CYANIDIN-*3-O*-GLUCOSIDE IS AN IMPORTANT ANTHOCYANIN IN SEVERAL CLONES OF *VITIS VINIFERA* L. PINOT NOIR FRUITS AND RESULTING WINE FROM MICHIGAN AND NEW ZEALAND.

Gerard A. LOGAN[1*], G. Stanley HOWELL[2], Muraleedharan G. NAIR[3]

[1*] Corresponding Author: Gerard A. Logan [Tel.: +64 6 974 8000, Ext.: 5832; Fax.: +64 6 974 8910; E-mail.: glogan@eit.ac.nz]. Lecturer in Viticulture, School of Viticulture & Wine, Faculty of Science & Technology, Eastern Institute of Technology, Gloucester Street, Private Bag 1201, Taradale, Hawkes Bay, New Zealand.
[2] G. Stanley Howell, Emeritus Professor, Program of Viticulture and Enology, Department of Horticulture, Michigan State University, East Lansing, Michigan 48824, USA.
[3] Muraleedharan G. Nair, Professor, Bioactive Natural Products and Phytoceuticals, Department of Horticulture and National Food Safety and Toxicology Center, Michigan State University, East Lansing, Michigan 48824, USA.

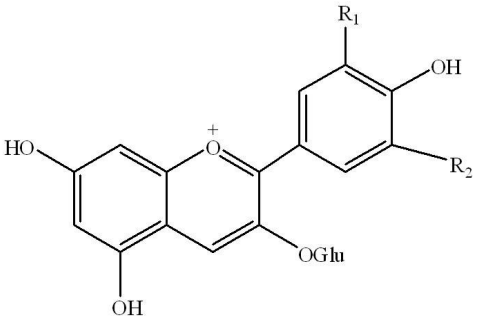
Abstract
In the cool winegrowing regions of Michigan, USA and Canterbury, New Zealand, *Vitis vinifera* L. Pinot noir is an economically important red winegrape cultivar. Both regions have problems with the color of Pinot noir wines based on anthocyanin concentration. Thus, anthocyanin concentration of *V. vinifera* L. Pinot noir fruit was investigated using three clones and two growing locations, Canterbury, New Zealand and Michigan, USA. Wines were made from Michigan sample vines, and analyzed for anthocyanin. Utilizing HPLC (High Pressure Liquid Chromatography) techniques, the five main anthocyanins (1-5), in the fruit and wine were identified and quantified based on cyanidin-3-*O*-glucoside (4, C3G), and total anthocyanin concentration in grapes and wine was compared. Both growing season and location showed significant effects on the C3G concentration in fruit ($p \leq 0.01$, and $p \leq 0.002$ respectively), and wine ($p \leq 0.005$, and $p \leq 0.04$ respectively). The clone of Pinot noir grown in both locations showed only non-statistically significant differences. Wines yielded lower ($p \leq 0.002$) anthocyanin concentration than the fruit at harvest. Data suggested growing region and winemaking extraction techniques are compounding limiting factors in the boundaries of anthocyanin concentration of Pinot noir wines. These results highlight the need to grow multiple clones at each location.

Keywords: Vitis vinifera; Pinot noir; clone; malvidin; peonidin; petunidin; cyanidin; delphinidin; vineyard; Michigan; New Zealand

Introduction

The red grape, *Vitis vinifera* L. Pinot noir, is important commercially throughout the world, including Burgundy, France, California, Oregon, and Michigan, USA and the South Island of New Zealand. Color is an important indicator of fruit maturity, and an imperative factor in wine quality as perceived by the consumer (Creasy and Logan 2003, de Freitas and Mateus 2004). The development of red color in grape berries occurs with an increase of a class of phenolic compounds known as anthocyanins (Figure 1).

Anthocyanins are highly significant compounds in nature, as they determine the color of many flowers and fruits (Holton and Cornish 1995, 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. Also, the biosynthesis of anthocyanin in maize (*Zea mays*), snapdragon (*Antirrhinum majus*) and petunia (*Petunia hybrida*) has been well established (Holton et al. 1995).



		R_1	R_2
1	Malvidin	OCH_3	OCH_3
2	Peonidin	OCH_3	H
3	Petunidin	OCH_3	OH
4	Cyanidin	OH	H
5	Delphinidin	OH	OH

Figure 1 Anthocyanins, -3-O-glucosides of malvidin (1), peonidin (2), petunidin (3), cyanidin (4) and Delphinidin (5) as found in V. vinifera L. Pinot noir.

