EFFECT OF ETHEPHON AND IRRIGATION DEFICIT ON COLOR ENHANCEMENT OF *VITIS VINIFERA* L. AS DETERMINED BY ANTHOCYANIN CONTENT OF WINE

Submitted by

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In partial fulfillment of the requirements

for the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall 1999

COLORADO STATE UNIVERSITY

November 8, 1999

WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY MARIANNE POXLEITNER ENTITLED: EFFECT OF ETHEPHON AND IRRIGATION DEFICIT ON COLOR ENHANCEMENT OF *VITIS VINIFERA* L. AS DETERMINED BY ANTHOCYANIN CONTENT OF WINE, BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

Committee on Graduate Work

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ABSTRACT

Three experiments were performed on Colorado grown grapevines to test irrigation deficit and/or ethephon application on the color enhancement of red wines. In the first experiment, drip irrigated Pinot noir vines were exposed to an irrigation deficit in the second half of the growing season. Vine water potential was monitored with a pressure bomb, and resulting wines were analyzed spectrophotometrically for anthocyanin content. No significant increase in anthocyanins was found between the wines from the control and treatment groups.

The second experiment used Sangiovese grapevines to test the application of a 750 ppm ethephon spray at eight weeks and nine weeks post anthesis against a control. Spectrophotometric analysis of the wines showed significant increases in anthocyanin content of 16.7% and 41.7% above the control for the eight and nine week post anthesis applications, respectively.

The third experiment utilized furrow irrigated Pinot noir vines to test the effects of ethephon on vines subjected to a late season water deficit. A pressure bomb was used to monitor vine water potential, a neutron probe was used to monitor soil moisture, and the wines were analyzed spectrophotometrically. A significant increase in anthocyanin content was found due to the ethephon application at nine weeks post anthesis to the vines subjected to the water stress.

A final experiment was conducted to observe the differences in color between Pinot noir and Merlot wines from different Colorado wineries. In addition, the color variation between different cultivars of wines from the Orchard Mesa Research Center was also investigated. Significant differences were found between the Pinot noir wines as well as the Merlot wines, and between the various cultivars analyzed.

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CHAPTER I

LITERATURE REVIEW/ INTRODUCTION

Anthocyanins are the red and purple pigments that provide color to grapes and red wines. Their biosynthesis in the grape, and their presence in wines, has been the subject of many studies world wide. The amount and composition of anthocyanins present in red grapes varies considerably with the species, cultivar, and environment, as well as the conditions during fermentation and aging of the wines (Mazza, 1995; Mancinelli, 1985).

Phenolics are a group of chemical compounds characterized by at least one aromatic ring with one or more hydroxyl groups attached (Stack, 1997). One group of phenolic compounds are the flavenoids which include the flavonols, flavonones, flavones, catechins, chalcones, as well as the anthocyanins (Mancinelli, 1985; Singleton, 1988). The basic chemical structure of the flavonoid is $C_6C_3C_6$, a structure that is derived from the condensation of 4 coumaryl-CoA and malonyl-CoA . This reaction is mediated by the enzyme chalcone synthase (Mancinelli, 1985).

There are five anthocyanidins found in the grape: delphinidin, petunidin, malvidin, cyanindin, and peonidin (Ribereau-Gayon, 1974). These algycones become anthocyanins as they are glycosylated by the addition of one, or two, glucose molecules at the 3' and or 5' positions respectively. In *Vitis vinifera* L. cultivars, only the 3-glucosides exist, while

V. labrusca L., *V. riparia* L., *V. rupestris* L., and their hybrids contain the 3,5-diglucosides or a mixture of 3- and 3,5-diglucosides (Hrazdina and Moskowitz, 1982). With the exception of a few of the red cultivars, notably Pinot noir, the pigments are partially acylated on the 6-hydroxyl of the 3-linked glucose by acetic acid, p-coumaric acid, or caffeic acid (Singelton, 1988). Fig. 1.1 shows the chemical structure of the 5 anthocyanidins present in grapes and red wine.

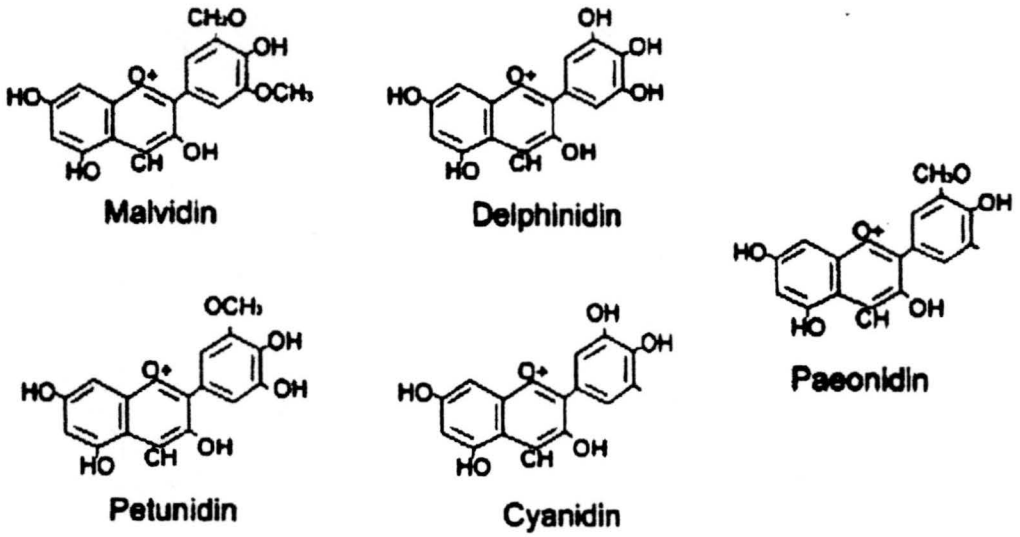

Figure 1.1 The five anthocyanidins present in *V. vinifera*.

The anthocyanins are fundamentally responsible for all of the color differences between grapes and the resulting wines. Anthocyanins also contribute to the organoleptic

and chemical qualities of wine due to their interaction with other phenolic compounds, proteins, and polysaccharides (Mazza, 1995). These polymeric pigments, sometimes called tannins, are formed by condensation reactions between anthocyanins and other phenolics early in the fermentation process. Therefore, wine color is actually an integration of contributions from polymeric and monomeric pigment forms of anthocyanins.

It has been established that color is the most important assessment factor in determining wine quality. The overall quality of a red wine can be estimated from the deepness of the red color alone. It has been shown that positive correlations exist between overall wine quality, flavor and the total pigment content (Singleton, 1982). However, since wine color is dependant on many variables, no precise chemical definition of color can be made (Somers and Evans, 1977). Thus color based analysis is difficult and a number of factors must be taken into account.

One factor that makes the analysis of wine color a challenge is the fact that wine cannot be diluted prior to spectral evaluation, for there is no proportionality between the dilution coefficient and optical density (Ribereau-Gayon, 1974). It has been found that a wine dilution factor of at least 50:1 is necessary before Beer's Law becomes operative for anthocyanins. Therefore, the measure of optical density must be made on undiluted wine utilizing the proper path length of 1mm (Somers and Evans, 1977).

Two other factors affecting wine color are pH and the amount of sodium metabisulfite added to fight microbial growth after fermentation. Somers and Evans (1975) found that variations in these two parameters may be so large that no relation is seen between wine color and the anthocyanin content of wines.

In mildly acidic solutions, such as wines, anthocyanins are in complex equilibrium between their red flavylium carbocation form and their colorless carbinol psuedobase form. As acidity is increased and the pH falls below 2.6, the equilibrium will be shifted so that the anthocyanins will exist entirely in their red flavylium form (Singleton, 1988). Therefore, one of the problems in assessing red wine color is found in comparing wines with widely differing pH values (Timberlake, 1982). Experimentally, these differences can be overcome by lowering the pH of the wine samples to less than one, thereby shifting all of the anthocyanins to their colored form. Fig. 1.2 illustrates the ionization of the anthocyanins at low pH values.

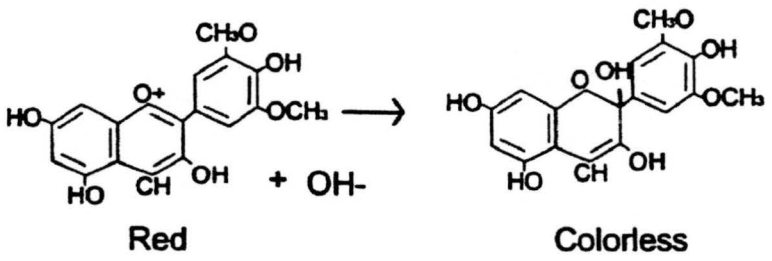

Figure 1.2 Effect of pH on ionization and color of anthocyanins.

Further to the influence of wine pH, the anthocyanin equilibria is affected by sulfur dioxide added after fermentation to fight microbial growth (Hrazdina and Moskowitz, 1982). Anthocyanins are instantly decolorized following the addition of SO_2 at normal red wine pH. Bisulphite ions condense with anthocyanins as shown in Fig. 1.3. This reversible reaction decreases overall red wine color by rendering anthocyanins colorless (Ribereau-Gayon, 1974). In contrast to anthocyanins, polymeric pigments are insensitive to SO_2 (Somers, 1982) and the residual color after such treatment of a red wine is due to polymeric pigment forms. Thus, treating wine samples with SO_2 prior to spectral evaluation provides the absorbance of the polymeric forms of anthocyanins and phenolics. Since acetaldehyde binds much more strongly to SO_2 than to the anthocyanins, the addition of excess acetaldehyde permits measurement of that portion of total anthocyanins which may have been decolorized by combination with SO_2 (Somers and Evans, 1977). This fact makes it possible to compare wines with different enological backgrounds when the amount of SO_2 added after fermentation is unknown.

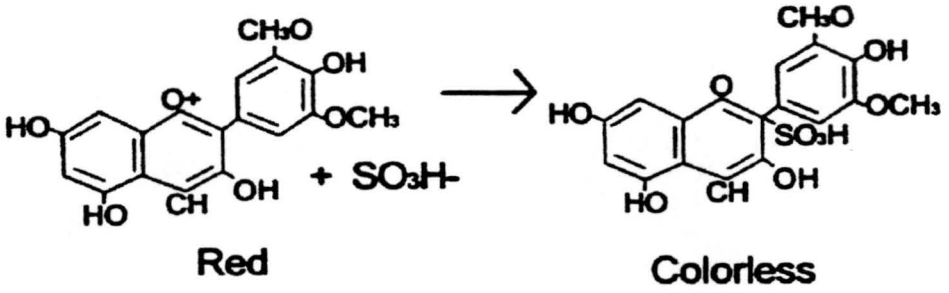

Figure 1.3 Reaction of an anthocyanidin with sodium metabisulphite to render the pigment colorless.

From the information presented above the importance of wine color as a means of determining wine quality, as well as the difficulties of determining wine color, are evident. Using the chemical equilibria principles above, the color of red wines from different treatments can be evaluated and the effects of the treatments on wine color determined. This is important because, historically, certain wines produced in Colorado have failed to attain adequate pigmentation. By manipulating grape vines to increase anthocyanin content as well as using the principles presented, various effects on the color of the resulting wines can be assessed.

Grapevine irrigation has been a practice in water limited regions since as early as 2900 B.C. (Grimes and Williams, 1990). According to Smart and Coombe (1983) the

effects of water deficit on berry ripening and the composition of juice are legion. Indeed, much work has been done to test the physiological effects of water deficit on grape vines.

