin concentration (y) is regressed against yield per vine (x) and plotted for data of year and location separated by clone, figure 3.53 was generated. This figure suggested minimal influence of clones regardless of their growing location or year in which they produced fruit. Further, it reinforced the results noted earlier regarding the lack of statistical differentiation between the clones of Pinot noir, as for all measurements in this work, when separated by clone, on the basis of absorbance, C-3-G concentration and total anthocyanin.

Figure 3.54 indicated of the behavior of anthocyanin concentration in the different years for all clone and location data based on linear assessment. Although 2005 was a more suitable growing season, it seems that anthocyanin concentration remains almost constant regardless of yield, but that in 2004 it actually increases slightly with yield. However, both lines of best fit still provide extremely poor regression values up to 0.2434, which does not provide the basis for any formal relationship.

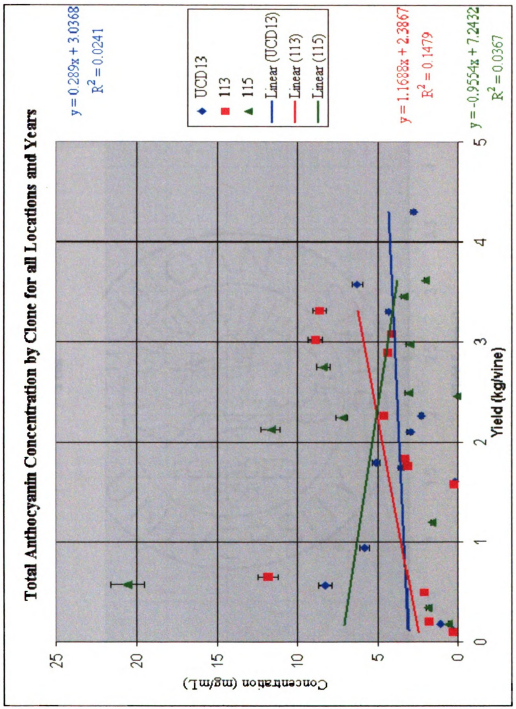


Figure 3.53: Total anthocyanin concentration of clones UCD13 (blue), 113 (red) and 115 (green) in 2004 and 2005, New Zealand and Michigan locations, expressed on the basis of vine yield (kg/vine). 5% error bars and lines of regression shown (regressions and equations in corresponding color) (p-values: UCD13=0.7544, 113=0.6348, 115=0.2042).

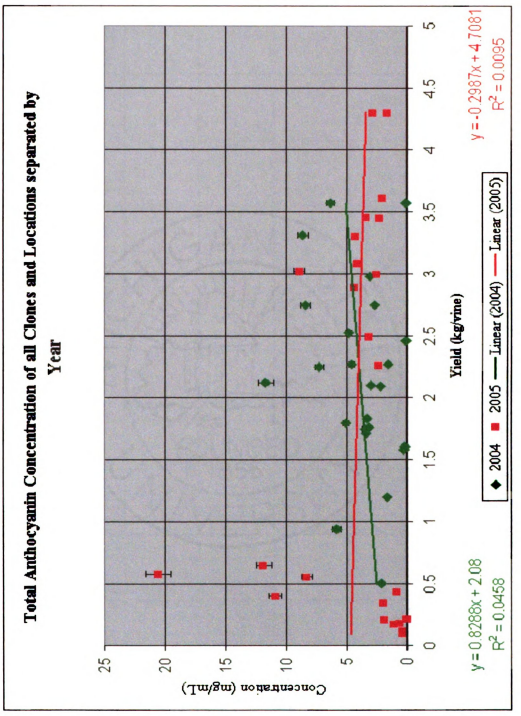


Figure 3.54: Total anthocyanin concentration of all clones and years, separated by location, expressed on the basis of vine yield (kg/vine). 5% error bars and lines of regression shown (regressions and equations in corresponding color) (p-values: 2004=0.2309, 2005=0.7133).

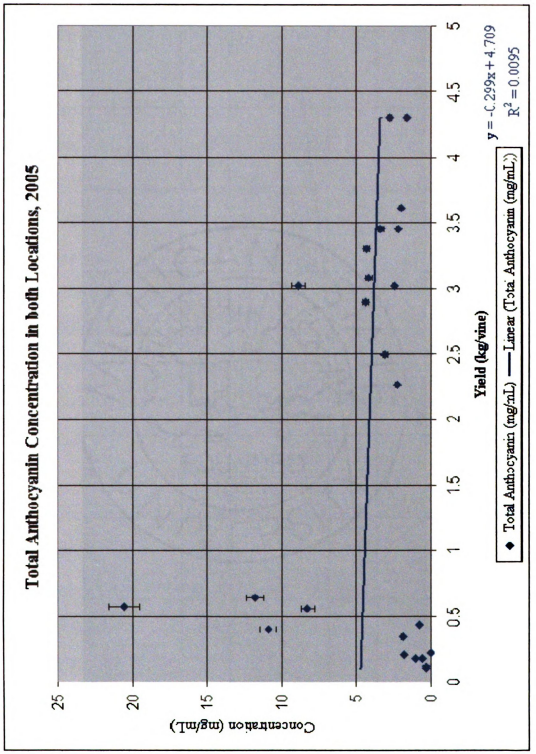


Figure 3.55: Total anthocyanin concentration of all clones and both locations for 2005 expressed on the basis of vine yield (kg/vine). 5% error bars and lines of regression shown (regressions and equations in corresponding color) (p-value: 2005=0.7133).

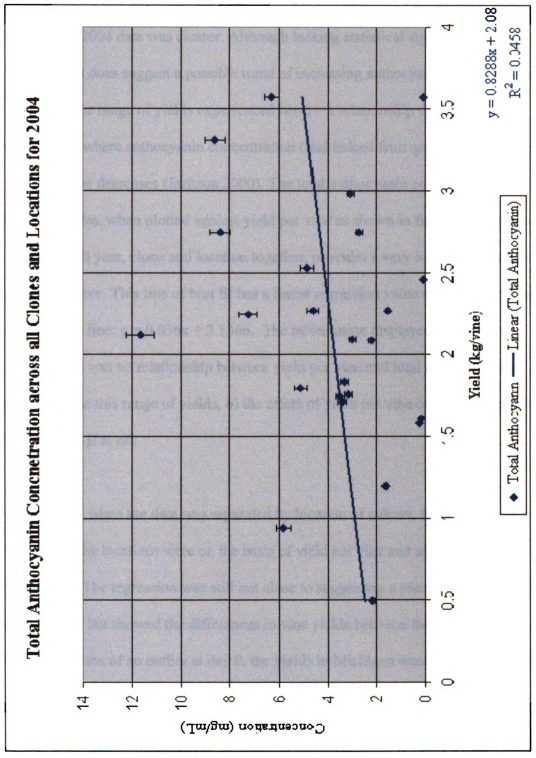


Figure 3.56: Total anthocyanin concentration of all clones and both locations for 2004 expressed on the basis of vine yield (kg/vine). 5% error bars and lines of regression shown (regressions and equations in corresponding color) (p-value: 2004=0.2309).

When the two experimental years were demonstrated separately, (figures 3.55 and 3.56) then the 2004 data was clearer. Although lacking statistical significance (R^2=0.0458), it does suggest a possible trend of increasing anthocyanin with increasing yield (within the range of yields experienced here) – a relationship thought to be the inverse of this where anthocyanin concentration (and indeed fruit quality) should increase as yield per vine decreases (Jackson 2000). The total anthocyanin concentrations of different samples, when plotted against yield per vine as shown in figure 3.57, displaying all data for each year, clone and location together, provides a very loose fit for a linear relationship curve. This line of best fit has a linear regression value of 0.0163, and an equation of the line: $y = 0.036x + 3.1366$. The information displayed suggests two things: a) there was no relationship between yield per vine and total anthocyanin concentration in this range of yields, b) the effect of yield per vine on total anthocyanin was very small if at all.

Finally, when the data was separated by location of culture, figure 3.58 suggests how different the locations were on the basis of yield per vine and anthocyanin concentration. The regression was still not close to suggesting a precise relationship in either location, but showed the differences in vine yields between the two locations – with the exception of an outlier at day 0, the yields in Michigan were more consistent than in New Zealand. New Zealand data also show the expected relationship between yield per vine and anthocyanin concentration. As the vine yields increase, the concentrations of anthocyanins decreases linearly with $y = -0.733x + 4.413$. The data

shown for Michigan does also decrease, but with a very shallow negative curve ($y = -0.0978x + 5.6549$).

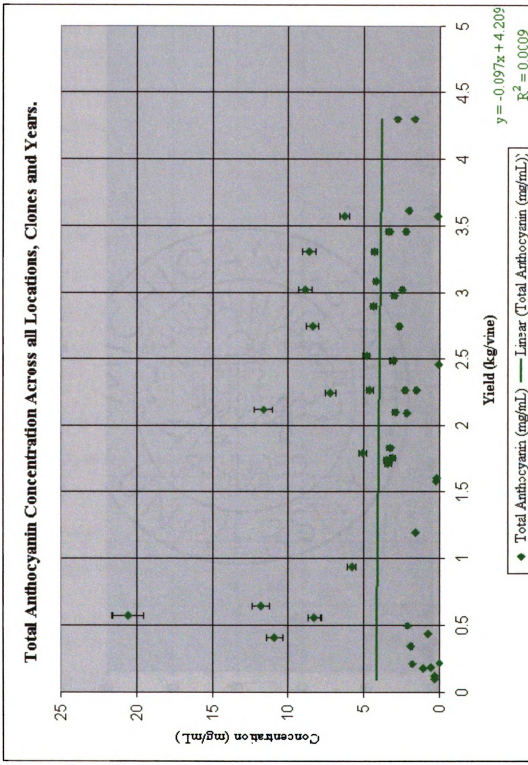


Figure 3.57: Total anthocyanin concentration of all clones, locations and years expressed on the basis of vine yield (kg/vine). 5% error bars and lines of regression shown (regressions and equations in corresponding color) (p-value=0.7133).