There are many factors involved in the final concentration of anthocyanins in the skins of winegrapes. The concentration in grape berries is primarily controlled by vine genes and photosynthesis, although vineyard environment (Cortell et al. 2007a), vintage (Intrigliolo and Castel, 2008), and cultural techniques play an important role (Boss et al. 1996, Bindon et el. 2008, Guidoni et al. 2008). The biosynthesis of anthocyanins in berries begins at veráison, and continues until harvest or fruit physiological maturity, whichever occur first (Esteban et al. 2001). Environmental factors that affect anthocyanin biosynthesis include sunlight, temperature and soil type (Cortell et al. 2007a) - all are functions of vineyard growing location and vintage (Wilson, 1998, Anderson et al. 2008). Cultural techniques affecting anthocyanin development include canopy management, yield, pruning method and nutrient availability (Spayd et al. 2002, Guidoni et al. 2008). Effects of vine habitat on concentration of anthocyanins in berries include precipitation and evaporation, which affect vine physiology and berry composition (Esteban et al. 2001, Bindon et al. 2008, Intrigliolo and Castel, 2008). Similarly, there are many factors affecting the concentration of anthocyanins in wines of *V. vinifera* L. Pinot noir (Cortell et al. 2007b). Such factors include fermentation (length, temperature, pH, TA), oxidation, SO_2 and complex chemical reactions occurring during wine aging (Boulton, 2001, Sacchi et al. 2005, Soar et al. 2008).

To determine the behavior of anthocyanin concentration present in *V. vinifera* L. Pinot noir fruit at harvest, and the resultant wine, in response to different vineyard environments and vintages, an approach was applied to measure the fruit from veráison to harvest, and young wine, on the basis of anthocyanin concentration. Therefore, the ripening fruit was sampled during the developmental period and compared on the basis of different clones during two seasons in separate hemispheres. Wine was made from the remaining treatment fruit and analyzed in the same way. The results of our approach are presented in this manuscript.

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 and production of anthocyanins 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 *V. vinifera* 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-sandy-loam soils with grass inter-rows, a few kilometers eastward from the shores of Lake Michigan at N 42.08° W 86.35°, 220 meters above mean sea level. The site has an average growing season length of 165 days, yielding 1200 growing degree days (base 10°C), 950 mm annual rainfall, with a mean temperature all year of 11.30°C (Michigan Automated Weather Network).

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 *V. vinifera* L. 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°. 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 12.90°C (NIWA Science, New Zealand).

Plant material
Vines used in this experiment were five-year-old (during the first year of the trial), *Vitis vinifera* L. Pinot noir, clones Dijon 113 (113), Dijon 115 (115) and University of California at Davis' clone 13 (UCD13), grafted to rootstock 101-14 Millardet et de Grasset. Each vine was pruned to a two-cane vertical shoot positioning system (VSP), in a commercially viable manner, planted 1.2 x 2.4 meters in New Zealand, and 1.8 x 2.6 meters in Michigan. 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 growing season, the vines were treated similarly with regard to canopy management, spray application and viticulture. At veráison, one vine from each clone was chosen as the whole cluster sample vine, and this remained the sample vine for the entire experiment. Vines were uniformly thinned at veráison, 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. In New Zealand, the vines were covered with netting to prevent bird damage, and irrigated using 1.4 liter/hour drippers for one hour per day when required by low rainfall. Neither technique was required in Michigan. Sampling began at this time, considered day 0, then continued with another sample at day 30, and lastly a sample at day 70 (harvest). Each sample consisted of two full clusters (one apical, one basal) removed randomly from each sample vine, once per clone, at each of the above sampling dates.

The growing season canopy management included: 1) Leaf removal from both sides of the vines fruiting zone that was conducted by hand to around 60% fruit exposure. This was repeated once when lateral leaves grew in place of primary leaves. 2) Shoot tucking. 3) Removal of dead/damaged shoots/leaves from canopy. 4) Normal canopy sprays including insecticides and fungicides applied at suitable periods.

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. The vines in New Zealand were arranged in groups of three vines for each clone, in one row. One sample vine was selected from each clone and used for all three sampling dates. Sample vines and treatments applied in Michigan were identical in all New Zealand based vines.

Field data obtained during experiments from vines other than sample vines, included: 1) cluster number per vine, 2) vine yields (kg/vine), 3) Soluble solids (°Brix), and 4) pH. During the experiments, general weather data from each geographic location were logged by outside sources (Michigan Automated Weather Network in Michigan, USA, and Lincoln University in Canterbury, New Zealand). The weather data included in the study were 1) growing degree days (GDD), 2) maximum and minimum air and soil temperatures (°C), 3) daily precipitation (mm) and 4) wind speed (m/s^{-1}), 5) light energy.