In 1989, Matthews and Anderson investigated the sensitivity of reproductive development to water deficits in Cabernet franc vines by altering the supply of irrigation water in a drip irrigated vineyard. Midday leaf water potentials indicated that significant water deficits were imposed when water was withheld before and after verasion. When water was supplied at two times the standard production rates, fruit growth and cluster initiation were increased. The data showed that yield increases of 30%- 40% can be obtained by increasing irrigation. Vine water status was seen to decrease until verasion in all treatments, but no differences were seen between treatments until after fruit set. Water deficits imposed before or after verasion inhibited yield and fruit expansion but early deficits were more inhibitory on these characteristics.

Studies done in Australia (Smart et al., 1974) analyzed the effects of trickle and furrow irrigation on yield and growth of Shiraz grapevines. Daily trickle irrigation applied to meet the evaporative demand of the vines produced similar yields to furrow irrigated vines receiving slightly more water. Decreasing the daily trickle application by half decreased yield by 16%. Water stress until verasion significantly reduced berry size, and it was determined that this was the yield component most sensitive to water stress.

Grimes and Williams (1990), conducted research in California to establish plant

based water status measurement techniques and critical values that determine irrigation regime adequacy. Differential irrigation quantities were supplied in several increments ranging from 40%-120% of the expected non-stressed crop evapotranspiration (ET_{crop}). Midday measurements of leaf water potential, crop water stress index, and stomatal conductance were closely associated with imposed water deficits and the resulting grape yields. All plant based water status reflecting measurements were found to be highly correlated. Grape yields followed declining water availability linearly over the range of available data, with a 50% reduction in ET_{crop} resulting in a 26% yield loss.

In 1983, Kliewer et al. discovered that irrigated vines had yields 25% higher than non irrigated vines by increasing both berry weight and berry number per cluster. The rate of shoot elongation was reduced by water stress before any differences were detected in predawn leaf water potential. Water stress had no effect on the number of primary leaves produced per shoot, or leaf growth at specific nodes. On a mild day with a maximum temperature of 30.5 °C and minimum air water potential of -143 MPa, stomatal conductance of irrigated vines increased to a maximum at 1600 hours, whereas stomatal conductance for the non-irrigated vines reached a maximum at 1000 hours, then declined as stomata began to close.

In 1974, R.E. Smart conducted experiments to test diurnal patterns of leaf water potential and stomatal resistance under different irrigation conditions. The ambient

environment, especially solar radiation, had a dominating effect on leaf water potential. Water stress induced stomatal closure was observed at leaf water potentials of -13 bars, although shoot growth rate was inhibited before negative tensions became that large.

Research conducted on potted Concord grape vines by Liu et al. (1978) showed that as leaf water potential reached -16 bars, stomatal closure was essentially complete and photosynthesis was minimal. A prolonged (longer than two weeks) and/or severe stress (leaf water potential less than -16 bars) led to large increases of ABA content and incomplete recovery of photosynthesis despite reopening of stomata on restoration of plant water status.

In addition to the physiological studies above, Hardie and Considine (1976) performed water stress experiments on container grown grapevines during different stages of development. It was discovered that the water deficits increased the production of anthocyanins in red wine cultivars.

In 1990, Williams and Matthews presented data from previous experiments showing that the effects of water deficits in the vineyard on fruit composition are reflected in the composition of the subsequent wine. Wines had greater color and total phenolic concentrations when made from vines that experienced early or late season water deficits compared to vines irrigated weekly.

Prichard (1998) designed an experiment to evaluate the timing and severity of water deficits on the fruit and wine quality of Cabernet Sauvignon grapevines. It was discovered that imposing irrigation practices that deliver 70% of the water necessary to maintain favorable Cabernet Sauvignon vine status resulted in an increase in wine color density of approximately 50%.

An alternative to using irrigation deficit to enhance anthocyanin content of wines is the application of 2-chloroethyl phosphonic acid, commonly called ethephon, which has been implicated in enhancing grape color. When applied to the leaves it is translocated to the fruit where it degrades to ethylene, chloride, and phosphate (EPA, 1998). Ethephon is a plant growth regulator responsible for controlling different phases of plant growth and development by application to various growth sites. Ethephon has no long-term toxilogical effects and is approved by the Environmental Protection Agency for use on grapes (Extonet, 1998). Discrepancies exist as to the proper concentrations, timing, and methods of application necessary to illicit the desired effect of increased berry and wine color.

Work done by Weaver and Pool at the University of California at Davis (1971) tested the application of ethephon at 200 to 2,000 ppm to the grape cultivars Tokay, and Emperor, and different application times of 1,000 ppm to Carignane grapevines. For Tokay it was determined that the color was more intense due to the 2,000 ppm application

than the lesser concentrations, whereas the Emperor vines responded best to the 1,000 ppm concentration. The most effective application date for color enhancement of all cultivars was about 2 weeks after initiation of coloration. Overall, it was determined that using ethephon to increase berry color in problem cultivars could be of great assistance to growers.

Hale et al. (1970), performed experiments to test the effects of both ethylene and ethephon on the ripening of grapes in Australia. Ethylene hastened the start of ripening of Doradillo grapes when it was applied for 10 days starting midway through the slow growth phase. Ethephon applied to Shiraz grapes showed the same effect but, when it was applied during the end of the first rapid growth phase or at the start of the second rapid growth phase it delayed ripening. These results stressed the importance of the slow growth phase on berry development and the timing of ethephon application.

Work performed by Jensen et al. (1982) discovered that ethephon enhanced color development in the red cultivars Flame Seedless, Cardinal, Red Malaga, Queen, Tokay, and Emperor. The rates required were one half to one pint of ethephon per acre for all cultivars except Tokay, which required between one to two pints per acre. It was also determined that concentrated application from twenty-five to fifty gallons per acre, or dilute applications of 200 to 500 gallons per acre were equally effective. The most favorable treatment time was determined to be at the beginning of color development.

Work was performed at the University of California at Davis (Wicks, 1976) to analyze the effects of exposing grape clusters to light and dipping them in ethephon on the anthocyanin development and composition of Tokay, Cardinal, Emperor, and Ribier grapes. The levels of total anthocyanins in the skins of the Ribier and Emperor berries dipped in the ethephon was significantly greater than in the skin of the control fruit, however, the anthocyanin levels in the Tokay berries did not differ significantly between the ethephon treated and control fruit. The increase in pigment due to light was significant for Emperor and Tokay but not for Ribier, and neither ethephon nor light treatments produced consistent changes in total phenolics. A positive linear correlation was found between total anthocyanins and total phenolics in the skins of both Emperor and Ribier grapes.

Red wine color is of great interest to viticulturists and enologists in the state of Colorado due to the fact that red wines produced in the state have less than ideal color compared to wines from other regions. Developing means of increasing the anthocyanin content of the grapes and red wines would increase the market value of the wines benefitting the growers and wineries. From the scientific information compiled above, experiments were designed to try and increase the anthocyanin concentrations of Colorado grown Pinot noir and Sangiovese grapes and wines. It was hypothesized that water stress and ethephon application could be used to enhance wine pigmentation.

CHAPTER II

IRRIGATION DEFICIT

Introduction

An experiment was designed to test the effects of irrigation deficit on the color of wines produced from Colorado grown Pinot noir grape berries. The variety Pinot noir was chosen because it has a history of developing poor color in the state of Colorado. It was hypothesized that exposing the grapevines to a late season irrigation deficit would increase the anthocyanin content in the berries, and that this increase would be reflected in the wine color.

Materials and Methods

An irrigation deficit experiment was performed on seven year old, drip irrigated, Pinot noir vines located at the Colorado State University, Orchard Mesa Research Center in Grand Junction, Colorado. The vines were planted in a five by ten foot spacing and were trained in a double trunk bi-lateral cordon system on a seven wire vertical shoot positioned trellis.

The vines were chosen from a completely randomized block of 20 test cultivars. The experiment consisted of groups of four vines in four rows, for a total of 16 vines. The treatments were randomly assigned to consist of two groups of vines to be the control,

and two groups of vines to receive the water stress treatments, for a total of eight unstressed, and eight stressed vines.

On July 17, 1998 the emitters on the vines to receive the water stress were plugged, and the vines received no further irrigation for the rest of the growing season. Moreover, to ensure that the vines did not receive any auxiliary water, the emitters on the adjoining vines were plugged as well.

Vine water status was monitored weekly throughout the experiment using a pressure bomb. Leaf water potentials were determined at midday using leaves with approximately the same age, size and vine placement. To reduce the effects of transpiration from the leaf surface, in the time between removing the leaf from the vine and placement in the pressure bomb, each leaf was enclosed in a small sandwich bag before being cut from the vine (Liu et al., 1978; Matthews et al., 1987; and Grimes and Williams, 1990). The leaf and bag were then situated in the pressure bomb chamber and the leaf water potential determined in negative bars.

In addition to the pressure bomb data, temperature data were also collected. Maximum and minimum temperatures, as well as precipitation, were recorded daily from a weather station on the grounds of the Orchard Mesa Research Center.

The Pinot noir grapes from the control and water stressed treatments were harvested on September 23, 1998. Richard Hammon, the enologist and viticulturist, at the Colorado State Orchard Mesa Research Station made wine from each of the treatments.

The wines were evaluated at the Colorado State University campus in Fort Collins, Colorado. The protocol chosen to evaluate the anthocyanin content of the wines was the sodium metabisulphite method originally outlined by Somers and Evans (1977); this method is the most popular and widely used protocol for analyzing the anthocyanin content of wines, and it has many benefits (Singleton, 1988). From five spectrophotometric measurements the values of color density, color hue, total anthocyanins, degree of ionization of anthocyanins, ionized anthocyanins, degree of ionization of anthocyanins after abolishing the effect of SO_2, free SO_2, and total phenols, were determined.

The spectrophotometric analysis was performed on a Beckman 640DU-UV visible spectrophotometer equipped with computer based analytical software and temperature control as follows: A 100µl wine sample was added to 10ml 1M HCl. After 3-4 hours the absorbance was measured at 520nm and 280nm in a 10mm cuvette and the values corrected for dilution. Next, 20µl of 10% acetaldehyde was added to a fresh 2.0ml wine sample and after 45 minutes the absorbance was measured in a 1mm cuvette at 520nm. Then, 0.33ml of fresh wine was pipetted into the 1mm cuvette and the absorbance taken at 420nm and 520nm. To the above sample, 5µl of 20% sodium metabisulphite solution was added and mixed by inversion. After one minute the absorbance was taken at 520nm. All absorbances were converted to correspond to a 10mm pathlength (i.e. x10).

The above protocol was replicated ten times for each of the treatments and then the following mathematics were performed on the collected absorbance readings. The 'E' value represents the absorbance value corrected for the 10mm pathlength.

- Wine color density = $E_{420} + E_{520}$
- Wine color hue = E_{420} / E_{520}
- Total anthocyanins (mg/l) = $20 (E^{HCl}_{520} - 5/3\ E^{SO2}_{520})$
- Degree ionization of anthocyanins
  $= (E_{520} - E^{SO2}_{520}) / (E^{HCl}_{520} - 5/3\ E^{SO2}_{520}) \times 100$
- Ionized anthocyanins (mg/l) = $20 (E_{520} - E^{SO2}_{520})$
- Free SO_2 (mg/l) = $3.84 (E^{CH3CHO}_{520} - E_{520}) / E_{520} - E^{SO2}_{520}$
- Degree ionization anthocyanins after removal of SO_2 effect
  $= (E^{CH3CHO}_{520} - E^{SO2}_{520}) / (E^{HCl}_{520} - 5/3\ E^{SO2}_{520}) \times 100$
- Total phenolics = $E_{280} - 4$

Results

As of July 17, 1998 the drip irrigated Pinot noir vines had received 107 gallons of water per vine. The vines subjected to irrigation deficit conditions received no additional water after this date. The control vines, however, received an additional 121 gallons of water per vine in that growing season. Therefore, the drought stressed vines received only 47% of the water applied to the control vines.