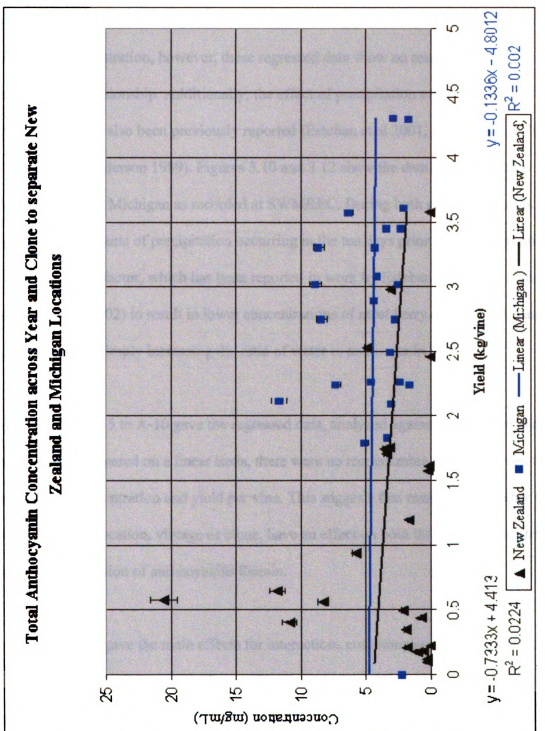


Figure 3.58: Total anthocyanin concentration across year and clone to separate New Zealand and Michigan locations, expressed on the basis of vine yield (kg/vine). 5% error bars and lines of regression shown (regressions and equations in corresponding color) (p-values: NZ=0.0393, MI=0.7166).

As previously reported by Matthews and Anderson (1989), berry components such as anthocyanins normally decrease with increasing yield, as a result of the decreasing concentration, however, these regressed data show no resemblance to this demonstrated relationship. Additionally, the effect of precipitation on anthocyanin concentration has also been previously reported (Esteban et al 2001, Kennedy et al 2002, Matthews and Anderson 1989). Figures 3.10 and 3.12 show the data of precipitation across vintages in Michigan as recorded at SWMREC. During both growing seasons, data reports large amounts of precipitation occurring in the ten days prior to harvest (figures 3.10 and 3.12), a factor, which has been reported in work by Esteban et al (2001), and Kennedy et al (2002) to result in lower concentrations of most berry constituents, namely anthocyanins by simply increasing the ratio of water to anthocyanin in the berry.

Figures A-5 to A-10 gave the regressed data, analyzed against polynomial curves. As with data compared on a linear basis, there were no recognizable trends between total anthocyanin concentration and yield per vine. This suggests that many factors, other than simply growing location, vintage or clone, have an effect on both the final yield of fruit, and the concentration of anthocyanins therein.

Table 3.6 gave the main effects for interactions commonplace to the work. It also complements tables 3.7 and 3.8. Vineyard interactions are mostly not significant (n.s.), as are some of the treatment interactions. However, average cluster weight and total anthocyanin in New Zealand x Michigan, and UCD13 x 113 x 115 interactions are significant which indicates differences in location, and clone on the basis of cluster

weight and anthocyanin concentration, possibly due to the precipitation and irrigation effects addressed earlier.

Main Effects of Vineyard Data, Environmental Conditions and Total Anthocyanin

Location	Av. Cluster Weight (g)	Av. Yield per vine (kg)	°Brix	Av. Max. Temperature (°C)	Av. GDD	Av. Precip. (mm/day)	Total Anthocyanin (mg/mL)
NZ	50.7	1.51	23.1	20.1	803	1.3	66.4
MI	82.6	2.47	23.9	25.3	1446	2.4	93.5
	*	n.s.	n.s.				n.s.
F	1.49						

Year	Av. Cluster Weight (g)	Av. Yield per vine (kg)	°Brix	Av. Max. Temperature (°C)	Av. GDD	Av. Precip. (mm/day)	Total Anthocyanin (mg/mL)
2004	48.1	1.44	23.1	22.1	1066	2.2	77.9
2005	85.2	2.55	23.9	23.3	1184	1.5	82
	*	n.s.	n.s.				n.s.
F	1.51						

Clone	Av. Cluster Weight (g)	Av. Yield per vine (kg)	°Brix	Total Anthocyanin (mg/mL)
UCD13	93.6	2.83	23.2	42.9
113	72.9	2.17	23.9	53.4
115	98.1	2.96	23.4	63.6
	*	n.s.	n.s.	n.s.
F	0.34			

Location x Year	3.04
Location x Clone	0.17
Year x Clone	0.21

Table 3.6 Main effects plot for total anthocyanin concentration, vineyard and harvest parameters, with interactions.

Least Square Means

EFFECT	Pr > |t|	LOCATION	CLONE	YEAR	ESTIMATE	STANDARD ERROR	t VALUE
Year	0.113			2004	0.06584	0.04014	1.64
Year	0.0023			2005	0.1355	0.04014	3.38
Location	0.1044	NZ			0.06602	0.04014	1.64
Location	0.0012	MI			0.1353	0.04014	3.37
Year x Location	0.1597	NZ		2004	0.08085	0.05677	1.42
Year x Location	0.3717	MI		2004	0.05103	0.05677	0.9
Year x Location	0.3683	NZ		2005	0.05139	0.05677	0.91
Year x Location	0.0002	MI		2005	0.2196	0.05677	3.87
Clone	0.0683		113		0.091	0.04916	1.85
Clone	0.0086		115		0.1328	0.04916	2.7
Clone	0.1162		UCD13		0.07817	0.04916	1.59
Year x Clone	0.3345		113	2004	0.06755	0.06952	0.97
Year x Clone	0.3035		115	2004	0.07205	0.06952	1.04
Year x Clone	0.4076		UCD13	2004	0.05792	0.06952	0.83
Year x Clone	0.1041		113	2005	0.1144	0.06952	1.65
Year x Clone	0.0069		115	2005	0.1936	0.06952	2.78
Year x Clone	0.1612		UCD13	2005	0.09843	0.06952	1.42
Location x Clone	0.2872	MI	113		0.07455	0.06952	1.07
Location x Clone	0.2759	MI	115		0.07634	0.06952	1.1
Location x Clone	0.4996	MI	UCD13		0.04717	0.06952	0.68
Location x Clone	0.1267	NZ	113		0.1074	0.06952	1.55
Location x Clone	0.0081	NZ	115		0.1893	0.06952	2.72
Location x Clone	0.1207	NZ	UCD13		0.1092	0.06952	1.57

Table 3.7 Least square means of fixed effects for the mixed model of data generated by total anthocyanin concentrations in fruit from veraison (day 0) to harvest (day 70).

Differences of Least Square Means

EFFECT	LOCATION	CLONE	YEAR	LOCATION	CLONE	YEAR	Pr > |t|
Year			2004			2005	0.2309
Location	MI			NZ			0.2263
Year x Location	MI		2004	NZ		2004	0.7133
Year x Location	MI		2004	MI		2005	0.0196
Year x Location	MI		2004	NZ		2005	0.0178
Year x Location	NZ		2004	NZ		2005	0.9984
Year x Location	NZ		2004	MI		2005	0.0393
Year x Location	MI		2005	NZ		2005	0.0397
Clone		113			115		0.5494
Clone		113			UCD13		0.8542
Clone		115			UCD13		0.4345
Year x Clone		113	2004		115	2004	0.9636
Year x Clone		113	2004		UCD13	2004	0.7227
Year x Clone		113	2004		115	2005	0.6348
Year x Clone		113	2004		UCD13	2005	0.2042
Year x Clone		113	2004		115	2005	0.7544
Year x Clone		113	2004		UCD13	2005	0.8861
Year x Clone		115	2004		UCD13	2004	0.6677
Year x Clone		115	2004		113	2005	0.2205
Year x Clone		115	2004		115	2005	0.6893
Year x Clone		115	2004		UCD13	2005	0.4782
Year x Clone		UCD13	2004		113	2005	0.172
Year x Clone		UCD13	2004		115	2005	0.6815
Year x Clone		UCD13	2004		UCD13	2005	0.4236
Year x Clone		113	2005		115	2005	0.8711
Year x Clone		115	2005		UCD13	2005	0.3365
Location x Clone	MI	113		MI	115		0.9856
Location x Clone	MI	113		MI	UCD13		0.7744
Location x Clone	MI	113		NZ	115		0.739
Location x Clone	MI	113		NZ	UCD13		0.2471
Location x Clone	MI	115		MI	UCD13		0.7258
Location x Clone	MI	115		MI	UCD13		0.7676
Location x Clone	MI	115		NZ	113		0.7527
Location x Clone	MI	115		NZ	115		0.2544
Location x Clone	MI	115		NZ	UCD13		0.7394
Location x Clone	MI	UCD13		NZ	113		0.1441
Location x Clone	MI	UCD13		NZ	115		0.3529
Location x Clone	MI	UCD13		NZ	UCD13		0.5303
Location x Clone	NZ	113		NZ	115		0.4079
Location x Clone	NZ	113		NZ	UCD13		0.986
Location x Clone	NZ	115		NZ	UCD13		0.4179

Table 3.8 Differences of least square means of fixed effects for the mixed model of data generated by total anthocyanin concentrations in fruit from veraison (day 0) to harvest (day 70).