Winemaking
The fruit remaining after sample collection from the Michigan trial vineyard site were harvested by hand when the fruit reached a predetermined maturity of 22.0° Brix. Transport to the winery then occurred which took approximately two hours, following the completion of harvest. The fruit was placed in a cool room at 5.0°C for two hours prior to processing. All of the fruit was crushed and de-stemmed separately using a small-scale crusher/de-stemmer. The resulting must, placed in 30 liter food grade plastic drums were given a ten-day cold soak at 5.0°C with 50ppm of SO_2 and a CO_2 blanket (Parley et al. 2001) added to each container to avoid oxidation of anthocyanins. During the ten-day cold soak, the vessels were plunged daily to ensure skins remained moist and viable for color extraction.

Following the cold soak period, the pre-fermentation must was warmed to 25.0°C by placing vessels in a warm room (at 25.0°C) for 12 hours prior to the initiation of fermentation. Musts were then adjusted for sugar and acid, to ensure resulting alcohol uniformity across clones. Musts were inoculated with "Lalvin Wadenswil 27" (*Saccharomyces cerevisiae)* yeast; selected based on a desire

for uniformity across batches preventing over expression of oak, fruit or floral characters. Fermentations took 3 weeks at 25.0 ℃ to complete to dryness. During this time the fermentations were monitored and the caps were plunged daily. The wines were then drained off the skins when complete (<1% residual sugar) using muslin cloth and funnel. Wine samples were then poured into 100mL Schott bottles and stored at 5.0℃ until analysis.

Analytical methods

Fruit extract preparation

Cluster samples were kept in a fridge which slowly cooled the fruit to 5℃ immediately after collection, before being weighed. Berries were then removed from the rachis and the seeds extracted before remaining skin/flesh/juice were puréed and analyzed for soluble solids and pH. This fruit purée was then acidified to pH 3 using 3N HCl (300 µL) to stabilize anthocyanins, before being frozen to -20℃, weighed and immediately lyophilized (FTS Systems, Inc., Stone Ridge, NY). The lyophilized samples were weighed and New Zealand samples transported to Michigan lab where all the samples were stored at -20℃ until analysis. An aliquot (5 g) of the powdered and lyophilized fruit purée was re-hydrated to fresh weight basis with reverse osmosis (RO) H_2O and adjusted to pH 3 using 3N HCl (300 µL), centrifuged at 1,675 g for 30 min, decanted and then filtered through 0.45µm using a syringe filter into HPLC vials. The total volume of the supernatant containing anthocyanins was recorded for each sample.

Wine sample preparation

Wine samples of 100mL each, had ethanol and water removed slowly using a rotary-evaporator (Buchi, R-22, Brinkmann Instruments, New York), with water bath set at 40℃, in a round-bottom flask rotating at approximately 60RPM. Dried samples were immediately re-hydrated by weight, with reverse osmosis (RO) H_2O and adjusted to pH 3 using 3N HCl (300 µL). Wines were then filtered through 0.45µm using a syringe filter into HPLC vials, as were the fruit extract samples, which were both analyzed immediately following the re-hydration.

HPLC analysis of anthocyanins

Total anthocyanin concentration in Pinot noir grape and wine samples was determined on the basis of cyanidin-3-glucoside (C3G) concentration in the sample (Chandra et al. 2001, Wada and Ou 2002). The standard, C3G, was prepared according to the published procedures from Nair's laboratory (Zhang et al. 2005, Chandra et al. 2001). Individual anthocyanins were identified from chromatograms and calculated on the basis of C3G concentration in samples (Chandra et al. 2001, Wada et al. 2002, Price et al. 1995). The pure C3G was dissolved in H_2O (pH 3) (mg/mL H_2O), and a serial dilution was prepared to yield: 0.50, 0.25, 0.125 and 0.0625 mg/mL respectively. Each standard solution was injected in triplicate and the data means used to construct the standard curve.

The acidic water extracts of fruit and wine samples (pH 3) were injected in triplicate and analyzed on a Waters Corp. HPLC column (Xterra C_{18}, 4 x 250 mm) at 40℃. The solvent system used was TFA/ H_2O (0.1:99.9, v/v), TFA/acetic acid/acetonitrile/H_2O (0.1/1/48.5/50.40, v/v) under step gradient conditions, and anthocyanins were detected at 520nm (PDA, Waters Corp.) at a flow rate of 0.8 mL/min (Waters 600 multi-solvent delivery system, Waters Corp.). Total anthocyanin concentration was calculated according to a standard curve, and expressed on a C3G basis. The concentrations were plotted against peak area to prepare the standard curve.

Statistical analysis

Statistical analysis including the MIXED procedure was conducted using SAS statistical software (version 9.1; SAS Institute, Cary, NC). Least significant differences (LSD) means separation and regression analyses were calculated using Microsoft Excel (version 2003; Microsoft Corporation, Redmond, WA). Probability values lower than 0.05 were accepted as significant.