Despite having received less than half as much water as the control, the drought stressed vines showed no visual symptoms of drought stress. There was no loss or burning of leaves, and the tendrils remained turgid.

Leaf water potential values were recorded weekly throughout the duration of the water deficit experiment using a pressure bomb. Fig. 2.1 graphically represents the data collected. Statistical significance was determined at $P<0.05$ between the treatments for each date and is represented by an asterisk on the graph. There was a marked decrease in the water potentials of both the stressed and unstressed vines from the beginning of the experiment through its completion.

Figure 2.2 contains the maximum and minimum temperature data as well as precipitation data collected daily from the research center.

The Pinot noir berries from the control vines and the vines subjected to the irrigation deficit were harvested on Sept. 23, 1998. At harvest the control berries not exposed to an irrigation deficit had a Brix° of 24.2%, and a pH of 3.37. The berries from the water stressed treatment had a Brix° of 23.6%, and a pH of 3.27. When harvest was complete the wines were made in three gallon carboys according to treatment.

Sub samples of the wines were taken and evaluated at three different dates using the sodium metabisulphite method outlined previously. The sub samples were taken approximately eight weeks apart on the following dates, February 9th, April 7th , and June 15th, 1999. Sub sampling was spaced as such in order to follow the maturation of the wines. Since the trends observed remained constant between sub samples only the data set collected from the third sub-sampling when the wine was the most mature will be presented here in Table 2.1. The first and second data sets can be found in Appendix A.

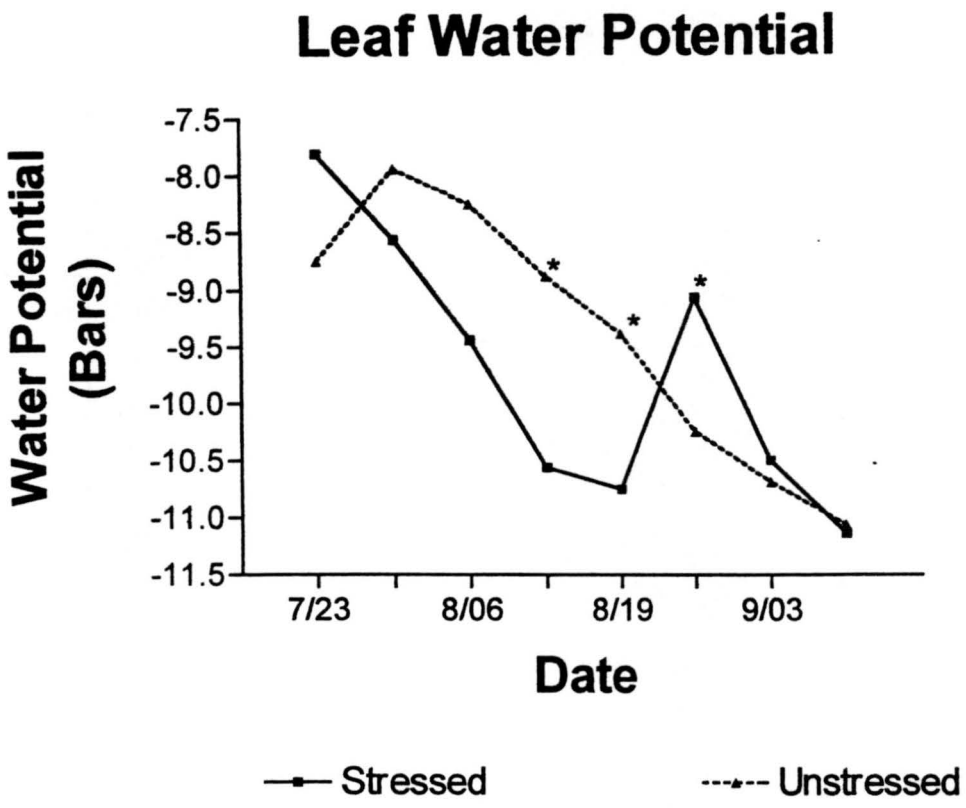

Figure 2.1 Graphical representation of pressure bomb data collected from Pinot noir vines subjected to an irrigation deficit and control. Leaf water potential is presented in -bars. Statistically significant differences are indicated by asterisks.

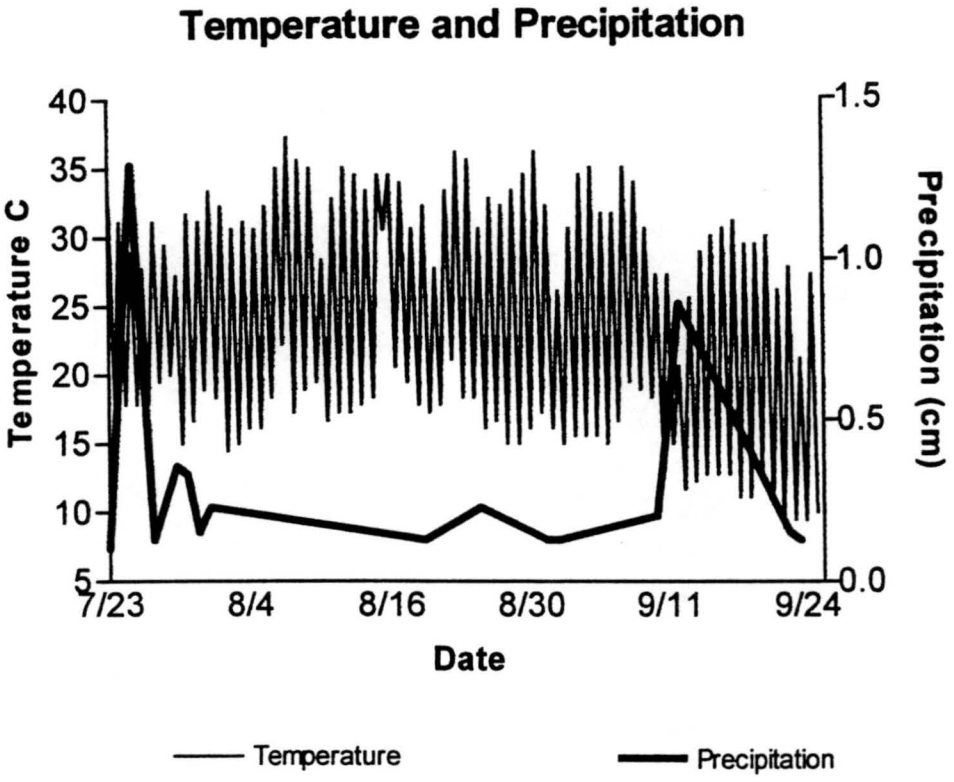

Figure 2.2 Daily maximum and minimum temperatures and precipitation data from the Orchard Mesa Research Center in 1998.

Table 2.1
Pinot noir irrigation deficit experiment trial III, 6/15/99. Analysis of wine color based on spectrophotometric properties.

Wine Color Analysis for Pinot Noir Irrigation Deficit Experiment								
	Color Density	*Color Hue*	*Total Antho-cyanins (mg/L)*	*Degree Ionization Anth. (%)*	*Ionized Antho-cyanins (mg/L)*	*Free SO_2 (mg/L)*	*Degree Ionization No SO_2 (%)*	*Total Phenolics (Abs. Units)*
Unstressed	2.216b[z]	.7635a	83.32b	9.970a	8.304b	1.842a	14.74a	17.77b
Stressed	2.078a	1.128b	77.41a	10.22a	7.898a	2.888b	17.88b	15.56a

[z]Statistical analysis was performed using a Student's T-test with a $P<0.001$ on Prism 2 software. Means followed by different letters indicate statistical significance.

Discussion and Conclusion

The leaf water potential data presented in Fig. 2.1 were collected with a pressure bomb at midday (Matthews and Anderson, 1989) based on the principle that grapevine water status is known to remain constant after solar noon until mid afternoon when the vine's water status begins to recover (Grimes and Williams, 1990; Smart, 1974). It is important to remember that the greater the negative water potential, the greater the stress on the vine. As can be seen in Fig. 2.1, at the beginning of the experiment the xylem water potential in the vines that would be exposed to the water stress was less than that of the vines in the control group. By the second week of the experiment the stressed vines had begun to decrease in water potential while the unstressed vines showed some recovery. For the next three weeks the stressed vines and the unstressed control vines both continued to show decreases in leaf water potential, but the rate of decrease in the stressed vines was greater than that of the well watered vines. By the fourth week of the experiment the stressed vines had decreased in water potential to -10.75 bars, while the unstressed vines had decreased to only -9.5 bars. In the following week the stressed vines had a dramatic increase in water potential, while the unstressed vines continued their decreasing trend. By the seventh and final week of the experiment both the stressed and unstressed vines had decreased to the same water potential of -11 bars.

According to Matthews et al. (1987) it is not unusual for well watered vines to experience water stress due to a three fold increase in water consumption as the season progresses (Hardie and Considine, 1976). As the evaporative demand exceeds the roots

ability to deliver water the vine, water stress can occur as is seen with the ever decreasing water potential of the unstressed control group. The water potential of the stressed vines followed a predictable trend downward as the soil water was depleted by the vines. The aberrant spike in water potential of the stressed vines on 8/26 could be attributed to the ambient environment around the vines such as irradience, temperature, and relative humidity all which impact evaporative demand (Hardie and Considine, 1976). From Fig. 2.2, one can observe that 0.25cm of rain was received on 8/26, this precipitation occurred as the water potential values were being collected for water stressed vines. This precipitation probably decreased the evaporative demand on the vines due to a higher relative humidity, perhaps explaining the spike in plant water potential in the stressed vines on that date.

There is known to be a close direct relationship between leaf water potential and stomatal conductance in grape vines (Kliewer et al., 1983). Stomatal aperture, transpiration, and photosynthesis, all decrease in plants subjected to differing degrees of water stress (Van Zyl, 1987). As water becomes limited within the vine the stomatal aperture is decreased in order to reduce transpiration, CO_2 fixation is thereby reduced, slowing photosynthetic activity.

Discrepancies in the literature exist as to the exact value at which complete stomatal closure and the subsequent cease in photosynthesis occurs. Van Rooyen et al. (1980) state that stomatal aperture and photosynthesis start to decrease by a leaf water potential of -5 bars, and complete stomatal closure and a cease in photosynthetic activity

occurs by -12 bars. It has been proposed that stomatal closure occurs as early as -10 bars (Kliewer et al., 1983) or as late as -16 bars (van Zyl, 1987). These discrepancies can be explained by the grapevine's inherent ability to adapt to its environment thus affecting the water potential at which stress occurs (van Zyl, 1987). Therefore, with a final leaf water potential of -11 bars one can infer that at least a moderate amount of water stress was imposed on the Pinot noir vines subjected to both treatments. However, the water stressed treatment sustained lower leaf water potentials compared to the control treatment for a majority of the experiment. As a result of the water stress, the stomatal aperture and photosynthetic rate were both decreased thereby affecting the vines metabolism, theoretically causing an increase in the anthocyanin content of the berries.

