

**MINERAL SPRAYS TO ENHANCE COLOUR DEVELOPMENT  
IN  
RED WINE GRAPES**

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## Summary and Recommendations

Calcium and phosphorus sprays were applied on 4 occasions to Cabernet Sauvignon vines on a site near Red Cliffs, Victoria, from December 19, 2003, to January 11, 2004. Measurements conducted on berries collected from early February through to harvest on March 11 indicated that the total amount of phenolics and anthocyanins were increased by the application of calcium and phosphorus, although the increases were quite small (2-3%). Vine N and P status, as indicated by the concentration of those nutrients in the petioles at flowering, were high and variable across the site, reduced the extent of the response, and in the case of N probably reduced the ability of vines on the site to achieve high levels of phenolics across the board.

- The extent of the response to the sprays was probably limited by timing, and any further exploration of the potential role of Ca and P sprays should encompass an application period from fruit set onwards, and be conducted on a site where the petiole N and P levels range from deficient to adequate.
- A clear gap exists in the knowledge base regarding the management of vine nutrient, particularly N and P, status to maximise potential anthocyanin synthesis and manage cropping levels profitably.

The spectral properties of the wines made from the grapes from vines sprayed with calcium and phosphorus were not different from the spectral properties of the wines made from grapes from unsprayed vines, but a distinct preference for the latter group of wines over the former was apparent. The basis of this preference may lie in the perturbation of the vines' mineral nutrition by the additional minerals supplied as sprays.

- The identification of the changes caused by the additional minerals may afford Cabernet Sauvignon growers greater means of controlling grape quality. The differences in FTIR spectra would appear to provide a reasonable starting point for such an investigation.

## Introduction

In recent years red wine grape quality has been increasingly assessed in terms of colour in addition to soluble solids as a basis for payments to growers, and much effort has been invested in trying to develop methods of determining grape berry colour quickly and accurately. This effort reflects the wine industry's belief that red grape colour is positively related to final red wine quality. The class of compounds in red grapes responsible for colour are the anthocyanins, which are located in the berry skin. Consistent high levels of anthocyanins have been generally difficult to achieve in red wine grape varieties growing in the Sunraysia/Riverland region. The fact that some grape growers can more or less consistently produce grapes with higher colour levels than others in the region means that it is biologically possible, but agronomically difficult to reproduce. Much R&D activity aims to enhance colour levels by reducing berry size using various means (e.g. irrigation, pruning and crop thinning).

Grapes are not unique in having fruit skin anthocyanin levels as an important quality parameter. Anthocyanins contribute to skin and/or flesh quality in plum, cranberry, raspberry and blueberry, for example. In apple fruit significant negative correlations between the final anthocyanin and total flavonoid concentrations in the skin on the one hand and fruit N, P, K, Ca and Mg concentrations earlier in fruit development on the other have been found (Awad and de Jager, 2002). Partly consistent with these observations on apples, Cabernet sauvignon and Merlot berry anthocyanin levels were observed to be decreased by increasing N supply (Keller and Hrazdina, 1998; Hilbert *et al.*, 2003). These more recent observations validate observations made by Kliewer on Emperor table grapes (Kliewer, 1977).

Recently, total anthocyanin levels in two red-skinned apple cultivars were found to be enhanced by application of mineral sprays containing phosphorus, calcium and nitrogen (Gómez-Cordovés *et al.*, 1996; Larrigauduere *et al.*, 1996; Li *et al.*, 2002). The degree of response (*i.e.* total anthocyanins with mineral sprays divided by total anthocyanins without mineral sprays) was greater than the degree of response reported recently for Cabernet sauvignon sprayed with ethanol at veraison (Chervin *et al.*, 2001) (1.3-2.5 for apples *c.f.* 1.3 for grapes).

The term anthocyanin covers a large number of compounds, and it is possible that the responses observed in apples may not involve the types of anthocyanins responsible for colour in red wine grapes. However, comparison of analytical data indicate that there is some commonality: enhanced levels of cyanidin derivatives were noted in apple skins in response to mineral sprays (Gómez-Cordovés *et al.*, 1996) and this was one class of anthocyanins shown recently to be increased by ethanol sprays in Cabernet sauvignon berry skins (El Kereamy *et al.*, 2002).