Results and Discussion

Vineyard factors

Despite constant cluster numbers throughout the experimental units, the yield per vine in both locations during 2005 was more than twice that which occurred in 2004 (Table 1). Although marginally reduced in 2005, soluble solids remained largely unaffected despite the yield having doubled on the vines in 2005 in both Michigan and New Zealand (Table 1).

Table 1 - Michigan (MI) and New Zealand (NZ) harvest data for both 2004 and 2005 growing seasons.

Year	Clone	MI	Yield/vine (kg)	Tonnes/Hectare	Brix
2004	UCD13		1.47 a	2.6 a	23.9 a
	113		1.51 a	2.7 a	24.8 a
	115		1.47 a	2.6 a	24.1 a
2005	UCD13		3.06 a	5.4 a	22.4 a
	113		3.98 a	7.1 a	24.4 a
	115		3.38 a	6.0 a	24 a
Year	Clone	NZ	Yield/vine (kg)	Tonnes/Hectare	Brix
2004	UCD13		1.52 a	2.7 a	21.9 a
	113		0.44 a	0.7 a	22.2 a
	115		1.76 a	3.1 a	21.7 a
2005	UCD13		1.8 a	3.2 a	24.5 a
	113		1.2 a	2.1 a	24.1 a
	115		1.7 a	3.0 a	23.9 a

Values with different letters indicate significant ($p \leq 0.05$) differences between samples means

The weather data recorded at SWMREC (Benton Harbor, MI) showed there was about half as much rain, warmer air and soil temperatures in 2005 than in 2004 (Table 2). Additionally, the accumulated growing degree days (GDD) (base 10°C) in 2005 at harvest were over 250 GDD higher than those accumulated by harvest date in 2004. Further, the growing season in Michigan showed greater light energy in both years than in New Zealand (Figure 2). The light energy in Michigan accumulated at a higher rate than the accumulated light energy for New Zealand.

Table 2 - Michigan (MI) (1st May – 25th September) and New Zealand (NZ) (1st November – 25th March) weather data, logged as hourly data, and the 145 daily averages taken, in the period pre-veráison to harvest.

Year	Av. Max. Temp. (°C)	Av. Min. Temp. (°C)	Av. Temp. (°C)	Av. Soil Temp. (°C at 10cm)	Precipitation (mm) (day 0-70)	Precipitation (mm) (day 30-70)	Light energy MJ/m2	GDD (base 10°C)
MI								
2004	24.3 b	13.3 b	18.6 b	21.2 b	176.3 b	117.8 c	3267 b	1320 b
2005	26.3 c	14.7 b	20.3 c	22.7 c	183.8 b	143.7 d	3465 c	1573 c
NZ								
2004	19.92 a	9.6 a	12.95 a	14.63 a	131.8 a	64.1 a	2020 a	812 a
2005	20.37 a	10.2 a	13.32 a	14.25 a	134.2 a	101.8 b	2054 a	795 a

Values with different letters indicate significant ($p \leq 0.05$) differences between samples means

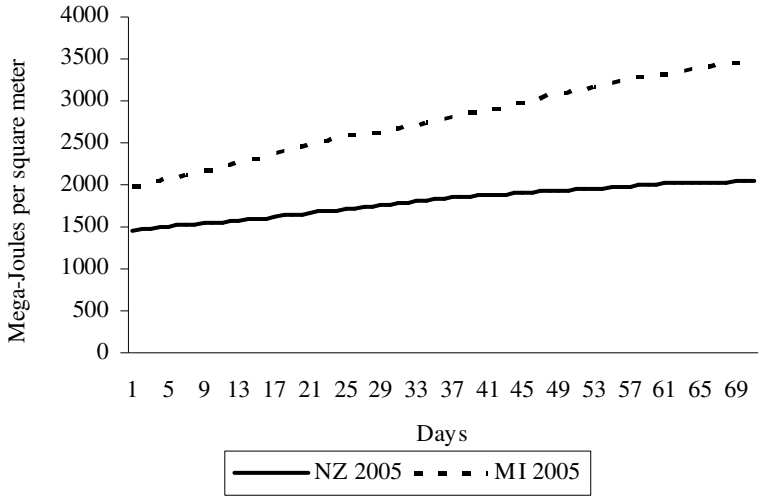


Figure 2 - Accumulated light energy (MJ/m^2) experienced in both New Zealand (NZ) and Michigan (MI) vineyards during 2005 growing season recorded daily (day 0=veráison, day 70=harvest).

The New Zealand harvest data for both 2004 and 2005 (Table 1) showed that yield was similar between years, except for variations experienced with clone 113 in both years, which could have been due to the clone 113 fruit ripening earlier than other clones and starting to experience berry shrivel, alternatively this clone may have experienced lower fruit-set than other clones in New Zealand during 2004.