The data presented in Table 2.1 illustrate the differences in color found between the control and irrigation stress treatments according to eight parameters based on spectrophotometric data collected from the wine. The values for color density decreased from 2.216 in the control to 2.078 for the water stressed treatment. It is known that anthocyanins absorb maximally at 520nm, and the polymeric pigment forms absorb maximally at 420nm (Singleton, 1988). Since the color density parameter is the additive effect of the two absorbances ($E_{420} + E_{520}$), it can be concluded that the irrigation stress treatment decreased either the amount of anthocyanins absorbing at 520nm, or decreased the amount of polymeric pigment forms absorbing at 420nm, or both. From looking at the color hue parameter which is ratio of the absorbances at 420nm and 520nm (E_{420} / E_{520}) the relationship between the polymeric pigments absorbing at 420nm and the anthocyanins

absorbing at 520nm can be made. The color hue increased from 0.7635 for the unstressed control treatment to 1.128 for the water stressed treatment. This indicates that there were more anthocyanins in the polymeric pigment form compared to free anthocyanin in the water stressed treatment. The lower the color hue of a wine, the more red it will appear, whereas a higher the color hue indicates that the wine is more brown.

In accordance with color hue and color density data, the values for total milligrams of anthocyanins present in the wine decreased from 83.32mg/L in the unstressed control to 77.41mg/L in the water stressed treatment. Since anthocyanins are directly responsible for wine color this reduction between the control and the experimental treatment indicates that the wine from the water stressed vines was less red than the wines from the control treatment regardless of the equilibrium between free and polymeric anthocyanins.

The parameter that measures the degree of ionization of anthocyanins makes it possible to compare wines with different pH values despite the fact that wine color is highly influenced by this chemical property (Timberlake, 1982). There was no statistically significant difference in the degree of ionization of anthocyanins between the treatments. This indicates that both treatments had approximately the same percentage of anthocyanins in the colored flavylium ion form at wine pH despite the fact that wines from the unstressed and stressed treatments had pH's of 3.84 and 3.55 respectively. However, there was a statistically significant difference between the milligrams per liter of ionized anthocyanins present in the wine. The water stressed treatment had 7.89 mg/L of ionized anthocyanins whereas the unstressed treatment had 8.304 mg/L ionized anthocyanins.

This higher concentration of ionized anthocyanins in the unstressed control treatment, which had a higher color density, was unusual since a higher pH shifts the equilibrium toward the colorless pseudo base form.

The concentration of free SO_2 present in the wine is a measurement of the sulfur dioxide added to the wine after fermentation to fight microbial growth. Since SO_2 decolorizes anthocyanins through a condensation reaction (Ribereau-Gayon, 1974) these values are important when analyzing wine color. The control treatment contained only 1.842 mg/L of free SO_2 whereas the stressed treatment contained 2.888 mg/L of free SO_2. The free SO_2 level is higher in the stressed treatment because the amount of SO_2 added after fermentation is random and based on the judgement of the enologist. Therefore, even if the wines contained the same concentration of anthocyanins, the unstressed treatment would appear more colored due to the excess bleaching that had occurred by the SO_2 in the stressed treatment.

The next parameter, the degree of ionization discounting SO_2, can be used to determine the percentage of anthocyanins in the wine that would be in their ionized colored form regardless of the amount of SO_2 that was added after fermentation. The unstressed control treatment showed 14.74% ionization and the water stressed treatment showed 17.88% ionization. Therefore, despite the differing levels of SO_2 added to the wine, the stressed treatment would have a larger percentage of its anthocyanins in the colored form. This parameter makes it possible to compare wines when the amount of anti-microbial SO_2 added is unknown. Without this parameter it would be impossible to

determine if the wine color were representative of the anthocyanin concentration or if the wine had undergone significant color loss in the aging process.

The final parameter is a measurement of the total phenolics present in the wine, determined from a spectrophotometric reading at 280nm, the peak absorbance of phenolics minus four, which is a value used to eliminate the effect of ultra-violet absorbing non-phenolics (Somers and Evans, 1977). The phenol content of young red wines can be extremely varied. Phenolic compounds such as tyrosol, gallic acid, caffeic acid, tannins, catechins, and anthocyanins are just a few of the phenols present in most red wines. These phenolic constituents which are all derivatives of phenylalanine, are responsible for not only the color, but also the astringency, bitterness, and oxidation level of different wine. Since the phenolic content of wines is so varied and is dependent on so many exogenous factors like environment and enological practices it is difficult to analyze what phenolic constituents make up the total phenolic parameter measured here. The stressed treatment had a phenolic concentration equal to 15.56 absorbance units whereas the unstressed control treatment had a higher value of 17.77 absorbance units. These values are in agreement with the measurements for total anthocyanins with the unstressed control treatment containing more than the stressed treatment. This is expected since anthocyanins are a type of phenolic compound and are included in the measurement.

From the leaf water potential data presented it was concluded that the vines subjected to both treatments sustained a moderate degree of water stress. The vines in the water stressed treatment had lower water potentials throughout some of the experiment

indicating a longer duration of water stress then the control vines. From the color analysis of the wine it is obvious that the wines made from the stressed vines did not attain a higher concentration of anthocyanins as one might have hypothesized. It is known that late season irrigation deficits are know to cause yield decreases (Smart et al., 1974), but these yield decreases are not associated with a decrease in cluster number (Kliewer et al., 1983). The decrease in yield is due to a reduction in berry size which leads to a greater skin area to volume ratio (Hardie and Considine, 1976). Since anthocyanins are present in the berry skins, the greater skin area to berry volume ratio can lead to higher anthocyanin concentrations in the resulting wines. This clearly was not the case in this particular experiment.

Examples have been cited where reduced carbohydrate availability in water stressed vines caused poor color development in the berries (Hardie and Considine, 1976). Low carbohydrate availability could be related to a reduction in photosynthesis caused by the closing of the stomata and reduction of CO_2 absorption and assimilation. Overall, it cannot be concluded that field grown Pinot noir grapevines subjected to a late season irrigation deficit produce berries that result in wines with higher anthocyanin content and increased color.

CHAPTER III

ETHEPHON APPLICATION

Introduction

Based on previous research, an ethephon experiment was designed to evaluate the effect of two application times of ethephon on the color enhancement of Sangiovese wines made from grapes grown in Colorado. It was hypothesized that foliar applications of 750ppm ethephon, eight and nine weeks post anthesis would increase the anthocyanin content of the berries and this increase would be reflected in the color of the resulting wines.

Materials and Methods

The ethephon application experiment was performed on seven year old drip irrigated Sangiovese vines located at the Colorado State University, Orchard Mesa Research Center. The vines were planted in a five by ten foot spacing and were trained in a double trunk bi-lateral cordon system on a seven wire vertical shoot positioned trellis. The 24 vines used in the experiment were selected from a group of Sangiovese vines planted as the last two vines in a number of rows as well as those belonging to a variety trial. The vines were selected based on health, vigor, and the amount of fruit present.

A completely randomized design was used consisting of three treatments,

replicated eight times, for a total of 24 experimental units. Treatment one was the control, treatment two was applied on 10 August, 1998 and treatment three was applied on 17 August, 1998. The spray dates were based on time elapsed since anthesis. According to Hale et. al. (1970) ethephon applied eight to nine weeks post anthesis is most beneficial at increasing berry color. Therefore, treatment two was applied eight weeks after anthesis on 10 August, 1998, and treatment three was applied nine weeks after anthesis on 17 August, 1998. A backpack sprayer was used to apply the 750ppm ethephon spray to each vine. The ethephon spray consisted of Ethrel, the commercial form of ethephon, Triton X, a surfactant to aid in the formulations adherence to the vines, and 5% ethanol to help dissolve the Ethrel (Extonet). The vines were sprayed until the point of dripping using approximately 650ml of the ethephon solution per vine.

The berries from the control vines as well as the treated vines were harvested on 26 October, 1998. The wines for each treatment were made at the Orchard Mesa Research Center by Richard Hammon.

The wines were then evaluated on the Colorado State University campus. The protocol used to assess the anthocyanin content in the wines was the sodium metabisulphite method of Somers and Evans (1977) that was presented in detail in Chapter 2.

Results

Neither of the ethephon application dates caused any visual variation in the grapevines or berry clusters prior to harvest on 26 October, 1998. At harvest the control berries that had not received ethephon had a Brix° of 21.5, and a pH of 3.20. Treatment two that received the ethephon application on 10 August, 1998, had a Brix° of 22.0, and a pH of 3.24, while treatment three that received the ethephon application on 17 August, 1998, had a Brix° of 22.1, and pH of 3.24.

After harvest, the grapes from the three treatments were made into wine in three gallon carboys and stored until the sub-sampling took place. The wines were sub-sampled and evaluated using the sodium metabisulphite method of Somers and Evans (1977) on 15 February, 7 April , and 15 June, 1999. The third data set that represents the most mature wine is presented in Table 3.1, the other two data sets can be found in Appendix C.

Table 3.1 Sangiovese ethephon application experiment trial III, 6/15/99. Analysis of wine color is based on spectrophotometric properties.

Wine Color Analysis for Sangiovese Ethephon Application Experiment								
	Color Density	*Color Hue*	*Total Antho-cyanins (mg/L)*	*Degree Ionization Anth. (%)*	*Ionized Antho-cyanins (mg/L)*	*Free SO_2 (mg/L)*	*Degree Ionization No SO_2 (%)*	*Total Phenolics (Abs. Units)*
Control	1.656a[z]	1.086b	60.93a	9.12a	5.444a	7.154c	25.98b	16.10c
8/10/98	1.842b	1.070b	87.06b	8.163a	7.10ab	5.943b	20.79a	15.24a
8/17/98	2.317c	0.983a	104.5c	9.604ab	9.036b	4.518a	20.82a	18.34b

[z]Statistical analysis was performed using a one way ANOVA and Tukey's mean separation test. Different letters indicate statistical significant differences. Refer to Appendix D for relevant statistical information.

Discussion and Conclusion

From the color data presented in Table 3.1 conclusions can be made about the wines in relation to the experimental treatments performed on the grapes. The color density which is the additive absorbance at 420nm and 520nm ($E_{420} + E_{520}$) increased from 1.656 in the control to 1.842 in treatment two, and finally to 2.317 in treatment three. Since free anthocyanins absorb maximally at 520nm, and polymeric anthocyanins absorb maximally at 420nm (Singleton, 1988) it can be concluded that there was an increase in either the concentration of free anthocyanins or polymeric anthocyanins, or both with the two application times. The color hue data that represents the ratio of polymeric anthocyanins to free anthocyanins (E_{420} / E_{520}) shows a trend opposite to the color density data. The color hue data did not differ statistically from 1.086 for treatment one, the control, to 1.070 for treatment two, but did decrease significantly to 0.983 for treatment three. When the data for color density and color hue are analyzed together a clearer picture of the anthocyanin composition of the wines can be made. The decrease in the ratio of polymeric anthocyanins and free anthocyanins between the 10, August and the 17, August ethephon applications indicates that there was either an increase in free anthocyanins absorbing at 520nm, or a decrease in polymeric anthocyanins absorbing at 420nm. From the color density data that shows a definite increase in the additive absorbance of the two kinds of pigments it can be concluded that the decreasing ratio is due to a larger concentration of free anthocyanins in the wine from treatment three.

The total anthocyanin concentrations of the wines supports the above conclusion by showing that the control treatment had the lowest anthocyanin concentration with only 60.93 mg/L. The ethephon application in treatment two increased the concentration significantly to 87.06 mg/L, and the ethephon application for treatment three increased the ethephon concentration even further to 104.5 mg/L. The difference between the anthocyanin concentration in treatment one (control) and treatment two represents an increase in anthocyanins of 16.7%, whereas the difference from treatment one to treatment three represents an increase in anthocyanins of 41.7%. This tremendous difference in anthocyanin content is visually evident in the photograph of the wines presented in Appendix E.