Figure 3.59 Pinot noir trial plot at SWMREC Vineyards, Benton Harbor, Michigan.

Figure 3.60 Pinot noir trial plot at Lincoln University Vineyard, Canterbury, NZ.

CHAPTER 4

THE IMPACT OF CELLAR FACTORS ON THE COLOR AND ANTHOCYANIN PROFILE OF PINOT NOIR TABLE WINES IN MICHIGAN, USA.

In the cool climate winegrowing region of Michigan USA, *Vitis vinifera* L Pinot noir is an economically important red winegrape cultivar. The region has problems with the final color of young Pinot noir wines based on anthocyanin presence and concentration. The anthocyanin concentration of *V. vinifera* Pinot noir fruit at harvest and resulting wine was investigated using the fruit and wine of three clones. Utilizing HPLC techniques, the five main anthocyanins in the fruit and wine (labeled 1-5) were identified based on a cyanidin-3-glucoside standard, and total anthocyanin concentration was compared. Spectrophotometric methods were used to analyze the samples absorbance to give an indication of color perception by the human eye. Both growing season and clone of Pinot noir were significant effects on the absorbance and anthocyanin concentration of fruit and wine extract samples ($p \leq 0.005$, and $p \leq 0.04$ respectively). Clones performed differently in each season leading to questions regarding the importance of growing multiple clones in each location. Climate of growing region and winemaking extraction techniques are compounding limiting factors in the boundaries of anthocyanin concentration and final color of Pinot noir wines.

INTRODUCTION

The color of a wine is one of the very first attributes a consumer perceives about the overall quality of the product (Creasy and Logan 2003). Therefore many purchase decisions are made on the basis of color, resulting in a direct impact on winery income or success. The color of a wine comes from the extraction of compounds in grape skins during fermentation, known as anthocyanins. Anthocyanins are extremely important compounds in nature, as they determine the color of many flowers and fruits (Zhang et al 2005), and in the case of *Vitis vinifera* L. Pinot noir account for the color in both the fruit and resulting wine (Kennedy et al 2002).

There are many factors involved in the final concentration of anthocyanins in the wine. The initial concentration found in the fruit at harvest when the berry has stopped synthesizing anthocyanins, is the total anthocyanin available to the winemaking process. Further effects on the final concentration of anthocyanins in the fruit include vineyard habitat (sunlight, temperature and soil), canopy management techniques, yield, pruning method, and nutrient availability (Esteban et al 2001, Spayd et al 2002).

During winemaking, there are many limiting factors that reduce the concentration of anthocyanins in the final wine. Such factors include fermentation (length, temperature, pH, TA), oxidation, SO_2 and complex chemical reactions occurring during wine aging.

In an attempt to measure and compare the color and anthocyanin concentration present in the fruit at harvest, and the resultant wine, the study reported here was

constructed. The goals of the experiment were to; 1) determine the concentration of anthocyanins present in the fruit for extraction into the wine, 2) analyze, using controlled vinification techniques the resulting color and anthocyanin concentration in the wine after fermentation, and 3) compare the effects on the above parameters between different clones, and vintages of Pinot noir.

MATERIALS AND METHODS

Vineyard Site

The vineyard site for this experiment was situated at Benton Harbor, Michigan, USA. The site was chosen to evaluate local growing conditions on the expression of color in Pinot noir wines. The vineyard is part of the Michigan State University's Southwest Michigan Research and Extension Center (SWMREC). The vines for the experiment were located on north-south oriented rows of the SWMREC block. The vines are located on the lower West coast of the State of Michigan, planted on Kalamazoo-silt-loam soils with grass inter-rows, a few kilometers inland, eastward from the shores of Lake Michigan at N 42.0841° W 86.3570°, 220 meters above mean sea level. The site has an average growing season length of 165 days (with 165 days being the optimum for winegrapes), yielding 1200 growing degree days (base 10°C), 950mm annual rainfall, with a mean temperature all year of 11.30°C (Glen Creasy 2005, personal communication).

Plant Material

Vines used in this experiment were *Vitis vinifera* L. Pinot noir, clones 113, 115, UCD13. Each vine was pruned to a two-cane vertical shoot positioning system (VSP), in a commercially viable manner. The vines were selected based on their similarity across the experiment, which includes a visual inspection for pest damage, diseases or other factors that could limit its ability to produce, ripen or mature fruit with minimal damage. During the budburst-veraison phase, the vines were treated similarly with regard to canopy management, spray application and viticulture. One vine from each clone was chosen as the whole cluster sample vine, and the remaining vines were harvested for winemaking purposes. Vines were uniformly thinned at veraison, with fruit removed to a level of 30 clusters per vine, equally of apical and basal clusters on fruiting shoots, and all the second-set fruit was removed. Fruit sampling occurred at harvest, at the same time as remaining fruit is harvested for winemaking.

Canopy management included: 1) Leaf removal from both sides of the vines fruiting zone, that was conducted by hand to around 60% exposure of fruit as occurs in commercial vineyards. This was repeated once when lateral leaves grew in place of primary leaves. 2) Shoot tucking to keep desired shoots behind foliage wires and prevent damage from machinery. 3) Dead plant material removal from the canopy to prevent disease inoculum build-up. 4) Normal canopy sprays including insecticides and fungicides applied at suitable periods similar to commercial vineyard management.

Field Design

The vines for this study were arranged by clone in a randomized block design, set out in three groups of ten vines of one clone per block at the SWMREC vineyards at Benton Harbor, Michigan, USA. One vine from each clone was selected as the fruit sampling vine. Treatments were applied to all vines similarly, and fruit harvested for the winemaking process was taken from all vines separated only by clone.

Field data to be obtained during experiments include: 1) cluster number per vine, 2) vine yields (kg/vine), 3) °Brix, and for winemaking purposes 4) titratable acidity (g/L), and 5) pH. During the experiments general weather data was being logged by outside sources (Michigan Automated Weather Network in Michigan, USA). This weather data would include; 1) Growing degree days (GDD), 2) Maximum and minimum air and soil temperatures (°C), 3) precipitation (mm), and 4) Wind Speed (m/s^{-1}).

Sampling

The sampling protocols are broken down into several groups for ease of use. Each set specifies a different area of the work and protocols used therein. The protocols are given as follows;

Sample Preparation

- Weigh each of the two-cluster samples separately (1 sample per clone)
- Removed rachis (carefully removed berries)
- Removed seeds (crushed berries gently, without crushing seed)

- Weighed the skin/juice/flesh for each sample (two clusters (apical and basal))
- Add 3 N hydrochloric acid 'drop-wise' until pH 3 is reached (used a Fisher Scientific accumet® model 10 pH meter)
- Purée in a blender for two minutes (used a Virtis Research Equipment, model 30 blender)
- Pour out onto freeze drying tray, rinsing blender with a 20 mL of distilled water each time, washing thoroughly between samples.
- Record fresh weights
- Freeze dry
- Weigh powder
- Place into sealed plastic Glad© "zip-lock" bags after tare weight is established
- Weigh bag and powder
- Store immediately at -20°C until required for shipping and analysis by HPLC and spectrophotometry.

Winemaking Protocols

- Grapes harvested by hand at 22.0° brix.
- Swift transport to the winery occurs; approximately two hours.
- Bins of fruit placed in the cool room at 5.0°C for two hours prior to processing.
- Fruit crushed and de-stemmed separately.
- Must given ten-day cold soak at 5.0°C with 50ppm sulfur dioxide and carbon dioxide blanket added to each container (Parley et al, 2001).

- Plunged daily
- The vessels for the vinification were 30 liter food grade plastic drums.
- Following cold soak, must warmed, adjusted for sugar and acid, to ensure alcohol uniformity across clones.
- Must then placed at 25.0°C for 12 hours prior to yeast inoculation with "Lalvin Wadenswil 27" yeast; selected based on desire for uniformity across batches and not over expression of oak, fruit or floral characters.
- Fermented for 3 weeks at 25.0 °C with daily cap plunging.
- Drain-off occurred when ferment was completed (<1% residual sugar) using muslin cloth and a funnel.
- Wine samples poured into Schott bottles and stored at 5.0°C until analysis.
- Samples dried with a rotary-evaporator, and made ready for packaging and shipping.

Experimental Conditions

The experiments undertaken were done to provide a basis for practical, commercial application in the future. Thus, the trial blocks had the treatments imposed with as many of the normal viticultural inputs in place so that the results would be reproducible in a regular commercial vineyard. There are three experiments, each using the same vineyard treatments, plant material and sites.

HPLC Analysis

HPLC Standards

The standards for the analysis of the anthocyanins in the Pinot noir fruit extract and wine samples were difficult to obtain and very expensive. For this reason, a pure extract of cyanidin-3-glucoside was obtained, and all peaks and results were compared on the basis of cyanidin-3-glucoside concentration in the fruit/wine sample (Chandra et al 2001, Wada and Ou 2002). The standard, (obtained from M. G. Nair at the National Food Safety and Toxicology Center, Michigan State University, East Lansing, Michigan) was pure, and re-hydrated with pure HPLC-Grade water spiked with HCl to pH 3.00 – to maintain continuity among samples. The standard produced as follows: a) 1.00mg cyanidin-3-glucoside powder was re-hydrated with 1.00mL of pH 3.00 water, and b) a serial dilution was prepared form the stock solution to yield: 0.500, 0.250, 0.125, 0.0625 and 0.03125mg/1.00 mL concentrations respectively. Each standard was injected in triplicate and used in the construction of the standard curve.