The weather for the New Zealand vineyard (Table 2) showed that the average temperature was fairly consistent between years, and growing degree days were down in 2005. The rainfall average was slightly up during the 2005 growing season. The data for growing degree days in New Zealand vineyard location were much lower than the data for Michigan Vineyard GDD (Table 2). During both growing seasons, Michigan data showed large amounts of precipitation in the ten days prior to harvest, whereas the New Zealand vineyard precipitation was well distributed throughout the growing season (Figure 2). Additionally, when accumulated precipitation for each vintage was compared on the basis of location the New Zealand vineyard experienced much lower precipitation than found in Michigan, especially in 2004.

The weather at both locations may have had an influence on the differential yields experienced between seasons with the same cluster number per vine, although pre-veráison cluster numbers may have been different between years. The accumulated growing degree days (Table 2), 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 (Jackson 2000, Reynolds et al. 1995), 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% in both locations (Table 2).

Further, the effect of light energy during the growing season has been shown to affect both the concentration and accumulation of anthocyanins in the skins of winegrapes. It has recently been reported that monomeric anthocyanins occurs with increased light, although when UV light was filtered out, they found no significant differences suggesting a possible need for the whole light spectrum (Spayd et al. 2002).

Anthocyanin concentration

The C3G concentration in the fruit during 2004 was generally higher in Michigan compared to New Zealand (Table 3). During 2005 however, concentration of C3G in New Zealand samples far exceeded that of Michigan samples that year. This effect of location on the concentration of C3G in samples during both years (Table 3), was highly significant ($p \leq 0.002$) in all Michigan samples, and at harvest in New Zealand samples. This suggested that other factors were involved in the concentration of C3G in the berry skin, such as the methylation of C3G to form Pn3G (Roggero et al. 1986), the biological activity of which has been found by Logan's lab (data not shown) to occur at different rates in the fruit in response to differing levels of vine vigor, which again suggested the

importance of vineyard growing environment on the anthocyanin activity during fruit ripening (Gerard Logan, 2008, unpublished data).

Table 3 - Individual anthocyanin (malvidin-3-glucoside (M3G), peonidin-3-glucoside (Pn3G), petunidin-3-glucoside (Pt3G), delphinidin-3-glucoside (D3G) and cyanidin-3-glucoside (C3G)) concentration (mg/g fresh weight) in the fruit at harvest for 2004 and 2005 growing seasons in both New Zealand (NZ) and Michigan (MI) grown fruit of clones UC Davis 13 (UCD13), Dijon 113 (113) and Dijon 115 (115).

			Anthocyanin Concentration (mg/g)					
2004	NZ	Clone	M3G	Pn3G	Pt3G	D3G	C3G	Total
		UCD13	137.4b	40.3a	12.4a	4.8a	9.7a	204.7b
		113	50.5a	26.3a	3.6a	1.7a	1.3a	83.3a
		115	44.1a	18.2a	2.3a	1.3a	1.8a	67.7a
	MI	UCD13	75.2a	25.8a	3.8a	0.9a	2.5a	108.4a
		113	113.7b	49.5a	7.7a	2.4a	6.0a	179.4a
		115	187.7b	86.3a	15.0a	4.0a	9.4a	302.4b
2005	NZ	UCD13	187.0b	55.6a	40.9a	14.3a	42.5a	340.4b
		113	275.4b	94.7a	47.2a	17.9a	48.4a	483.5b
		115	433.3b	147.0b	102.1b	37.5a	114.9b	834.9c
	MI	UCD13	70.1a	43.1a	2.7a	2.4a	2.0a	120.4a
		113	257.2b	67.8a	15.7a	5.3a	11.6a	357.6b
		115	76.2a	52.4a	3.0a	3.0a	2.4a	136.9a

Values with different letters indicate significant ($p \leq 0.05$) differences between samples means

However, the impact of location on the total anthocyanin profile (M3G, Pt3G, Pn3G, C3G and D3G) (Table 3) was not statistically significant ($p \leq 0.2$). Differences between the significance of the total anthocyanin profile compared with the individual C3G concentration indicated that while C3G was statistically different at harvest at the two locations, the total anthocyanin profile did not change enough as a result of the location to be statistically significant. This was largely due to the low concentration of C3G in the fruit, and the transient nature of the primitive pigment which only allows for snapshots of its concentration to be determined during phase III berry ripening. This location effect on the C3G however, was likely based on climatic differences (light energy, growing degree days, rootstock, soil and precipitation) between the two locations as discussed earlier in the text.

During 2004, both locations reported lower light intensities than found in the 2005 growing season (Table 2). Most of the data showed higher concentrations of anthocyanins at both locations in 2005 than in 2004, again suggested the involvement of other factors in this relationship such as a lack of degradation of primitive and terminal anthocyanin pigments possibly arising from lower berry surface temperatures with an increase in shade from increased vegetative growth.