Since it has already been established that the ethephon application timed nine weeks post anthesis on 17, August increased the anthocyanin content of the resulting wines by almost 42%, the remaining color parameters presented in Fig 3.1 can be used to analyze the chemical equilibria of wines. The degree of ionization of the wines which represents the percentage of the anthocyanins present in the ionized, colored form were similar for all treatments. This indicates that for all three treatments approximately the same percentage of anthocyanins in the wine were colored and therefore visibly red. When the same parameter was looked at in terms of milligrams per liter of ionized anthocyanins rather than percentage, the values were slightly different. There was no significant difference between the milligrams ionized in the control and treatment two, and no difference between treatment two and treatment three. There was however a

statistically significant difference between the milligrams of ionized anthocyanins in the control and treatment three. Since the percentage of anthocyanins that were ionized were similar between treatments, this increase is due to the significantly higher concentration of anthocyanins present in treatment three. If there are almost 42% more anthocyanins in treatment three than the control it follows that at similar pH's, treatment three would contain more milligrams per liter of ionized anthocyanins.

The concentration of free SO_2 present in the wines is important due to the ability of sulfur dioxide to bleach anthocyanins. Sulfur dioxide is added to wines routinely to stop the growth of microbial contaminants during the aging process. Since the SO_2 bleaches the anthocyanins, reducing the visible color of wines, it is helpful to be able to quantify the degree to which the color of the wines has been altered in the wine making process. In this experiment the concentrations of free sulfur dioxide decreased from 7.154 mg/L in the control to 5.943 mg/L in treatment two, and finally to only 4.51 mg/L in treatment three. This indicates that more of the anthocyanins present in the control wines had been bleached by SO_2 than in the treatment two and three wines, and that the treatment two wines had more bleached anthocyanins than the treatment three wines. Since it has already been established that there was a gradation in the concentration of anthocyanins from the control up to treatment three, it can be concluded that the visible color difference in Appendix E is due to the differences in anthocyanin concentration. However, the decrease in SO_2 concentrations from the control to treatment three is at least partially responsible for the color variation present in Appendix E.

The bleaching effect of sulfur dioxide on wine color is removed from the equation in the next parameter. The parameter entitled the degree of ionization without SO_2 describes the percentage of the anthocyanins that would be in the colored form if no SO_2 had been added to the wine. For this experiment there was a statistically significant difference between the control and treatments two and three, but not between treatments two and three themselves. These results are in agreement with the values obtained for free SO_2 levels in that the control contained the most free SO_2 so it is expected that if its effect were discounted the control wine would have a higher percentage of colored anthocyanins than the other two treatments. Despite this, the fact that the concentrations of anthocyanins were so much greater in treatments two and three these wines still appear more colored as in Appendix E due to the higher anthocyanin content.

The final parameter investigated in terms of wine color is the level of total phenolics present in the wines as determined by the absorbance at 280nm minus a factor of four to remove the effects of any non-phenolic constituents. In this experiment the total phenols expressed in absorbance units were highest for the wines from treatment three as one would expect since anthocyanins are included in this measurement. It is unexpected that the control wines had a higher level of phenols than the wines from treatment two. One would expect the levels of phenols to increase incrementally from the control to treatment three as was observed with the anthocyanin concentrations. However, the phenol content of wine can be influenced by enological factors such as the degree of maceration of the berries (Singleton, 1988), as well as cultural and environmental

differences experienced by the vines (Singleton, 1982). Since the components that make up the phenolic category are so varied it is impossible to know which component or components is responsible for the deviation in data without performing further biochemical analysis.

From the analytical color data presented above it can be concluded that the application of 750ppm ethephon to Sangiovese grapevines in Colorado markedly increases the anthocyanin concentration and the color of the resulting wines. While the effects of ethephon on many different berry growth characteristics has been examined it is still not known exactly how the ethephon is effecting the ripening process.

It is well known that grape berry growth follows a double-sigmoid growth pattern (Wicks, 1976; Boss et al., 1996; Crippen and Morrison, 1986; Hale et al. 1970) where initial berry enlargement is due to rapid cell division, followed by the slow growth or lag phase, and then the final stage of berry development characterized by verasion and ripening. The ripening stage includes changes such as berry softening, an increase in reducing sugars, a fall in acidity (Hale et al., 1970), accumulation of color, and an increase in berry volume caused by cellular enlargement. The ripening process is associated with a metabolic shift that occurs in grapes at the end of the slow growth phase. The phases of berry development can be loosely charted by counting the weeks from anthesis. The first growth phase occurs in the seven weeks post anthesis, followed by the slow growth phase in the eighth and ninth weeks (Boss et al. 1996), the final ripening stage begins in the tenth week and can continue until the sixteenth week post verasion or longer.

The effect of ethephon is assumed to be due to the stimulatory effect of the ethylene gas released (Wicks, 1976). In most fruits the ripening phenomenon is attributed to auxin induced ethylene production (Hale et al., 1970). However, grapes are a non climacteric fruit (Boss et al. 1996) and have never been observed to undergo any acceleration of ripening when exposed to auxins as in other fruits (Hale et al., 1970). In grapes, as the ripening process begins at the end of the lag phase, auxin levels decrease and ethylene is produced. Since endogenous auxin levels in grapes are antagonistic to ethylene action, a balance must be reached when the stimulatory effects of ethylene can overcome the inhibitory effects of auxins (Wicks, 1976). This effect was also observed by Hale et al. in 1970 when it was discovered that ethephon application in the first stage of berry growth delayed ripening, but application at eight or nine weeks post anthesis hastened the ripening and coloring process. This led them to conclude that an auxin-ethylene relationship was involved in the regulation of grape ripening.

Since the two application times of ethephon in this experiment were eight and nine weeks post anthesis it can be hypothesized that the berries were in their lag phase of growth and auxin levels were declining. This decrease in auxin levels can be used to explain the difference in anthocyanin concentration between the two application times. If the auxin concentration continued to decrease between the eighth and ninth week, the ethylene released from the ethephon would be more effective at accelerating the ripening and enhancing the coloring process.

From the data presented above it can be concluded that ethephon applied to Sangiovese grape vines grown in Colorado at eight and nine weeks after anthesis is effective as a means of increasing the color of the resulting wines. The application of 750ppm was more effective at increasing wine color at nine weeks post flowering than at eight weeks, most likely due to natural biochemical changes occurring in the fruit.

CHAPTER IV

IRRIGATION DEFICIT AND ETHEPHON APPLICATION

Introduction

An experiment was performed to test the combined effects of irrigation deficit and one application date of ethephon on the color enhancement of Colorado grown Pinot noir vines. It was hypothesized that combining the color enhancement effects of both irrigation deficit and ethephon application would increase the anthocyanin content of the berries, thereby increasing the color of the resulting wines.

Materials and Methods

The experiment utilized one row of 15 furrow irrigated Pinot noir vines located at the Colorado State University Orchard Mesa Research Center in Grand Junction Colorado. The vines were nine years old and planted in a ten by five foot spacing and were trained in a double trunk bi-lateral cordon system on a seven wire vertical shoot positioned trellis with an irrigation furrow along each side of the row.

To initiate the irrigation deficit experiment a dam was built on July 15, 1998 after the seventh vine to prevent irrigation of the subsequent 8 vines. The progression of the dry down experiment was monitored using a neutron probe for weekly soil moisture

evaluations. Four permanent neutron probe access tubes were installed, two in the irrigated portion of the row, and two in the non-irrigated portion of the row. Soil moisture levels were recorded weekly at 30.5, 61.0, and 91.4 cm depths from the start of the experiment until the middle of September. The three values were then added to obtain a value representing inches of moisture per meter of soil since the soil water was most likely extracted equally from all soil depths by the well developed root system (Van Rooyen, Weber and Levin, 1980). An unpaired T-test was performed and no statistically significant differences were found at the $P<0.05$ level.

The vine's water status was measured weekly during the duration of the experiment using a pressure bomb. Leaf water potentials were determined as described in Chapter II.

In addition to the irrigation treatment, the entire row of 15 vines was treated with a 750ppm ethephon spray on 17, August. The spray date was chosen based on work by Hale et. al. (1970) stating that ethephon applied 8 to 9 weeks post anthesis is most beneficial. The 17, August application date reflected a 9 weeks post anthesis treatment. A backpack sprayer was used to apply 650ml of the ethephon spray to each vine. The spray contained 750ppm Ethrel, the commercial form of ethephon, Triton X, a surfactant to aid in the formulations adherence to the vine, and 5% ethanol. There were not enough vines available to perform a factorial experiment to test the individual effects of irrigation deficit and ethephon application.

The Pinot noir berries from the experiment were harvested on September 23, 1998. The grapes from the irrigated control group, and the unirrigated treatment one group were made into respective batches of wine by Richard Hammon at the Colorado State University Orchard Mesa Research Center.

Results

The neutron probe values representing soil moisture are presented graphically in Fig. 4.1. The values for the soil beneath the stressed vines that received no water after July 15th remained fairly constant and show a slight increase over the duration of the experiment. The values for the soil beneath the un-stressed vines that received two additional furrow irrigations after July 15th also remained fairly constant with an increase at the beginning of August. Leaf water potential data was also collected weekly throughout the experiment to monitor the vine's water potential using a pressure bomb. Fig. 4.2 graphically presents the pressure bomb data collected, an unpaired T-test was used to determine statistical significance between treatments at $P<0.05$. The water potentials of both the stressed and unstressed vines show great fluctuation over the course of the experiment, with an overall decrease in water potential for both treatments.

There were no visual symptoms of drought stress on the vines subjected to the irrigation deficit stress. There were also no visible effects of the ethephon application on the vines or berries prior to harvest.

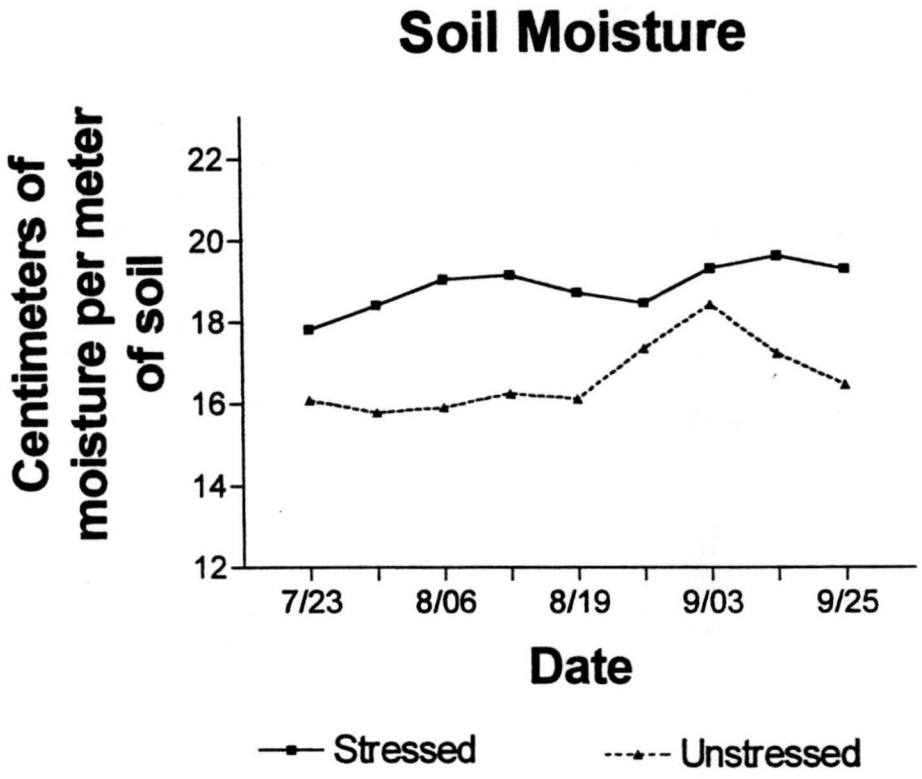

Figure 4.1 Graphical representation of neutron probe data collected from access tubes located in Pinot noir vines representing inches of moisture per meter of soil beneath water stressed and unstressed vines. No statistically significant differences were found.