Sample Preparation for HPLC.

The preparation for the HPLC phase of sample analysis began with either freeze dried grape extract samples, or the rotary-evaporated wine samples, in accordance with storage and shipping protocols. Each sample was compared on a per milliliter basis for concentrations to avoid errors between fruit extract and wine samples. This technique was more suitable for a representation of the fruit after crushing and processing to pre-ferment status at the winery, which was then compared directly on this bases of per milliliter (or per 1000 liters for a winery) to the wine produced from that fruit.

Solvents Used in HPLC Analysis

Solvents required for HPLC analysis of the anthocyanins in Pinot noir fruit and wine samples were as follows:

Solvent A ➔ 0.10% triflouroacetic Acid

99.90% HPLC-Grade Water

Solvent B ➔ 0.10% triflouroacetic Acid

1.00% Acetic Acid

48.50% Acetonitrile

50.40% HPLC-Grade Water

Solvent Gradient ➔

Time	Flow	%A	%B	%C	%D
0.01	1	80	20	0	0
26	1	40	60	0	0
35	1	80	20	0	0

Table 4.1 Solvent Gradient used in the HPLC analysis of all fruit and wine extract.

HPLC Configuration

Analysis of anthocyanins occurred using HPLC (Waters Corp., Milford, MA) with a PDA detector (Waters Corp., Milford, MA) and the techniques of published procedures (Seeram et al, 2002). The column used in the experiment is a Waters Chromatography Xterra C^{18} 150mm, 3.50μm particle size column. The solvents were used by percentages (v/v) under gradient throughout the entire 40 minute sample run (table 4.1). During this time the column was slightly heated and maintained at 40.0°C for the entire sample set. The mobile phase flow rate of 0.80 mL/min was used for all standards and samples, using 20μL injection aliquots for each. Peaks were detected at

520nm (Zhang et al, 2004). Blank samples of pure HPLC-Grade methanol were run every 10 samples to ensure the column remained clean and clear of debris build-up. Samples were injected in triplicate and the average peak areas were used in the data.

Spectrophotometric Analysis

Spectrophotometry was used to compare the results obtained with HPLC. The analysis used samples prepared at the same time as the HPLC samples – these were re-hydrated by weight with pH 3.00 HPLC-Grade water. The samples were then filtered through a 0.45μm syringe filter before being loaded into 10.00mm path-length quartz crystal cuvettes for analysis. Each sample was read at 520nm for anthocyanins and 700nm for sample purity / contamination. Dilution factor of 3.00 was required as most red wines were too dark when using 10.00mm path-length cuvettes. The dilution factors were corrected by simple multiplication, and recorded. The values obtained at 700nm should be very small, which would show little to no interference. The cuvettes were rinsed with distilled water and dried between samples to avoid contamination. Reference cuvette was filled with HPLC grade water to zero absorbance between samples. All measurements were taken on the same spectrophotometer to reduce experiment error where possible.

Statistical Analysis

Statistical analysis was conducted using SAS version 9.1 (SAS Institute Inc.). Methods utilized include the General Linear Model, Mixed Model and Analysis of Variance for total anthocyanin concentration per milliliter of fruit/wine extract where

appropriate. LSD Means separation was calculated for all of the comparisons on the basis of total anthocyanin concentration per milliliter of fruit/wine extract by SAS 9.1 also. Regression for standard curve and relationships between yield and soluble solids, year and location, clone and wine, for total anthocyanin concentration was calculated using Microsoft Excel. Finally 5% error bars and regression of anthocyanin concentration versus yield calculations were completed using Microsoft Excel.

RESULTS AND DISCUSSION

Vineyard Production Data

The data of cluster sampling and lab preparatory work conducted for each of the sampling dates is displayed in tables A-1 to A-4. Harvest data for the Michigan trial vines is displayed in table 4.2 representing 2004 and 2005 respectively. Despite constant cluster numbers throughout the experimental units, the yield per vine during 2005 is more than twice that which occurs in 2004 (table 4.2). This larger average cluster weight was associated with reduced TA at harvest in 2005 compared with 2004. Although marginally reduced in 2005, brix remained largely unaffected despite the yield having doubled on the vines in 2005. The pH was elevated considerably in 2005 fruit in each clone when compared to 2004 pH data.

The weather at SWMREC may have had an influence on these parameters; table 4.3 shows daily averages for 1st May through 25th September. These data show there was about half as much rain, warmer air and soil temperatures, and lower wind speeds in 2005 than in 2004. Additionally, the accumulated growing degree days (base 10°C) in 2005 at

harvest were over 250 higher than those accumulated by harvest day in 2004. This table for accumulated growing degree days, being a combination of units to describe the climate suitability for growth, based on average daily temperatures above 10°C, and taking into account time above that temperature, gives a direct comparative basis to show how 2005 was a more suitable year in which to photosynthesize and ripen fruit, than in 2004, by almost 16% (Jackson 2000, Reynolds et al 1995).

Further, the effect of light intensity during the growing season has been shown to effect both the concentration and accumulation of anthocyanins in the skins of winegrapes. Studies undertaken by Spayd et al (2002), reported an increase in monomeric anthocyanins with light intensity, although when UV light was filtered out, they found no significant differences suggesting a possible need for the whole light spectrum. This effect is noticeable in these experiments, as figures A-16 and A-18 (chapter 3) give the daily and accumulated daily light intensity readings taken at SWMREC for the duration of the study. During 2005, the concentration of anthocyanins – mainly comprised of malvidin-3-glucoside as previously reported in Pinot noir (Boulton 2001, Gao et al 1997, Jackson 2000) was higher at harvest. This relationship between increasing malvidin-3-glucoside concentration and lower light intensities, has been previously reported (Keller and Hrazdina 1998). During this work, it was also reported that cyanidin-3-glucoside was significantly reduced in shaded environments, however, lower light intensity in 2004 still produced similar concentrations of cyanidin-3-glucoside. This suggested that other factors were involved in the production and final concentration of cyanidin-3-glucoside,

such as light intensity and precipitation before harvest (chapter 3) a statement that is resounded throughout this work.

A: 2004

Clone	Fruit Weight (kg)	Vines	Yeild per Vine (kg)	Tonnes per Hectare	Average Cluster Weight (g)	Brix	TA g/L	pH
UCD13	45.69	31.00	1.47	2.64	49.00	23.90	10.50	3.15
113	24.15	16.00	1.51	2.71	50.00	24.80	8.50	3.35
115	51.48	35.00	1.47	2.64	49.00	24.10	9.60	3.21

B: 2005

Clone	Fruit Weight (kg)	Vines	Yeild per Vine (kg)	Tonnes per Hectare	Average Cluster Weight (g)	Brix	TA g/L	pH
UCD13	94.91	31.00	3.06	5.49	102.00	22.40	6.34	3.50
113	63.61	16.00	3.98	7.13	133.00	24.40	4.81	3.42
115	118.16	35.00	3.38	6.06	113.00	24.00	5.00	3.51

Table 4.2 Michigan harvest data including crop-load, total fruit weight, vine number and cluster weights with fruit harvest parameters for both 2004 (A) and 2005 (B) harvest seasons. Data is compiled for the entire experimental area on the basis of clone.

Year	Max. Air Temp. (°C)	Min. Air Temp. (°C)	Precipitation (mm)	Max. Soil Temp @ 5.08cm (°C)	Min. Soil Temp @ 5.08cm (°C)	Max Wind Speed (m/s)	GDD Base 10°C
2004	24.3	13.3	3.04	25.4	18.5	8.5	1320
2005	26.3	14.7	1.83	27.4	19.7	7.9	1573

Table 4.3 Weather summary for 2004 and 2005 at SWMREC vineyard location in Benton Harbor, Michigan. The data are calculated in the form of daily averages from 1st May – 25th September each year. Growing degree data for the same range are shown.

Additionally, the effect of precipitation on anthocyanin concentration has also been previously reported (Esteban et al 2001, Kennedy et al 2002, Matthews and Anderson 1989). Figures A-19, 21, 23, 25 and 30 (chapter 3) show the data of precipitation across vintages in Michigan as recorded at SWMREC. During both growing seasons, data reports large amounts of precipitation occurring in the ten days prior to harvest (figures A-19 and A-21), a factor, which has been reported in work by Esteban et al (2001), and Kennedy et al (2002) to result in lower concentrations of most berry constituents, namely anthocyanins by simply increasing the ratio of water to anthocyanin in the berry. Further, studies of Matthews and Anderson (1989) reported the effect of water on vine physiology. They showed reproductive development in *V. vinifera* was very sensitive to vine water status both in the current season, and the effects of the following season. The work also showed the relationship between yield and precipitation – higher precipitation resulting in higher yields (reduced concentration of berry components). This was suggested in the work reported here, where during 2005, the precipitation was much lower than in 2004, in the maturation period before harvest (figure A-30), and the concentration of anthocyanins was higher in 2005 than 2004, suggesting that precipitation had an effect on anthocyanin concentration by dilution. This suggested how important precipitation was on berry composition, including that of anthocyanins, and is again similar to the results reported by Matthews and Anderson (1989).