When the data for both locations were separated by year, the effects of location differences were magnified for C3G (Table 3) which further described how clones performed erratically based on location of growth with regard to C3G concentration.

The total anthocyanin profile of both years combined however did not change enough to draw statistically significant differences from the two locations, leading to questions regarding the importance of C3G in fruit and wine color development. The C3G behaviour reported here agrees with data of Cortell et al (2007a), who found that vintage was significant ($p<0.0001$), although site was not in this case, which supports the lack of correlations found to date.

Individual anthocyanins were identified from chromatograms (Figure 3) based on previously published literature (Chandra et al. 2001, Wada et al. 2002, Price et al. 1995). Anthocyanins identified in this work on the basis of elution time were as follows; 1 (malvidin-3-glucoside (M3G) 8.2 min), 2

(peonidin-3-glucoside (Pn3G) 7.6 min), 3 (petunidin-3-glucoside (Pt3G) 6.1 min), 4 (cyanidin-3-glucoside (C3G) 5.4 min) and 5 (delphinidin-3-glucoside (D3G) 4.0 min).

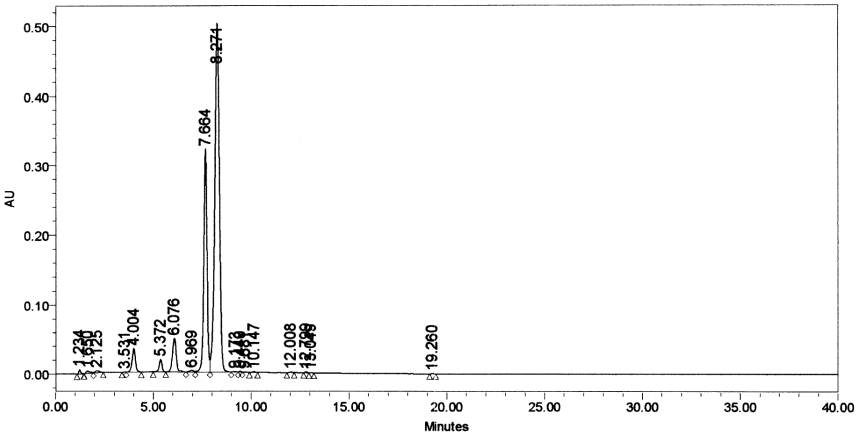


Figure 3 - Chromatogram of 2004 Michigan Pinot noir fruit purée of clone 115 at day 70 / harvest. Peaks from R-L: 1 (malvidin-3-glucoside (M3G) 8.2 min), 2 (peonidin-3-glucoside (Pn3G) 7.6 min), 3 (petunidin-3-glucoside (Pt3G) 6.1 min), 4 (cyanidin-3-glucoside (C3G) 5.4 min) and 5 (delphinidin-3-glucoside (D3G) 4.0 min)

The individual anthocyanin concentrations varied between location and growing season (Table 3). Similarities in patterns, however, existed between the 2004 Michigan-grown fruit and that of 2005 New Zealand-grown fruit data although New Zealand-grown fruit yielded higher concentrations of anthocyanins in each case. Rainfall average (in mm/day) was down during the 2005, compared to the 2004, growing season (Table 2), and the total growing degree days in New Zealand vineyard location are much lower than the total for the Michigan Vineyard (Table 2). These factors may have contributed to the differences observed for the total anthocyanin concentrations in our experiments (Table 4).

Table 4 - Total anthocyanin concentration by HPLC (mg/g (fresh weight without seed)) in fruit at harvest for both New Zealand and Michigan in 2004 and 2005.

NZ	Clone	2004	2005	MI	Clone	2004	2005
	UCD13	204 b	340 b		UCD13	108 a	120 a
	113	83 a	483 b		113	179 a	357 b
	115	67 a	834 c		115	302 b	136 a

Values with different letters indicate significant ($p \leq 0.05$) differences between samples means

Additionally, the effect of precipitation on anthocyanin concentration has also been previously reported (Matthews et al. 1989, Esteban et al. 2001, Kennedy et al. 2002, Intrigliolo and Castel 2008). During both growing seasons in Michigan, experienced large amounts of precipitation in the ten days prior to harvest, especially in 2005 (Figure 4) which resulted in lower concentrations of most berry constituents, namely anthocyanins, by increasing the ratio of water to anthocyanin in the berry (Esteban et al. 2001, Kennedy et al, 2002).