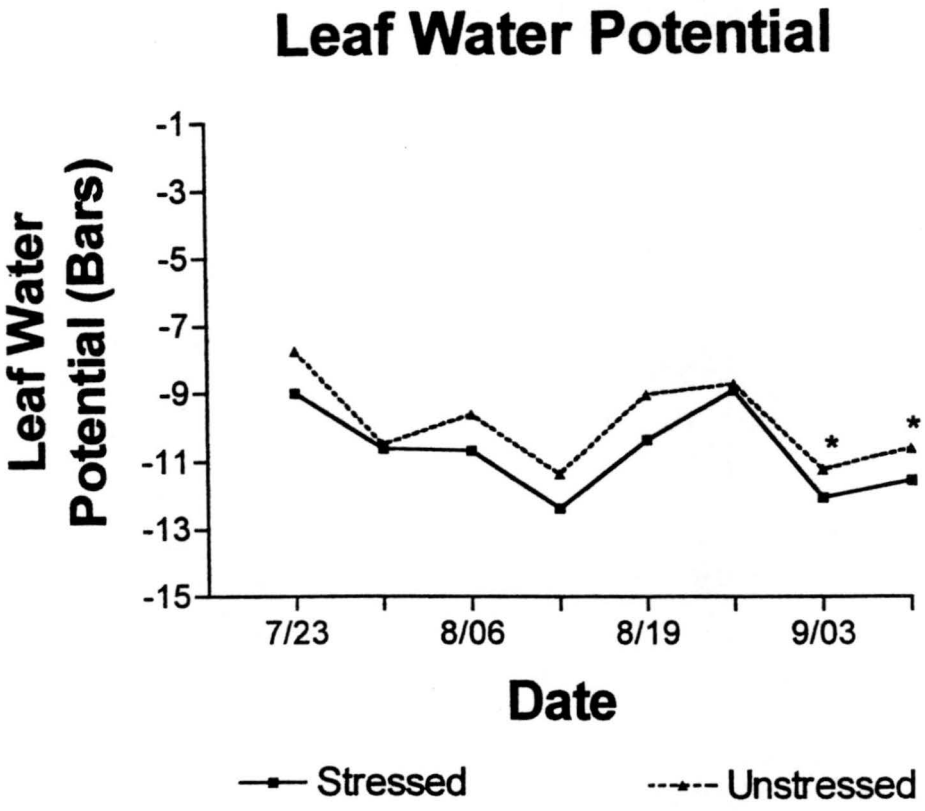

Figure 4.2 Leaf water potential data collected from Pinot noir vines subjected to an irrigation deficit and controls. Statistically significant differences are indicated by asterisks.

The Pinot noir berries from the control and irrigation deficit treatments all of which had received one ethephon application were harvested 23, September, 1998. At harvest the unstressed control berries had a Brix° of 23.8%, and a pH of 3.28. The berries subjected to the irrigation stress had a Brix° of 24.4%, and a pH of 3.22.

The grapes from the two treatments were made into wine in three gallon carboys. Sub samples of the wines were taken on 9 Feb., 7 April , and 15 June, all in 1999, and their color composition analyzed using the sodium metabisulphite method of Somers and Evans (1977). The color analysis from the third sub-sampling representing the most mature state of the wine is presented in Table 4.1. The data sets from the first and second wine analyses are in Appendix C.

Table 4.1 Color analysis of wines from Pinot noir irrigation deficit experiment and ethephon application. Analysis of wine color based on spectrophotometric properties.

Wine Color Analysis for Pinot Noir Irrigation Deficit Experiment with Ethephon							
	Color Density	*Color Hue*	*Total Antho-cyanins (mg/L)*	*Degree Ionization Anth. (%)*	*Ionized Antho-cyanins (mg/L)*	*Degree Ionization No SO_2 (%)*	*Total Phenolics (Abs. Units)*
Unstressed	3.098a[z]	0.864b	82.15a	18.95a	15.58a	18.92a	17.04a
Stressed	3.871b	0.764a	109.4b	19.19a	20.95b	22.08b	19.64b

[z]Statistical analysis performed using T-tests with a $P<0.001$. Means with different letters indicate statistical significance.

Discussion and Conclusion

The neutron probe data presented in Fig. 4.1 represents the soil moisture data in inches of moisture per meter of soil for the duration of the experiment. The T-tests performed to compare the treatment means at each date showed no statistically significant differences in the soil moisture between the two treatments at any of the sampling dates. Unfortunately, a statistically significant increase in soil moisture was detected via a T-test with $P<0.05$ in the stressed vines between the beginning and the end of the experiment . This is opposite from what was expected to occur.

Since the vines in this experiment were furrow irrigated there is no way to determine the exact amount of water either treatment received. From the neutron probe data collected it appears as if no water deficit was achieved in the stressed treatment. The reliability of neutron probe data can be questioned as a means of monitoring soil moisture at this site. It is possible that the neutron probe is not sensitive enough to pick up the reduction of soil moisture in this experiment, or there are errors in the data due to poor calibration of the instrument.

The leaf water potential data collected via a pressure bomb is presented in Fig. 4.2. This data was collected at midday (Matthews and Anderson, 1989) with the leaves placed in plastic bags to reduce the effects of transpiration (Liu et al., 1978; Matthews et al., 1987). Rather than exhibiting a constant decrease from the beginning to the end of the experiment, the leaf water potentials varied from week to week. The same trends were

observed in both the unstressed and stressed vines. From the beginning of the experiment to the first week, both the stressed and unstressed vines exhibited a decrease in xylem water potential. There was a slight increase in the unstressed vine water potential in the second week of the experiment while the stressed vines remained consistent through the third week. In both experimental groups a decrease in water potential is observed the third week followed by an increase in water potential by the fourth week. This increasing trend continued in the fifth week, followed by a decrease in the fifth week. By the seventh and final week of the experiment the xylem water potential for the stressed vines was slightly less than that of the unstressed vines. Overall, the water potentials for both of the experimental groups were much less than at the onset of the experiment.

The fluctuations in the water potentials of both groups of vines is most easily explained by changes in the ambient environment from week to week. Even though the sampling was performed at the same time of day and leaves of the same age size and location were used changes in irradience levels caused by cloud cover, and slight variations in temperature have the potential to alter the vines evaporative demand (Hardie and Considine, 1976). As was described in Chapter II, leaf water potentials less than -10 bars have a great impact on the vines physiology. The stomatal aperture is reduced as the vines water potential decreases thereby reducing the amount of CO_2 absorbed and reducing photosynthetic rates (Kliewer et al. 1983; van Zyl, 1987). Therefore, with final leaf water potentials of -11 bars for the unstressed vines and -11.5 bars for the stressed vines it can be concluded that both the stressed vines and unstressed vines were subjected

to a moderate water stress despite the fact that the unstressed vines received more water via the irrigation furrow.

The color analysis data for the wines from the two treatments is presented in Table 4.1. The color density value that describes the wines additive absorbance at 420nm and 520nm increased from 3.098 in the unstressed vines to 3.871 in the stressed vines. Since free anthocyanins absorb maximally at 520nm and polymeric anthocyanins absorb maximally at 420nm (Singleton, 1988) it can be concluded that either the concentration of free anthocyanins or polymeric anthocyanins or both, was increased in the water stressed treatment. The color hue parameter allows further investigation into the relationship between the two forms of pigments by representing the ratio of the absorbances at 420nm and 520nm. Since the color hue was greater in the unstressed wines at 0.864 than in the stressed vines at 0.764 it can be concluded that there were proportionally more free anthocyanins in the stressed wines than in the unstressed wines.

The data representing the total anthocyanin content in milligrams per litter of wine support the color density and color hue data by showing that the stressed wines had 109.4mg/L and the unstressed wines had only 82.15mg/L anthocyanins. Therefore, the wines from the stressed treatment contained almost 25% more anthocyanins than the unstressed treatment.

The data collected for the degree of ionization of anthocyanins shows that there was no difference between the percent of ionization of the wines from the two treatments. This means that at wine pH both treatments contained the same percentage of

anthocyanins in their ionized colored form. Since the pH of the wine from the unstressed control treatment had a pH of 3.62, and wine from the stressed treatment one had a pH of 3.44 one would expect that the stressed treatment would have a higher percentage of ionization. When the values for ionization of anthocyanins calculated in milligrams per liter are scrutinized it is observed that the wines from the stressed treatment contained a significantly higher concentration of ionized anthocyanins than the wines from the unstressed group. This is easily explained by the fact that the stressed wines contained 25% more anthocyanins than the unstressed wines, so they would contain proportionally more ionized anthocyanins even if the two wines contained similar percentages of ionization.

The next parameter explains the degree of ionization of the wines despite the amount of SO_2 added after fermentation. Since SO_2 is added to wine in the enological process, and is known to bleach anthocyanins, it is important to know the percentage of wine that has been rendered colorless by SO_2 addition. In this experiment it was discovered that 22.08%, and 18.92% of the stressed and unstressed wines respectively would have been in their ionized colored form if no SO_2 had been added to the wine. Since these values are only slightly higher than values for degree ionization of anthocyanins it can be concluded that SO_2 added after fermentation had a negligible effect on wine color.

The final parameter describing wine color is the amount of total phenolics in the wines presented in absorbance units. In this experiment the wines from the stressed

treatment had a total phenolic content of 19.64 absorbance units, and the wines from the unstressed treatment had a total phenolic content of 17.04 absorbance units. Since the phenolic make up of wines can vary greatly and can consist of many different compounds the exact meaning of this parameter is difficult to interpret. The fact that the stressed treatment contained more phenolics than the unstressed treatment can be explained by the fact that the stressed treatment had more anthocyanins than the unstressed treatment which would be incorporated into these values.

From the above color analysis it can be concluded that the wines that were exposed to the water stress had significantly more anthocyanins and enhanced color compared to the wines from the well watered control vines. The irrigation deficit seems to have had the desired effect despite the fact that the neutron probe data was inconclusive and the leaf water potential data indicated that there was very little difference in the water status of the vines in the two treatments.

The irrigation deficit experiment performed on drip irrigated Pinot noir vines and presented in Chapter II resulted in no increase in anthocyanin content and therefore had no color enhancing properties. It can be assumed that the increase in color seen in response to the irrigation deficit in this experiment is due to the joint effects of the water stress and the ethephon application.

As was already stated there is known to be a close direct relationship between leaf water potential and stomatal conductance in grape vines (Kliewer et al., 1983). Stomatal aperture, transpiration, and photosynthesis all decrease in plants subjected to differing

degrees of water stress (van Zyl, 1987). Stomatal closure is caused by an increase in the concentration of ABA, and it is known that ABA is produced in response to water deficits in most plants (Salisbury and Ross, 1992), including the *Vitis* species (Smart and Coombe, 1983). This increase in ABA concentration as a result of water stress has been implicated in the enhanced development of anthocyanin pigments in grape skins (Smart and Coombe, 1983). One of the ways ABA acts in plants is in the specific activation and deactivation of certain genes (Salisbury and Ross, 1992). It was discovered by Boss et al. (1996) that the accumulation of anthocyanins at verasion in grapes coincides with the increase in expression of all seven genes in the anthocyanin biosynthetic pathway. It was, therefore, determined by researchers that induction of anthocyanin synthesis is triggered by regulatory genes. It is possible that ABA is somehow involved in this regulatory process.