Spectrophotometric Absorbance

Figure 4.1 gives the absorbance of late season fruit (harvest), and that of the resulting wine. It appears that regardless of the absorbance value for the fruit at harvest, the wine samples will almost without exception yield a similar absorbance between clones and seasons. No relationship between harvest fruit absorbance and final wine produced could be established for this data. However, the differences between the harvest fruit (Day 70) absorbance and the final wine produced were statistically very significant with $p \leq 0.005$ (table 4.4). The data collected for harvest fruit, during the experiments do not include the weight of seeds as these were removed to avoid large amounts of tannin in the samples. As a result, the values for absorbance and anthocyanin concentration in the harvest fruit, could be up to 21% lower, on a per weight basis. However, as most differences between harvest fruit and resulting wine were greater than 50%, the differences still exist at the reported levels.

Practically speaking, lower absorbance in the wine compared to the fruit is a problem for the winemaking, especially in cooler climates where total anthocyanin in Pinot noir is limited, leading to even lower colored wines than can be achieved in slightly warmer climates.

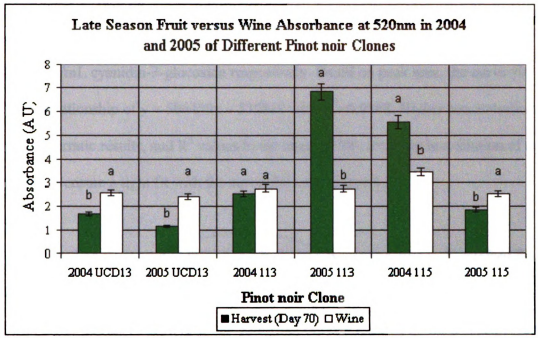


Figure 4.1 Effects of winemaking on absorbance at 520nm of clones UCD13, 113 and 115, showing Harvest date and Wine samples (p=0.05).

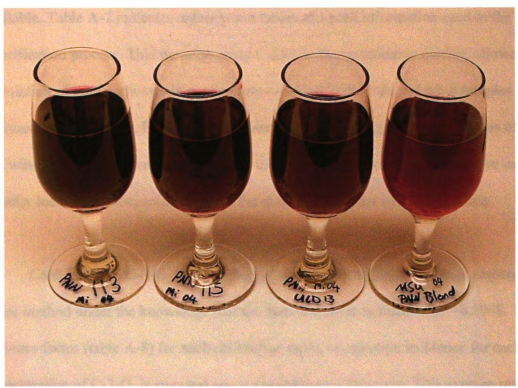


Figure 4.2 Michigan Pinot noir wines from 2004; clones 113, 115, UCD13 respectively alongside a Spartan Cellars blend of Pinot noir from 2004 (Note: Blend not cold-soaked).

Anthocyanin Concentration

The standard curve established for cyanidin-3-glucoside (C-3-G) is displayed in figure 4.3. The curve was constructed using concentrations of 0.0625, 0.1250, 0.2500 and 0.5000 mg/mL cyanidin-3-glucoside respectively. Based on peak area, the curve yielded a linear relationship of $y = 596489x - 530819$ with $R^2=0.9968$. Higher concentrations produced erratic results, and R^2 values lower than 0.6700, resulting in a dilution of many samples to ensure a tight fit with the curve shown in figure 4.3.

Based on this curve of C-3-G, the five anthocyanins present in *V. vinifera* Pinot noir (including C-3-G) could be visually identified from fruit and wine sample chromatograms. However each anthocyanin can only be marked by magnitude, receiving labels 1-5, as the standards for the remaining four anthocyanins (1, 2, 3 and 5) were not available. Table A-7 indicates anthocyanin labels and peak information used in the identification process. This iteration using C-3-G as the quantitative marker, allowed for calculation of total anthocyanin concentrations found in the fruit and wine samples analyzed in this manner. Examples of chromatograms resulting from the analysis of fruit and wine samples are given in figures A-1 to A-4, where peaks mentioned above can be visually located, anthocyanin number 1 the most predominant by concentration.

Calculation of C-3-G concentration is achieved by using the average response factor method under the knowledge that the standard curve is linear to $R^2=0.9968$. The response factor (table A-8) for each calibration point, or injection incidence for each concentration of C-3-G, is summed and averaged across the curve. This average response

Figure 4.3 Standard curve of cyanidin-3-glucoside concentration (mg/mL) is prepared using HPLC standards of 0.0625, 0.125, 0.25 and 0.50 mg/mL respectively, and prepared on the basis of peak area.

factor is then multiplied by the area for each required peak, yielding a concentration of C-3-G in mg/mL. Tables A-9 to A-12 provide results of C-3-G concentration calculations for each of the samples analyzed.

Calculation of total anthocyanin (table A-13 and A-14) in a given sample simply requires the response factor for C-3-G to be multiplied by the peak area value of the identified peaks, and then completed by adding the five concentration values together. This produces a total anthocyanin concentration based on C-3-G concentration in the sample.

As with the absorbance results for the effect of winemaking, the concentration of C-3-G, and total anthocyanin were also significantly greater in the harvest fruit samples than in the wines produced from that fruit (figure 4.4 and 4.6). This result was anticipated, considering the final color of the grape skins is still a deep red after fermentation and pressing – in other words, all of the anthocyanin was not extracted from the skins by fermentation or maceration procedures as already known (Jackson 2000). Further, work by Gao et al (1997) in which anthocyanin extraction was studied in Pinot noir using different vinification techniques, they found that the concentration of malvidin-3-glucoside decreased by at least 30% in the first two days during fermentation, and up to 62% after 7 days, findings that are consistent with this work at similar levels.

However the magnitude of the difference between anthocyanin available in the fruit at harvest and that extracted into the wine, is of concern winemakers, showing exactly how large the difference was, both physically and statistically.

Observation of figure 4.6 revealed while differences exist between the Harvest fruit anthocyanin concentration and that of the final wine, Pinot noir clone UCD13 seemed to extract a larger percentage of its total anthocyanins than any other clone. In 2004, clone UCD13 wine contained almost 74% of the concentration of anthocyanin that was present in the fruit, it performed well in 2005 also, however extraction was down slightly to about 59%. Unfortunately, some extraction values were as low as 28% of possible anthocyanin as witnessed in 2005 clone 113. To the best of the authors knowledge, there is no prior research involving the comparison of individual clones of Pinot noir (namely UCD13, 113 and 115) on the basis of anthocyanin concentration as reported in this work.

The final color of the wine seemed to be limited by extraction techniques not solely based on the location of vineyards as suggested in the early stages of this work. Figure 4.4 shows that in all samples, the extraction of C-3-G was limited, to less than 7% in the case of 2005 clone 113. However, unlike the absorbance values, the concentration was affected somewhat by the total amount in the fruit, as figure 4.4 shows, when the concentration of C-3-G in fruit increased or decreased, as did the concentration in the final wine – to differing magnitudes. This observation was also noticeable in the total anthocyanin concentration (figure 4.6). The total anthocyanin concentration of the fruit

and wine samples were significantly different at the 0.005 level of significance. This is similar to the effect of the C-3-G results, and again raises concerns about the current extraction techniques in winemaking.

The Least Square Means for the analysis of fruit and wine are displayed in table 4.4, and thus suggested the following: 1) Statistically significant differences do exist between clones of Pinot noir anthocyanin concentration at harvest ($p=0.0003$), 2) There is no direct relationship between the concentration of anthocyanins present in the fruit, and those extracted into the wine ($p=0.6541$), and 3) That the effect of harvest fruit anthocyanin concentration on final wine anthocyanin concentration is statistically significant ($p=0.0043$).

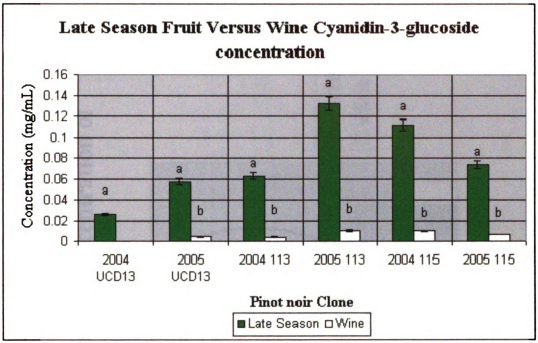


Figure 4.4 Impact of winemaking on cyanidin-3-glucoside concentration for each clone and year, separating harvest (day 70) fruit and wine resulting from that fruit (p=0.05).

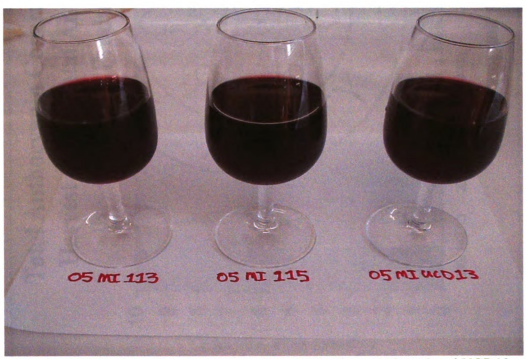


Figure 4.5 Michigan Pinot noir wines from 2005; clones 113, 115 and UCD13 respectively.

Figure 4.6: Total anthocyanin concentration of Michigan fruit at harvest compared with total anthocyanin concentration of wine produced from fruit for each clone and year ($p=0.05$).