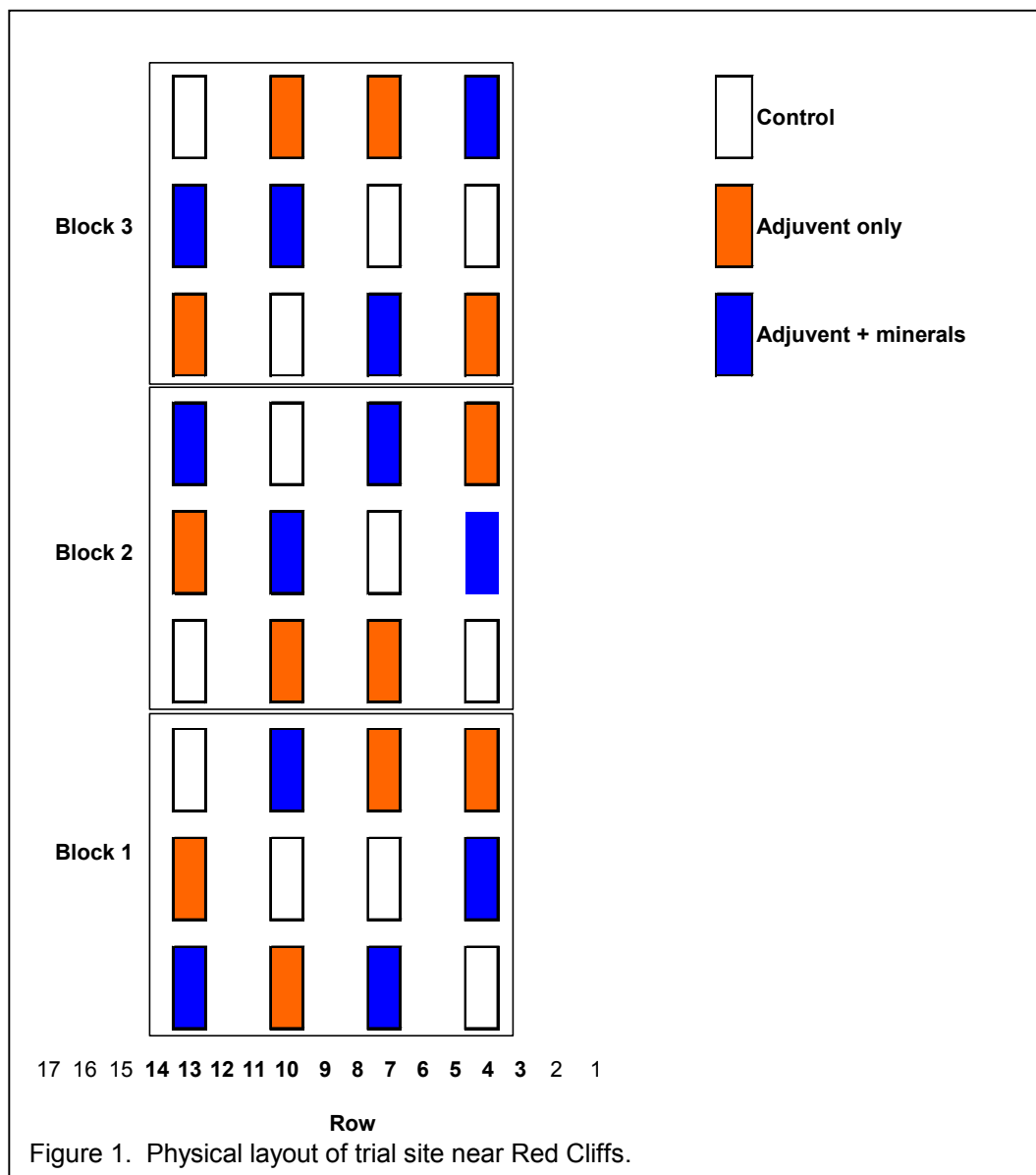
If this effect could be demonstrated in red wine grapes, it may ultimately expand the options that regional wine grape producers have available to control wine grape quality. This report describes a trial conducted during the

2003/04 grape growing season in the Sunraysia area to determine whether the type of mineral sprays used to enhance skin colour of red-skinned apples also affect colour development in red wine grapes.

### Materials and Methods

A commercial property in the Red Cliffs area was used. The site consisted of drip-irrigated Cabernet sauvignon on Schwarzmann rootstock. The rows ran north/south, and were 3m apart. Vine spacing within rows was 2m. Soil types ranged from Type 8 heavy phase through to Murray sand (Hubble and Crocker, 1941).

A randomised block design was set up on the site (Figure 1). Treatments were applied to 6-vine plots in each of 4 rows separated by 2 buffer rows. Plots within rows were separated by 2 vines. Within each row and block there were 3 plots, to which 3 treatments were applied. Thus there were 12 replications of each treatment across the site.