The fact that no increase in color was observed in the previous irrigation deficit experiment indicates that the combined treatments of irrigation deficit and a properly timed application of ethephon is responsible for the increase in anthocyanin content. It has been determined that ethephon applied in the lag phase of berry growth works effectively at increasing the anthocyanin content of grape berries (Hale et al., 1970). The application time of ethephon in this experiment corresponds with the end of the lag phase, by which time endogenous auxin levels are believed to be minimal (Wicks, 1976). Hale et al. (1970) came to the conclusion that an auxin-ethylene relationship was involved in the regulation of grape ripening and it is possible that the properly timed ethephon application and the subsequent release of ethylene in conjunction with the increased ABA levels in the

vines resulted in an increase in anthocyanin biosynthesis.

Since limited enzymatic studies of anthocyanin accumulation in grapes have been performed, little is known about the control mechanisms of this pathway (Boss et al., 1996). This, in conjunction with the fact that the exact mode of ethephon action is unknown, makes it difficult to pinpoint the mechanism or mechanisms that caused the increase in anthocyanin content of the berries exposed to both an irrigation deficit and an application of ethephon. The fact remains that Colorado grown Pinot noir vines exposed to these two factors did exhibit the desired color enhancement of the resulting wines.

CHAPTER V

COMPARISON OF COLOR IN COLORADO WINES

Introduction

An experiment was conducted to determine if differences in anthocyanin content and color existed between wines from different wineries, and between different wines from the same winery. It was hypothesized that different enological practices result in wines with varying anthocyanin content and color. The goal of the experiment was to determine if differences in color existed between the wines from different wineries. No attempt was made to compare overall wine quality.

Materials and Methods

Laboratory analyses were conducted to compare the color of Pinot noir and Merlot wines from several wineries in Colorado. Different wines made at the Colorado State University, Orchard Mesa Research Center (OMRC) were also evaluated. Wines for the Pinot noir and Merlot study were chosen based on commercial availability at the individual wineries and retail liquor stores while the OMRC wines were provided by Richard Hammond. The names of the wineries from which the samples were obtained will be kept confidential. In the following experiment the winery names will be alphabetically coded to maintain anonymity, each letter representing a different winery. If the same letter

is used in different experiments it represents the same winery in each study. The Pinot noir analysis was conducted on 1995 vintages from the OMRC and winery 'A' and 1997 vintages from wineries 'B' and 'C'. The Merlot analysis was conducted on 1995 vintages from the OMRC, and wineries 'B', 'D', and one 1996 vintage from winery 'E'. The analysis of wines from the OMRC compared Pinot noir, Shiraz, Sangiovese, Dolcetto, and Merlot wines from 1995.

The wines were evaluated at the Colorado State University campus in Fort Collins, Colorado. The protocol chosen to evaluate the anthocyanin content of the wines was the sodium metabisulphite method originally outlined by Somers and Evans (1977). A detailed description of the protocol is provided in Chapter II. From five spectrophotometric measurements the values of color density, color hue, total anthocyanins, degree of ionization of anthocyanins, degree of ionization of anthocyanins after abolishing the effect of SO_2, and total phenols, were determined. An analysis of variance followed by a Tukey's mean separation test ($P<0.05$) was performed on the above data.

Results

The results of the Pinot noir comparative analysis are presented in Table 5.1. Differences in color of the Pinot noir wines are evident. The winery 'B' Pinot noir had the lowest color hue, highest color density, the most anthocyanins per liter, and the highest percentage of ionization of anthocyanins compared to any of the other wines. Also, the

Table 5.1
Summary of comparative analysis performed on Pinot noir wines from Colorado wineries.

Pinot noir Comparative Analysis						
Winery	*Color Density*	*Color Hue*	*Total Antho-cyanins (mg/L)*	*Degree Ionization Anth. (%)*	*Degree Ionization No SO_2 (%)*	*Total Phenolics (Abs. Units)*
OMRC	2.908b[z]	1.158b	25.78a	28.80b	48.30c	21.21b
A	3.092c	1.389c	N/A	N/A	N/A	28.22d
B	4.665d	0.9888a	68.40c	31.66b	31.63b	26.57c
C	2.038a	1.151b	50.60b	8.278a	19.45a	17.67a

[z]Different letters indicate statistical significance of means as determined by Tukey's mean separation test.

'B' wine showed no increase in ionization upon removal of SO_2, indicating that none of the anthocyanins present had been bleached by additional SO_2 added after fermentation.

In contrast, the wine from 'A' had such a low anthocyanin concentration, that no values could be determined for total milligrams per liter of anthocyanins, or degree of ionization of anthocyanins.

The wine from 'C' had the second highest anthocyanin content with 50.60 mg/L, but the lowest degree of ionization of those anthocyanins. Therefore, even though the anthocyanins were present, only 8.3% of them were in their colored form in the wine. Removing the effects of SO_2 bleaching only increased the ionization to 19.45%, the lowest of the three determined values.

The wine from the OMRC showed adequate color density and color hue values, but due to low anthocyanin content and a high degree of bleaching by SO_2 wine color can be described as inferior to that of the 'B' Pinot noir.

The data from the Merlot comparative analysis is presented in Table 5.2. The wine from 'B' had the highest color density value as well as the highest anthocyanin content with 100.5 mg/L. The percent of ionization of the 'B' Merlot was the second lowest at only 17.16%, and could only have been improved to 21.35% if no SO_2 had been added.

The 'E' Merlot had the second highest color density value, as well as the second highest anthocyanin concentration with an impressive 92.13 mg/L. The

Table 5.2
Summary of comparative analysis performed on Merlot wines from Colorado wineries. Statistically significant differences determined at $P < 0.05$.

Merlot Comparative Analysis						
Winery	*Color Density*	*Color Hue*	*Total Antho-cyanins (mg/L)*	*Degree Ionization Anth. (%)*	*Degree Ionization No SO_2 (%)*	*Total Phenolics (Abs. Units)*
OMRC	4.119a[z]	1.036d	49.42a	21.78d	44.75c	24.87a
B	9.868d	.7281a	100.5d	17.16b	21.35a	34.11c
D	4.522b	1.014c	68.51b	11.70a	19.08a	28.54b
E	9.622c	0.8222b	92.13c	20.39c	26.20b	30.64b

[z]Different letters indicate statistical significance of means as determined by Tukey's mean separation test.

percent of ionized, or colored, anthocyanins was slightly higher than the 'B' wine at 20.39%, which could have been increased to 26.20% without the bleaching effects of SO_2.

The 'D' Merlot had a fairly low color density, and a moderate anthocyanin content that was diminished by the low ionization value of only 11.70%. The OMRC Merlot had the lowest color density, as well as the lowest anthocyanin content with only half of the anthocyanins as the 'B' wine. Even though the anthocyanin content was surprisingly low, the OMRC wine had the highest percentage of those anthocyanins ionized at 21.78%. This value could have been increased to as high as 44.75% if no additional SO_2 had been used.

Table 5.3 contains the data collected from the comparison of wines from the Orchard Mesa Research Center. Comparisons were made between Pinot noir, Shiraz, Sangiovese, Dolcetto, and Merlot wines. The final comparative study analyzed five different wines made at the Orchard Mesa Research Center to determine how anthocyanin content and color varied between cultivars. The Merlot had the highest color density value even though the Shiraz had by far the highest anthocyanin content with 86.3 mg/L. The Merlot had the next highest anthocyanin content with 49.42 mg/L. It is important to note that in the comparative Merlot study the same wine was ranked last with the lowest anthocyanin content. The Dolcetto came in third with 39.42 mg/L, while the Sangiovese and Pinot noir were tied for last place with anthocyanin contents of 21.84 mg/L and 25.78 mg/L respectively.

Table 5.3
Summary of comparative analysis performed on wines from the Orchard Mesa Research Center. Statistically significant differences determined at $P < 0.05$.

OMRC Comparative Analysis						
Wine	*Color Density*	*Color Hue*	*Total Antho-cyanins (mg/L)*	*Degree Ionization Anth. (%)*	*Degree Ionization No SO_2 (%)*	*Total Phenolics (Abs. Units)*
Pinot noir	2.908c[z]	1.158d	25.78a	28.80d	48.30b	21.21a
Shiraz	3.902d	1.006a	86.30d	10.44a	24.45a	22.88a
Sangiovese	2.745b	1.090c	21.84a	25.55c	24.73a	21.12a
Dolcetto	2.031a	1.319e	39.42b	10.27a	23.80a	21.18a
Merlot	4.119e	1.036b	49.42c	21.78b	44.75b	24.87b

[z]Different letters indicate statistical significance of means as determined by Tukey's mean separation test.

Discussion and Conclusion

From the data in Table 5.1 it is evident that there are statistically significant differences between the Pinot noir wines from the four Colorado wineries. The 'B' wine exhibited the best color and highest anthocyanin content while the wine from 'A' had such low concentrations of anthocyanins that comparisons could not be made. Pinot noir grapes are known to prefer cooler growing regions (Winkler et al., 1974) so the relatively hot climate of western Colorado could attribute to the poor coloration of Pinot noir berries from the region. The high number of cool microclimates in the area allows for great variation in pigment accumulation between vineyards. This in addition to the differing enological practices between the wineries could account for the variation between the wines studied.

Unlike the variety Pinot noir, Merlot grapes are less sensitive to heat, and usually produce adequately colored wines in Colorado. Consequently, the 'B' Merlot had the highest degree of color, with the 'E' Merlot not far behind (Table 5.2). The 'D' wine showed adequate anthocyanin accumulation but a low degree of ionization, while the OMRC wine showed poor anthocyanin content as well as a high degree of SO_2 bleaching.

From the analysis of the data in Table 5.3, conclusions about the wines from the Orchard Mesa Research Center can be made. The degree of ionization of anthocyanins was adequate in the twenty percent range for the Merlot, Sangiovese, and Pinot noir, but the Shiraz and Dolcetto were disappointing at 10.44% and 10.27% respectively. According to the degree of ionization discounting the effects of SO_2 all of the wines

except the Sangiovese lost approximately half of their color potential to SO_2 bleaching, indicating that less SO_2 could have been used.

The wines from the OMRC had surprisingly low anthocyanin concentrations in all cases except for the Shiraz. To magnify this problem further, a significant amount of the wine color was bleached by SO_2 added to the wine after fermentation. Sulfur dioxide addition after fermentation is a common practice used to control or eliminate microbial growth in the wine during aging. In this case the beneficial aspects of SO_2 addition were not balanced with the detrimental effect of anthocyanin bleaching.

From the data collected it can be concluded that there is indeed great color variation in Pinot noir and Merlot wines from various wineries in Colorado, as well as in the five different wines from the Orchard Mesa Research Center. These color differences are not due to one specific factor, but rather a combination of factors. The amount and concentration of the anthocyanins present in red grapes is known to vary considerably with the species, variety, maturity, seasonal conditions, production area, and yield of fruit (Mazza, 1995). The conditions of fermentation and aging of wine such as temperature, duration, and alcohol concentrations, affect the pigment concentrations in the wines as well (Mazza, 1995). Any one or all of these factors, therefore, could be responsible for the color variations observed between the wineries and varieties studied. This experiment illustrates the importance of enological practices on wine color, and stresses the need for continuity with factors such as SO_2 addition.