Least Square Means for Wine

EFFECT	SAMPLETYPE	ESTIMATE	STANDARD ERROR	DF	t VALUE	Pr>|t|
Sample Type	Harvest Data	0.07577	0.01257	8	6.03	0.0003
Sample Type	Wine	0.005948	0.01257	8	0.47	0.6541

Differences of Least Square Means

EFFECT	SAMPLE TYPE	SAMPLE TYPE	ESTIMATE	STANDARD ERROR	DF	t VALUE	Pr>|t|
Sample Type	Harvest Data	Wine	0.06992	0.01777	8	3.93	0.0043

Table 4.4 Least square means and differences of least square means of fixed effects for the mixed model of data generated by total anthocyanin concentrations in wine compared to that of harvest (day 70) fruit data.

CHAPTER 5
CONCLUSIONS

CONCLUSIONS

Summary

The growing conditions in 2005 were more suitable for good vine and fruit production. As a result, much of the data for 2005 were more favorable than 2004.

The differences expected between the New Zealand and Michigan growing locations were not statistically significant regardless of noted differences and inconsistencies between locations. Total anthocyanin concentrations were higher in New Zealand during 2005, and Michigan produced better results in 2004. C-3-G concentrations followed a similar pattern, yet these data seemed to closely associate with absorbance data – where higher C-3-G related to higher absorbance's, especially in the final wines.

Clone differences were noted during each year and growing location, however overall, the clone of Pinot noir was not a significant influence on either fruit or wine anthocyanin concentration, absorbance or C-3-G concentration. On the basis of these data clones performed in the manner expected when the meaning of the word clone is examined (A cell, group of cells, or organism that are descended from and genetically identical to a single common ancestor, such as a bacterial colony whose members arose from a single original cell (http://www.dictionary.com)).

The data for harvest fruit and final wine analysis were supportive. Statistically significant differences existed between the concentration of total anthocyanin in the fruit sample at harvest, and the wine ($p \leq 0.0050$). Some wines were extracting almost 74% of available anthocyanins in the case of UCD13 in 2004, whereas some were extracting as little as 28% as clone 113 displayed in 2005.

Further Research

The work reported here suggests several experiments that could delve further into the area of international location similarities and differences based on many factors of Pinot noir culture and vinification. The most prominent of these would be to continue to analyze the full anthocyanin profile of Pinot noir and track its changes throughout the ripening phase in the vineyard, and further still throughout the winemaking phase in the winery.

Another project could include documentation of total concentration or expected percentages of the different anthocyanins present in the different wine-grapes, and how this can be altered by either location of culture or manual control inputs in the vineyard to change the wine as desired.

Lastly, a determination of the effect of anthocyanin levels alone, on the gustatory perception of the final wine product of both experienced wine judges and novice wine drinkers, to determine the utmost effect of the anthocyanin profile on the final wine destined for consumers would be beneficial.

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APPENDIX

Table A-1: Fruit sampling data for lab analysis including cluster, rachis, fresh and dry weights (g) for year one, New Zealand.

2004 **New Zealand**

Week One

Sample	Cluster (x2) Weight (g)	Rachis (x2) Weight (g)	Fruit weight (g) (no seed)	Dry Weight (g)
Mariafield (UCD23)	238.2	11.8	189.3	18.9
UCD13	107.2	6.5	84.3	13.7
113	105.3	6.6	76.8	12.3
115	164.0	14.6	124.1	19.9

Week Two

Sample	Cluster (x2) Weight (g)	Rachis (x2) Weight (g)	Fruit weight (g) (no seed)	Dry Weight (g)
Mariafield (UCD23)	168.3	9.2	144.8	34.9
UCD13	116.3	6.9	101.3	26.6
113	117.2	5.7	100.2	24.4
115	198.7	33.9	147.5	33.6

Week Three

Sample	Cluster (x2) Weight (g)	Rachis (x2) Weight (g)	Fruit weight (g) (no seed)	Dry Weight (g)
Mariafield (UCD23)	114.1	6.9	75.3	20.2
UCD13	62.5	2.6	42.3	17.8
113	33.1	5.0	17.8	5.0
115	79.7	5.9	69.9	13.9

Table A-2: Fruit sampling data for lab analysis including cluster, rachis, fresh and dry weights (g) for year one, Michigan.

2004 **Michigan**

Week One

Sample	Cluster (x2) Weight (g)	Rachis (x2) Weight (g)	Fruit weight (g) (no seed)	Dry Weight (g)
Mariafield (UCD23)	NA	NA	NA	NA
UCD13	148.3	6.7	127.6	29.5
113	122.2	5.6	100.0	13.0
115	141.4	4.8	106.6	23.8

Week Two

Sample	Cluster (x2) Weight (g)	Rachis (x2) Weight (g)	Fruit weight (g) (no seed)	Dry Weight (g)
Mariafield (UCD23)	NA	NA	NA	NA
UCD13	119.5	4.3	95.8	31.8
113	220.6	7.0	175.9	43.0
115	149.6	3.3	129.8	32.4

Week Three

Sample	Cluster (x2) Weight (g)	Rachis (x2) Weight (g)	Fruit weight (g) (no seed)	Dry Weight (g)
Mariafield (UCD23)	NA	NA	NA	NA
UCD13	139.7	2.6	122.1	33.8
113	151.0	4.3	109.5	24.7
115	183.2	5.6	137.0	37.4

Table A-3: Fruit sampling data for lab analysis including cluster, rachis, fresh and dry weights (g) for year two, New Zealand.

2005 **New Zealand**

Week One

Sample	Cluster (x2) Weight (g)	Rachis (x2) Weight (g)	Fruit weight (g) (no seed)	Dry Weight (g)
Mariafield (UCD23)	14.6	1.8	10.8	0.6
UCD13	7.9	1.8	5.3	0.3
113	6.7	1.3	4.5	0.6
115	12.1	1.5	9.2	0.7

Week Two

Sample	Cluster (x2) Weight (g)	Rachis (x2) Weight (g)	Fruit weight (g) (no seed)	Dry Weight (g)
Mariafield (UCD23)	29.1	5.8	21.6	3.2
UCD13	11.8	1.5	9.7	1.5
113	13.9	4.3	8.9	1.4
115	23.0	2.0	19.5	2.6

Week Three

Sample	Cluster (x2) Weight (g)	Rachis (x2) Weight (g)	Fruit weight (g) (no seed)	Dry Weight (g)
Mariafield (UCD23)	26.9	3.7	21.0	4.8
UCD13	37.3	4.4	30.1	5.3
113	43.0	3.5	35.6	4.8
115	38.3	3.5	31.1	5.8

Table A-4: Fruit sampling data for lab analysis including cluster, rachis, fresh and dry weights (g) for year two, Michigan.

2005 **Michigan**

Week One

Sample	Cluster (x2) Weight (g)	Rachis (x2) Weight (g)	Fruit weight (g) (no seed)	Dry Weight (g)
Mariafield (UCD23)	NA	NA	NA	NA
UCD13	150.9	6.5	122.3	16.4
113	193.0	6.5	142.1	23.2
115	166.1	4.9	118.3	17.5

Week Two

Sample	Cluster (x2) Weight (g)	Rachis (x2) Weight (g)	Fruit weight (g) (no seed)	Dry Weight (g)
Mariafield (UCD23)	NA	NA	NA	NA
UCD13	220.3	8.4	178.7	33.2
113	205.4	6.9	178.5	28.4
115	240.9	7.0	216.9	29.8

Week Three

Sample	Cluster (x2) Weight (g)	Rachis (x2) Weight (g)	Fruit weight (g) (no seed)	Dry Weight (g)
Mariafield (UCD23)	NA	NA	NA	NA
UCD13	286.9	12.8	236.5	34.6
113	201.2	7.5	159.4	30.9
115	230.3	9.3	196.8	36.8

Table A-5 Absorbance of 2004 samples at 520nm (S1 = day 0, S2 = day 30, S3 = day 70).

Location	Clone	Sample	Absorbance (A)
NZ	MAR	S1	0.153
NZ	MAR	S2	2.625
NZ	MAR	S3	2.133
NZ	UCD13	S1	0.231
NZ	UCD13	S2	2.218
NZ	UCD13	S3	3.131
NZ	113	S1	0.298
NZ	113	S2	1.966
NZ	113	S3	1.441
NZ	115	S1	0.145
NZ	115	S2	1.734
NZ	115	S3	0.997
MI	UCD13	S1	4.050
MI	UCD13	S2	4.118
MI	UCD13	S3	1.672
MI	UCD13	WINE	2.581
MI	113	S1	3.113
MI	113	S2	8.021
MI	113	S3	2.535
MI	113	WINE	2.756
MI	115	S1	5.212
MI	115	S2	5.230
MI	115	S3	5.561
MI	115	WINE	3.453

Table A-6 Absorbance of 2005 samples at 520nm (S1 = day 0, S2 = day 30, S3 = day 70).

Location	Clone	Sample	Absorbance (A)
NZ	MAR	S1	0.036
NZ	MAR	S2	1.346
NZ	MAR	S3	4.803
NZ	UCD13	S1	0.014
NZ	UCD13	S2	0.905
NZ	UCD13	S3	4.742
NZ	113	S1	0.022
NZ	113	S2	0.801
NZ	113	S3	5.991
NZ	115	S1	0.032
NZ	115	S2	1.021
NZ	115	S3	8.221
MI	UCD13	S1	1.011
MI	UCD13	S2	2.232
MI	UCD13	S3	1.147
MI	UCD13	WINE	2.407
MI	113	S1	2.618
MI	113	S2	1.585
MI	113	S3	6.837
MI	113	WINE	2.746
MI	115	S1	1.596
MI	115	S2	1.258
MI	115	S3	1.860
MI	115	WINE	2.533

Table A-7 Individual anthocyanin identification for chromatograms, based on peak magnitude and retention time.