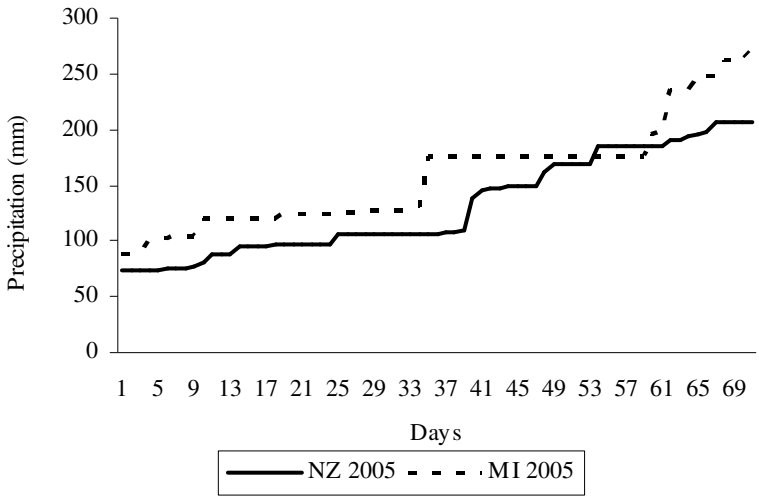


Figure 4 - Accumulated daily precipitation for both New Zealand (NZ) and Michigan (MI) vineyards in 2005 growing season (day 0=veraison, day 70=harvest).

The effect of precipitation recorded in Michigan (Table 2) during both seasons, suggested another reason for the lower anthocyanin concentration, when compared with New Zealand-grown fruit. Previous research on the effect of water on vine physiology has shown that the 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 primordia (Matthews et al. 1989). Also, a higher precipitation in the 14 days prior to harvest resulted in higher yields but a reduced concentration of berry components (Matthews et al. 1989). A similar trend of higher rainfall in the weeks leading up to harvest resulting in higher yields and lower concentrations of berry constituents, was also evident in our studies in Michigan and New Zealand. In 2005, the total precipitation was much lower in Michigan and slightly lower in New Zealand in the maturation period before harvest. The higher concentration of anthocyanins during 2005 in both locations (Table 4) suggested that precipitation had an effect on increasing yield and decreasing berry constituents including anthocyanins.

According to published reports, M3G was the most abundant anthocyanin in *V. vinifera* L. Pinot noir (Boss et al. 1996, Jackson 2000, Kennedy et al. 2002, Boulton 2001, Gao et al. 1997). However, in 2005, Michigan data (Table 3) showed that both UCD13 and 115 were noticeably lower in M3G than in New Zealand that year, and the concentration of Pn3G was only slightly lower than M3G in this case.

During both seasons, the concentration of anthocyanins, mainly comprised of M3G (1), as previously reported in Pinot noir (Boss et al. 1996, Jackson 2000, Kennedy et al. 2002, Boulton 2001, Gao et al. 1997, Cortell et al. 2008) was higher at harvest in New Zealand by 78% on average (by year and clone), than in Michigan. This is most notable when the comparison of the two locations accumulated light energies is examined (Figure 2). The data for Michigan showed greater light energy in both years, accumulating at a higher rate than the light energy for New Zealand (Table 2). In 2005, the concentration of anthocyanins at harvest in New Zealand was higher than in Michigan (Table 4). This relationship between increasing anthocyanin concentration and lower light energies has been previously reported (Keller and Hrazdina 1998), although the trend is not always followed as observed in the 2004 data.

The concentration of total anthocyanins among the three clones in both Michigan and New Zealand (Table 4) was not statistically significant when analyzed using the MIXED procedure (SAS 9.1). It appeared that there was no statistical difference between the clones based on C3G concentration and total anthocyanin concentration (Table 3). This result suggested that a) all clones are very similar on the basis of anthocyanin concentration, and b) those clones performed consistently throughout the experiment. However, the data provided indication of clone performance in a given location and year during the experiments.

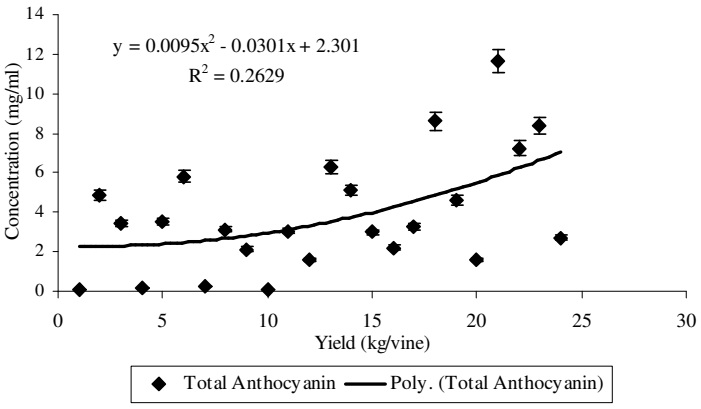


Figure 5 - Polynomial trend of 2004 total anthocyanin concentration of each purée sample regressed against yield for the respective vine in both Michigan and New Zealand