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APPENDIX A

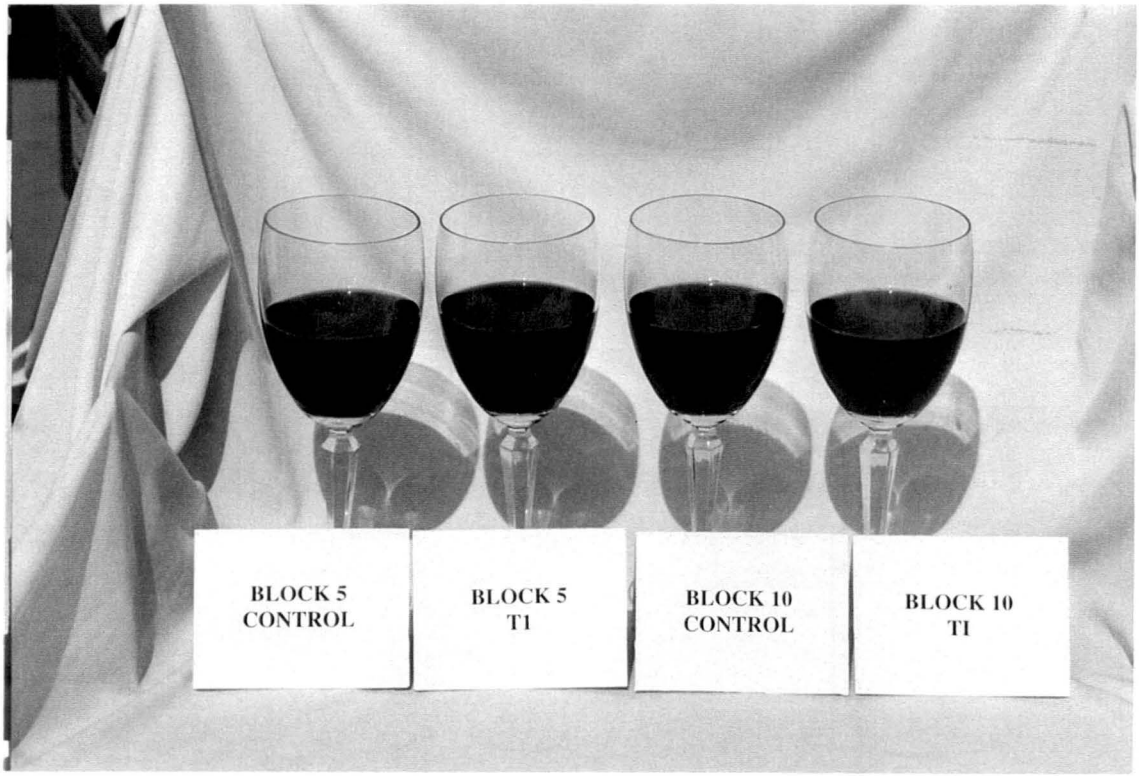
Table A.1 Statistical summary of trial I spectrophotometric data.

Pinot Noir Block 10 Irrigation Deficit Trial I - 2/4/99								
Treatment	*Color Density*	*Color Hue*	*Total Anthocyanin (mg/L)*	*Degree Ionization Anth. (%)*	*Ionized Anthocyanins (mg/L)*	*Free SO_2*	*Degree Ionization No SO_2 (%)*	*Tatal Phenolics (Abs. Units)*
Control	1.800 b	1.079 a	78.25 b	11.27 a	8.796 b	3.060 a	20.20 a	16.75 a
T 1	1.624 a	1.171 b	43.79 a	15.03 b	6.504 a	6.470 b	40.26 b	14.40 b

Table A.2 Statistical Summery for Trial II specrtophotometric data.

Pinot Noir Block 10 Irrigation Deficit Trial II- 4/7/99								
Treatment	*Color Density*	*Color Hue*	*Total Anthocyanin (mg/L)*	*Degree Ionization Anth. (%)*	*Ionized Anthocyanins (mg/L)*	*Free SO_2*	*Degree Ionization No SO_2 (%)*	*Tatal Phenolics (Abs. Units)*
Control	2.134 b	1.120 a	80.02 a	10.92 b	8.7206 b	2.032 a	16.68 a	17.88 b
T 1	1.953 a	1.163 b	78.52 a	9.685 a	7.554 a	3.690 b	18.96 b	15.23 a

APPENDIX B



Photograph of Pinot noir wines from the two irrigation deficit experiments. The two wines on the left labels Block 5 Control, and Block 5 TI are from the Irrigation Deficit and Ethephon Experiment in Chapter IV. The two wines on the right labeled Block 10 Control, and Block 10 TI are from the Irrigation Deficit Experiment in Chapter II.

APPENDIX C

Table C.1 Statistical summary of trial I spectrophotometric data.

Sangiovese Ethephon Experiment Trial I - 2/9/99								
Treatment	*Color Density*	*Color Hue*	*Total Anthocyanin (mg/L)*	*Degree Ionization Anth. (%)*	*Ionized Anthocyanins (mg/L)*	*Free SO_2*	*Degree Ionization No SO_2 (%)*	*Tatal Phenolics (Abs. Units)*
Control	1.120 a	1.016 b	84.87 a	6.174 b	5.240 a	N/A	N/A	14.15 b
T 1	1.208 b	1.044 c	105.5 b	5.152 a	5.432 a	10.73 b	19.48 a	13.01 a
T 2	1.589 c	0.9448 a	131.7 c	6.248 b	8.224 b	7.17 a	17.92 a	15.88 c

Table C.2 Statistical summary of trial II spectrophotometric data.

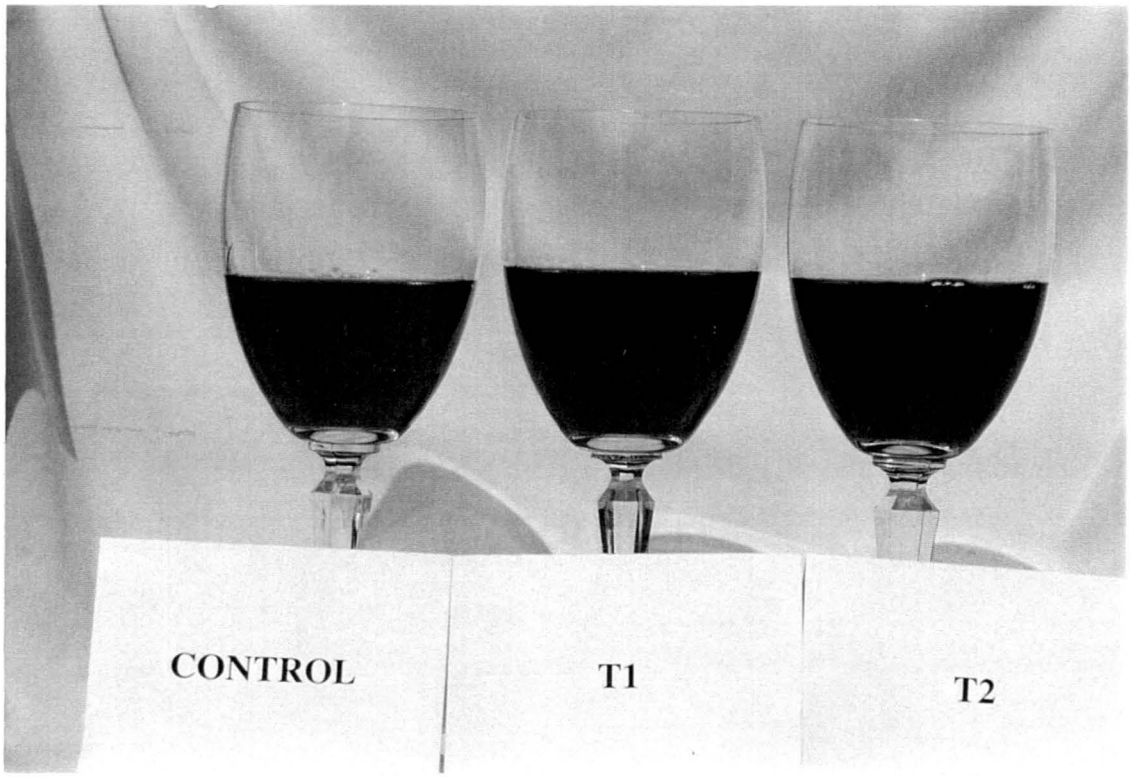
Sangiovese Ethephon Experiment Trial II - 4/13/99								
Treatment	*Color Density*	*Color Hue*	*Total Anthocyanin (mg/L)*	*Degree Ionization Anth. (%)*	*Ionized Anthocyanins (mg/L)*	*Free SO_2*	*Degree Ionization No SO_2 (%)*	*Tatal Phenolics (Abs. Units)*
Control	1.560 a	1.087 b	82.98 a	6.914 a	5.686 a	7.441 a	20.24 a	
T 1	1.708 b	1.097 b	95.27 b	6.314 a	6.008 b	8.395 a	20.07 a	15.65 a
T 2	2.092 c	1.000 b	122.3 c	7.259 ab	8.848 c	6.615 ab	19.45 a	17.19 c

APPENDIX D

Table D.1 Additional statistical information from one way ANOVA performed on data from Sangiovese Ethephon Application Experiment in Chapter III.

Color Parameter	Std. Deviation			Std. Error			Degrees of Freedom	Mean Squares	P value
	Control	TI	T2	Control	TI	T2			
Color Density	.0251	.0325	.0678	.0079	.0103	.0215	2	1.162	<0.0001
Color Hue	.0233	.0163	.0146	.0074	.0051	.0046	2	.0306	<0.0001
Total Anthocyanins (mg/L)	8.871	4.675	1.880	2.805	1.478	.5945	2	48.14	<0.0001
Degree Ionization (%)	1.502	.4913	.6267	.4749	.1554	.1982	2	5.375	0.0094
Ionized Anthocyanins (mg/L)	.3525	.3375	3.081	.1115	.1067	.9743	2	32.32	0.0006
Free SO2	.6917	.4571	.5555	.2187	.1446	.1757	2	17.41	<0.0001
Degree Ionization No SO2	3.790	1.038	.3737	1.199	.3281	.1182	2	89.34	<0.0001
Total Phenolics	.0808	.0753	.3082	.0256	.0238	.0975	2	25.51	<0.0001

APPENDIX E



Photograph of Sangiovese wines from Ethephon Application Experiment. The glass labeled TI corresponds to the wine from Treatment I applied on Aug. 10, 1998, and the glass labeled T2 corresponds to the wine from Treatment II applied on Aug. 17, 1998.

APPENDIX F

Table F.1 Statistical summary of trial I spectrophotometric data.

Pinot Noir Block 5 Ethephon Plus Irrigation Deficit Trial I- 2/4/99								
Treatment	*Color Density*	*Color Hue*	*Total Anthocyanin (mg/L)*	*Degree Ionization Anth. (%)*	*Ionized Anthocyanins (mg/L)*	*Free SO_2*	*Degree Ionization No SO_2 (%)*	*Tatal Phenolics (Abs. Units)*
Control	2.570 a	0.9272 b	102.1 a	14.59 a	14.80 a	1.045 a	18.53 a	17.25 a
T 1	3.479 b	0.7854 a	127.4 b	17.05 b	21.66 b	1.500 b	23.65 b	19.77 b

Table F.2 Statistical summary of trial II spectrophotometric data.

Pinot Noir Block 5 Ethephon Plus Irrigation Deficit Trial II- 4/7/99								
Treatment	*Color Density*	*Color Hue*	*Total Anthocyanin (mg/L)*	*Degree Ionization Anth. (%)*	*Ionized Anthocyanins (mg/L)*	*Free SO_2*	*Degree Ionization No SO_2 (%)*	*Tatal Phenolics (Abs. Units)*
Control	2.776 a	0.8955 b	86.6 a	17.67 a	15.22 a	0.6161 a	20.47 a	16.88 a
T 1	3.686 b	0.7948 a	115.5 b	18.56 a	21.39 b	1.095 b	23.76 b	19.02 b