Individual Anthocyanin Identification

Label	Peak Area Magnitude	Retention Time (minutes +/- 0.250)
1	Largest	8.20
2	Second Largest	7.60
3	Third Largest	6.10
4 (C-3-G)	Fourth Largest	5.40
5	Fifth Largest	4.00

Figure A-1 Chromatogram of 2004 Michigan clone 115 at day 0.

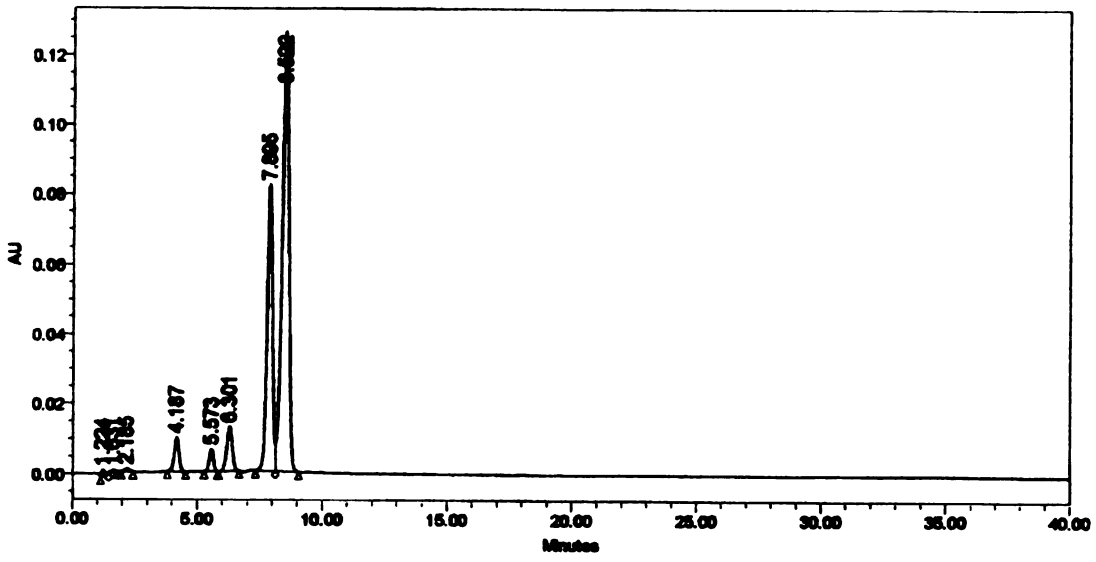

Figure A-2 Chromatogram of 2004 Michigan clone 115 at day 30.

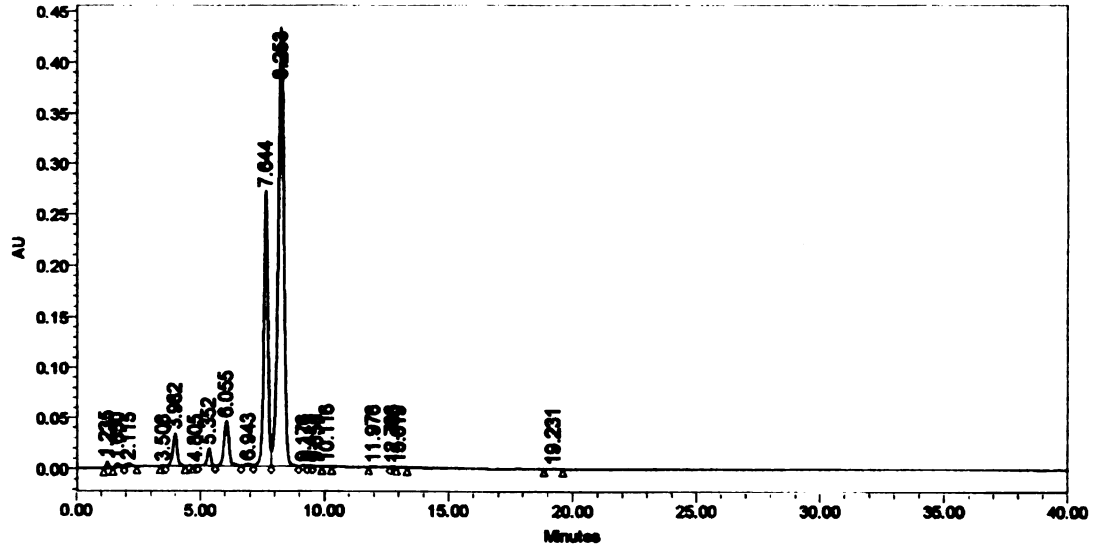

Figure A-3 Chromatogram of 2004 Michigan clone 115 at day 70.

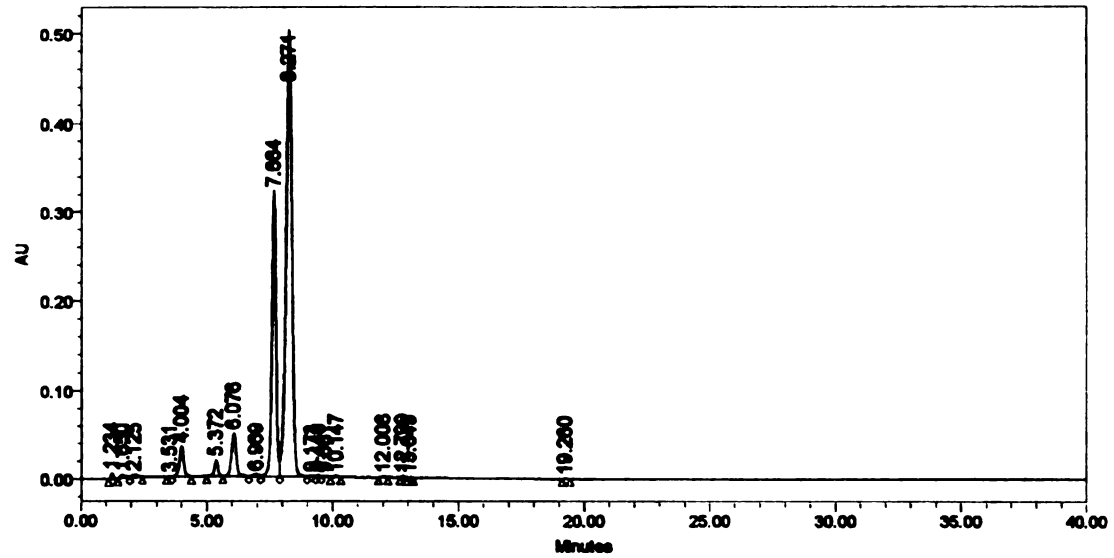

Figure A-4 Chromatogram of 2004 Michigan clone 115 wine.

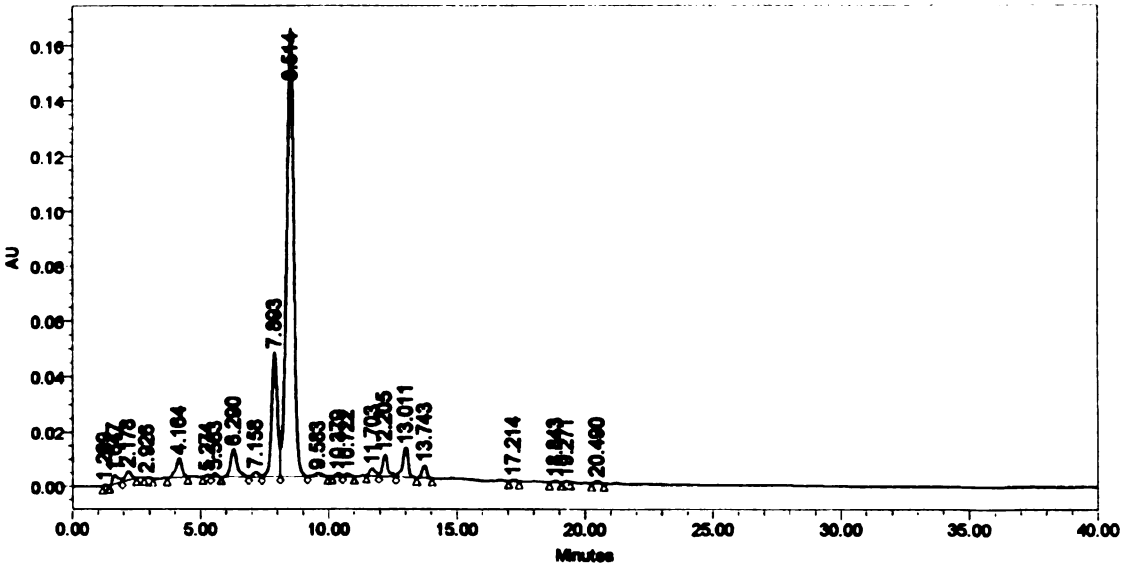

Table A-8 Response factor for cyanidin-3-glucoside standard.

C-3-G Standard Concentration (mg/mL)	Peak Area	Response Factor
0.0625	33042	1.89153E-06
0.125	724326	1.72574E-07
0.25	1232198	2.02889E-07
0.5	1852047	2.69972E-07

Average response factor for C-3-G = 6.34242E-07

Table A-9 Concentration of cyanidin-3-glucoside in 2004 New Zealand samples.