The plots were tagged at flowering on November 17<sup>th</sup>, and petioles from opposite the basal bunches were sampled from each plot to determine vine mineral nutrient status. The petioles were washed in 5 L of acidified de-ionised water with 2 drops of phosphate-free detergent to remove dust and spray materials, rinsed in several changes of de-ionised water, dried at 40°C for 5 days and ground to a fine powder. Total N was determined on a Leco CN 2000 and the other mineral elements were determined on a Spectroflame ICP following digestion of a 200 mg subsample in boiling HNO<sub>3</sub>.

Three treatments were applied: control (no spray), adjuvants only and adjuvants and minerals. The adjuvants only treatment consisted of wetter acidified to pH 1 with HNO<sub>3</sub>. The adjuvants and minerals treatment consisted of Seniphos (Phosyn PLC), which contains adjuvants and 40 g/L calcium and 310 g/L P<sub>2</sub>O<sub>5</sub> acidified with HNO<sub>3</sub> to pH 1, and was applied at a rate of 10 L ha<sup>-1</sup>. The treatments were applied by a commercial spray contractor using an over-the-row boom on a commercial spray unit (Silvan Turbomiser). The plots were marked at either end by flagging tape to allow the operator to turn the jets on and off at the start and end of each plot.

The treatments were applied on 4 occasions: December 19 and 28 and January 5 and 11.

Berry colour was assessed in 2 ways: non-destructively using a subjective rating over the course of veraison, and spectrophotometrically from the end of veraison to harvest.

The subjective assessment involved tagging 2 bunches on each of the 3 middle vines in each plot. Colour development was assessed visually by scoring each of 10 berries on each tagged bunch using a 3 point scale: 1 = berry still green; 2 = berry changing colour; 3 = berry completely changed colour. This assessment was conducted on January 12, 15, 19, 21, 23 and 28 and February 3.

From February 9 100-berry samples were collected from each plot on a weekly basis for colour and maturity measurements. Total soluble solids (°Brix) and titratable acidity were determined on the free-running juice of a 50 berry sub-sample on an Atago PR32 temperature-compensated digital refractometer and a radiometer automatic titrator, respectively. The 50 remaining berries were frozen overnight at -20°C, thawed, macerated and colour determined using the method of Iland *et al.* (2000)

Immediately prior to harvest, crop load was estimated by counting the number of bunches in a 22cm transect on either side of the trunk of the middle vine in each plot.

At harvest, ca. 10 kg of fruit were hand harvested from individual vines from the middle 3 vines of each 9-vine plot. Following subsampling for spectrophotometric colour measurements, replication was reduced to 4 per treatment by combining fruit from the same treatment from each of the 3

blocks down the row. The only form of replication at this point was row (n=4). Twenty kg of the composite fruit sample was then used for small-scale wine-making using the procedure described by Becker and Kerridge (1972). Twelve wines were made in total. There were no differences between the 12 lots of grapes with respect to total soluble solids (23.4°Brix), titratable acidity (4.1 g L<sup>-1</sup>) or pH (4.0). Across the board, ca. 4.1 g of tartaric acid L<sup>-1</sup> was added. The grapes were crushed on March 12, racked of on March 19 and again on March 23-24, and bottled on June 3. The wines were allowed to stand for 3 weeks before samples were taken for colour intensity measurements according to the method of Sommers and Evans (1977).

Additional to the colour measurements, FTIR analyses were conducted on Thermo Nicolet Avatar 370 FTIR.

The 4 wines from the Control treatment and the 4 wines from the Adjuvants + minerals treatment were tasted by 7 tasters in a preference testing design. This approach asks tasters to express a preference for one wine over another with respect to colour, fruit characters, tannins and overall appeal. The wines were presented as pairs from each row. Thus there were 4 pairs, each pair featuring a Control wine and an Adjuvants+minerals wine, both from the same row. Wines from the 2 groups were not presented in the same order. In addition to the preference testing, 4 of the tasters who were more skilled in sensory evaluation scored the 8 wines using the 20 point scale. This scale is divided into colour and clarity (3 points), bouquet (7 points) and palate (10 points).

The data were initially checked for outliers using Grubbs' test (Rohlf and Sokal, 1981). The data were then analysed as a randomised block design using Genstat 4.2 v6. Where necessary, data were transformed (square root or log<sub>10</sub>) to correct for non-normality. The design allowed for orthogonal contrasts to separate the effect of applying adjuvants [*i.e.* Control vs. (Adjuvants only+ Adjuvants and minerals)] from the effect of applying minerals [*i.e.* (Control + Adjuvants only) versus Adjuvants and minerals]. Significant differences between means were identified using least significant differences where a significant F ratio was detected. Chemometric data were analysed using Unscrambler<sup>®</sup> v7.8.