The concentrations of the individual anthocyanins (Table 3) yielded different profiles based on location and year, and the quantity of each anthocyanin was in agreement with published reports (Price et al. 1995, Bindon et al. 2008, Cortell et al. 2008). Although the differences shown for anthocyanin levels were significant at $P<0.05$, the data did not support a statistically significant trend based on growing location or season (Figure 5). However, the results suggested a trend of increasing anthocyanin concentration with increasing yield. This relationship did not agree with previous publications where the yield and anthocyanin concentration were reported to be inversely proportional (Jackson 2000). Finally, in our research when the data was separated by

location of culture, it showed differences in locations on the basis of yield per vine and anthocyanin concentration. New Zealand data also demonstrated the expected relationship between increasing yield per vine and decreasing anthocyanin concentration.

Wine anthocyanin concentration

The concentration of C3G, and total anthocyanin were significantly greater in the harvest fruit samples than in the wines produced from that fruit (Table 5). This result was anticipated, considering the final color of the grape skins is still a deep red after fermentation and pressing – 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 M3G 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.

Table 5 - Total anthocyanin concentration (mg/mL) in the ripening fruit (day 0, 30 and 70 (days from veráison)) and young wine (WINE) for 2004 and 2005 growing seasons in both New Zealand (NZ) and Michigan (MI) grown fruit of clones UC Davis 13 (UCD13), Dijon 113 (113) and Dijon 115 (115).

Year	Location	Clone	Sample Day	Total Anthocyanin mg/mL	Year	Location	Clone	Sample Day	Total Anthocyanin mg/mL
2004	NZ	UCD13	0	0.2a	2005	NZ	UCD13	0	0.3a
			30	3.5a				30	1.1a
			70	5.8b				70	8.3b
		113	0	0.2a			113	0	0.3a
			30	3.1a				30	1.8a
			70	2.1b				70	11.8b
		115	0	0.1a			115	0	0.6a
			30	3.1a				30	1.9a
			70	1.6b				70	20.6b
	MI	UCD13	0	6.3a		MI	UCD13	0	2.3a
			30	5.1a				30	4.3a
			70	3.0b				70	2.8b
			WINE	2.2a				WINE	1.7a
		113	0	3.3a			113	0	4.4a
			30	8.6a				30	4.2a
			70	4.6b				70	8.9b
			WINE	1.6a				WINE	2.5a
		115	0	11.7a			115	0	3.1a
			30	7.3a				30	2.0a
			70	8.4b				70	3.4b
			WINE	2.7a				WINE	2.2a

Values with different letters indicate significant ($p \leq 0.05$) differences between samples means

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 Table 5, 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.

However, work of Cortell et all. (2007b) showed similar levels of extraction of anthocyanins from Pinot noir fruit into young wines. Data they reported ranged from extraction values as low as 45.2% in low vigor vines of site B, up to 74.6% in high vigor vines of site A (Cortell et al. 2007b).

The final anthocyanin concentration 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. Additionally, it was determined that in all samples, the extraction of C3G was limited, to less than 7% in the case of 2005 clone 113 (data not shown). However, the concentration was affected somewhat by the total amount in the fruit, when the concentration of C3G in fruit increased or decreased, so did the concentration in the final wine – to differing magnitudes. This observation was also noticeable in the total anthocyanin concentration (Table 5). The total anthocyanin concentration of the fruit and wine samples were significantly different (p=0.005). This is similar to the effect of the C3G results, and again raises concerns about the current extraction techniques in winemaking.

The Least Square Means for the analysis of fruit and wine (data not shown) 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).

Conclusion

In conclusion, the total anthocyanin concentration of *V. vinifera* L. Pinot noir was observed to be higher in New Zealand during 2005 despite the lower light intensity. New Zealand fruit was also found to produce higher concentrations of C3G than Michigan in both years.

During the 2005 growing season, higher light intensity was proportional to anthocyanin concentration when compared with 2004 data for both Michigan and New Zealand. Both of these locations, the anthocyanin production by Pinot noir fruits varied between veráison and harvest. However, the rainfall event in Michigan before harvest may have contributed to this difference by location.
No statistical differences in anthocyanin concentration between location and growing season were found on the basis of clone throughout this study. Winemaking techniques limited the amount of anthocyanin that could be extracted from the berry skins to less than 7% of C3G, and 28% of total anthocyanin available in the fruit at harvest.
Our results suggest that C3G may in fact perform an important function in total anthocyanin concentration of *V. vinifera* L. Pinot noir prior to harvest, and post-fermentation, on the basis of location and growing season, and may be affected by various environmental and enological factors. This highlights the need to grow multiple clones of *V. vinifera* L. Pinot noir at each location, to account for both clone and seasonal impacts – those impacts being the basis for the regional differences attributed to regional terroir.

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