Clone	Sample	Injection 1	Injection 2	Injection 3	Average
		mg/mL	mg/mL	mg/mL	mg/mL
MAR	S1	0.0044	0.0045	0.0045	0.0045
MAR	S2	0.1150	0.1144	0.1139	0.1144
MAR	S3	0.0920	0.0920	0.0917	0.0919
UCD13	S1	0.0091	0.0092	0.0091	0.0091
UCD13	S2	0.0760	0.0758	0.0756	0.0758
UCD13	S3	0.1350	0.1390	0.1345	0.1362
113	S1	0.0080	0.0082	0.0082	0.0081
113	S2	0.0692	0.0695	0.0689	0.0692
113	S3	0.0620	0.0615	0.0613	0.0616
115	S1	0.0014	0.0014	0.0012	0.0013
115	S2	0.0654	0.0659	0.0660	0.0658
115	S3	0.0429	0.0357	0.0177	0.0321

Table A-10 Concentration of cyanidin-3-glucoside in 2004 Michigan samples.

Clone	Sample	Injection 1	Injection 2	Injection 3	Average
		mg/mL	mg/mL	mg/mL	mg/mL
UCD13	S1	0.0645	0.0630	0.0591	0.0622
UCD13	S2	0.0464	0.0497	0.0441	0.0468
UCD13	S3	0.0000	0.0261	0.0261	0.0261
UCD13	WINE	0.0000	0.0000	0.0000	0.0000
113	S1	0.0660	0.0660	0.0662	0.0661
113	S2	0.1371	0.1376	0.1382	0.1376
113	S3	0.0627	0.0627	0.0625	0.0626
113	WINE	0.0042	0.0039	0.0041	0.0041
115	S1	0.1723	0.1661	0.0000	0.1692
115	S2	0.1084	0.1089	0.1094	0.1089
115	S3	0.1118	0.1112	0.1112	0.1114
115	WINE	0.0102	0.0101	0.0096	0.0100

Table A-11 Concentration of cyanidin-3-glucoside in 2005 New Zealand samples.

Clone	Sample	Injection 1	Injection 2	Injection 3	Average
		mg/mL	mg/mL	mg/mL	mg/mL
MAR	S1	0.0000	0.0000	0.0000	0.0000
MAR	S2	0.0599	0.0600	0.0601	0.0600
MAR	S3	0.4316	0.4304	0.4301	0.4307
UCD13	S1	0.0000	0.0000	0.0000	0.0000
UCD13	S2	0.0870	0.0872	0.0871	0.0871
UCD13	S3	0.3472	0.3469	0.3463	0.3468
113	S1	0.0000	0.0000	0.0000	0.0000
113	S2	0.0000	0.1004	0.1006	0.1005
113	S3	0.4390	0.4392	0.4378	0.4387
115	S1	0.0000	0.0000	0.0000	0.0000
115	S2	0.1132	0.1132	0.1131	0.1132
115	S3	0.9242	0.9251	0.9208	0.9234

Table A-12 Concentration of cyanidin-3-glucoside in 2005 Michigan samples.

Clone	Sample	Injection 1	Injection 2	Injection 3	Average
		mg/mL	mg/mL	mg/mL	mg/mL
UCD13	S1	0.0000	0.0262	0.0259	0.0260
UCD13	S2	0.0834	0.0815	0.0814	0.0821
UCD13	S3	0.0573	0.0572	0.0570	0.0572
UCD13	WINE	0.0040	0.0042	0.0045	0.0043
113	S1	0.0288	0.0292	0.0290	0.0290
113	S2	0.0200	0.0200	0.0199	0.0199
113	S3	0.1323	0.1318	0.1321	0.1320
113	WINE	0.0084	0.0111	0.0113	0.0103
115	S1	0.0000	0.0169	0.0167	0.0168
115	S2	0.0399	0.0396	0.0398	0.0398
115	S3	0.0740	0.0741	0.0737	0.0739
115	WINE	0.0063	0.0066	0.0066	0.0065

Table A-13 Concentration of total anthocyanins in 2004 samples (mg/ml). (S1 = day 0, S2 = day 30, S3 = day 70).

Location	Clone	Sample	Total Anthocyanin
			mg/mL
NZ	MAR	S1	0.0974
NZ	MAR	S2	4.8447
NZ	MAR	S3	3.4042
NZ	UCD13	S1	0.1775
NZ	UCD13	S2	3.5006
NZ	UCD13	S3	5.8135
NZ	113	S1	0.2427
NZ	113	S2	3.1369
NZ	113	S3	2.1340
NZ	115	S1	0.0708
NZ	115	S2	3.0521
NZ	115	S3	1.6213
MI	UCD13	S1	6.2868
MI	UCD13	S2	5.0946
MI	UCD13	S3	2.9803
MI	UCD13	WINE	2.2053
MI	113	S1	3.3075
MI	113	S2	8.6029
MI	113	S3	4.6054
MI	113	WINE	1.5682
MI	115	S1	11.6610
MI	115	S2	7.2511
MI	115	S3	8.3792
MI	115	WINE	2.6930

Table A-14 Concentration of total anthocyanins in 2005 samples (mg/ml). (S1 = day 0, S2 = day 30, S3 = day 70).

Location	Clone	Sample	Total Anthocyanin
			mg/mL
NZ	MAR	S1	0.0000
NZ	MAR	S2	0.7661
NZ	MAR	S3	10.9172
NZ	UCD13	S1	0.3047
NZ	UCD13	S2	1.0753
NZ	UCD13	S3	8.2704
NZ	113	S1	0.3246
NZ	113	S2	1.8235
NZ	113	S3	11.8296
NZ	115	S1	0.5790
NZ	115	S2	1.9058
NZ	115	S3	20.5584
MI	UCD13	S1	2.3151
MI	UCD13	S2	4.3003
MI	UCD13	S3	2.8202
MI	UCD13	WINE	1.6525
MI	113	S1	4.3742
MI	113	S2	4.1526
MI	113	S3	8.8716
MI	113	WINE	2.5091
MI	115	S1	3.1049
MI	115	S2	2.0456
MI	115	S3	3.3685
MI	115	WINE	2.2386

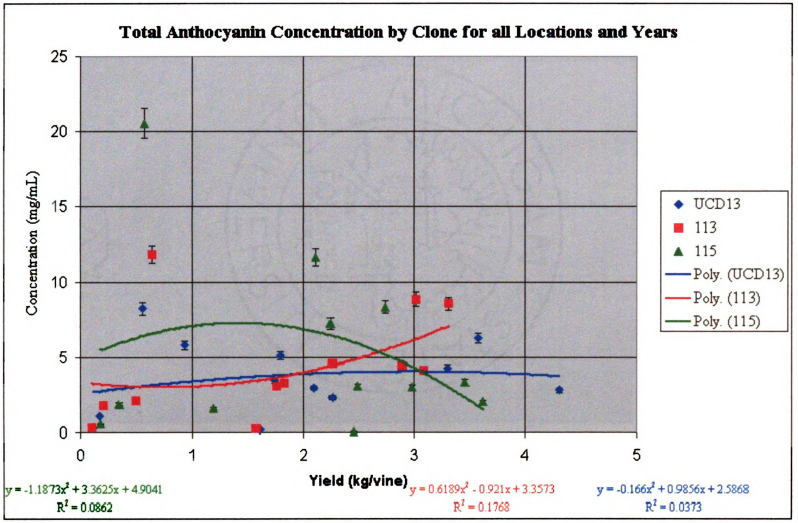

Figure A-5 Total anthocyanin regressed against yield per vine by clone for all years and locations.

Figure A-6 Total anthocyanin regressed against yield per vine by clone for 2005 in both locations.

Figure A-7 Total anthocyanin regressed against yield per vine for all clones, years and locations.

Figure A-8 Total anthocyanin regressed against yield per vine by clone and locations separated by year.

Figure A-9 Total anthocyanin regressed against yield per vine across all clones and locations for 2004.

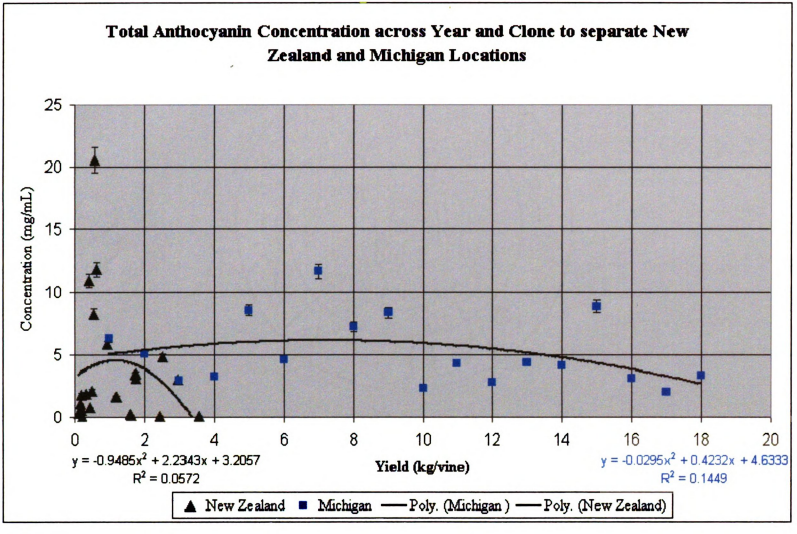

Figure A-10 Total anthocyanin regressed against yield per vine across year and clone to separate location.