## Results and Discussion

The site used in the trial showed large variation between plots in petiole nutrient status at flowering, particularly with respect to P (Table 1). The vines on the site would also be classified as generally having excessive N levels. These two features of the site are of importance with respect to flavonol accumulation because of the negative correlations between some macronutrients and apple skin anthocyanin (Awad and de Jager, 2002) and the negative impact that N supply has on flavonol accumulation in red grapes (Kliwer, 1977; Keller and Hrazdina, 1998; Hilbert *et al.*, 2003).

Berry biomass and sugar accumulation and titratable acidity depletion are presented in Figure 2. Mean berry biomass increased by ca. 110 mg berry<sup>-1</sup>

in the fortnight prior to harvest. Titratable acidity levels at harvest were less than 4 g L<sup>-1</sup> and soluble solids were around 23.3°Brix. Mean berry fresh weight at harvest was 0.97 g berry<sup>-1</sup>. The treatments applied had no impact on berry sugar accumulation, the depletion of organic acids or berry growth.

Table 1. Summary statistics for, and interpretation of, petiole analyses for Cabernet sauvignon sampled at flowering. Interpretations based on Robinson *et al.* (1997).

	Statistic				Interpretation		
	Minimum	Average	Maximum	CV	Minimum	Average	Maximum
N	0.82	1.36	1.75	16	adeq	high	high
P	0.13	0.26	0.44	32	def	adeq	adeq
K	1.0	2.0	2.8	20	marg	adeq	adeq
Ca	0.6	0.9	1.5	22			adeq
Mg	0.17	0.30	0.48	24	def	mar	adeq
S	0.05	0.07	0.12	22	No standards		
Na	0.005	0.009	0.013	20	>0.5% is toxic		
Al	5	11	18	28	No standards		
B	17	29	38	16	def	marg	adeq
Cu	18	33	56	23	>15 ppm indicative of spray residues		
Fe	6	13	23	35	>30 is adequate		
Mn	5	22	35	28	def	marg	adeq
Zn	20	37	64	27	marg	adeq	adeq

The onset of veraison occurred around January 10, and was completed by around February 5 (Figure 3). The treatments applied had no effect on the onset or duration of veraison.

Analysis of the colour measurements conducted on samples collected from February 9 through to harvest (March 11) indicate that berry total phenolics were maximal in mid February and that anthocyanins were maximal from mid February through to February 23 (Figure 4). Both total phenolics and total anthocyanins had declined significantly from those maxima by the time the fruit were harvested on March 11. Browning

anthocyanins were at a minimum on February 23, and increased marginally by harvest. The level of anthocyanins obtained on this site were *ca.* 30% lower than the mean weighted average for this variety in this region this season (Anon, 2004).

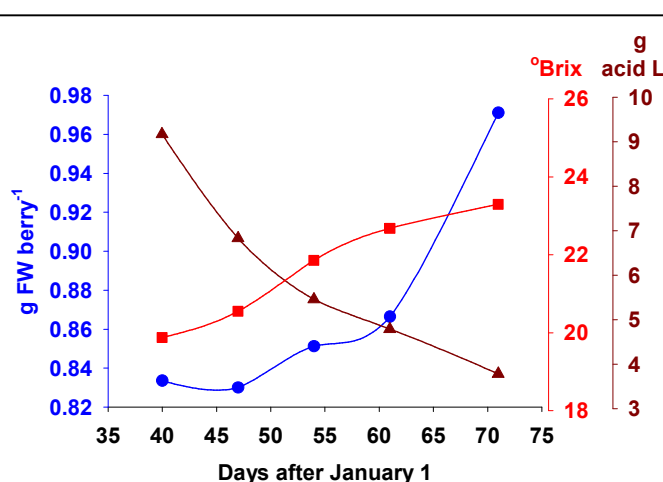


Figure 2. Time course of berry biomass and sugar accumulation and titratable acidity depletion for Cabernet sauvignon at Red Cliffs.

The spray treatments increased total phenolics and total anthocyanins expressed on a gravimetric basis (*i.e.* per gram berry fresh weight), but did not increase browning anthocyanins. The orthogonal contrasts indicated that the response was due to the application of minerals, and was not due to the application of adjuvants. Over the course of sampling for colour measurement, the application of minerals significantly ( $P=0.05$ ) increased total phenolics from 1.31 to 1.35 and anthocyanins from 0.97 to 0.99  $\text{mg g}^{-1}$  berry FW. The sprays were applied between December 19 and January 11 - after the period when the expression of flavonol synthase genes is high (Downey *et al.*, 2003). It is possible that calcium and phosphorus may have a greater impact, and possibly more significant in terms of final wine composition, if applied soon after fruit set when the expression of the genes involved in the flavonoid biosynthesis pathway is greater.

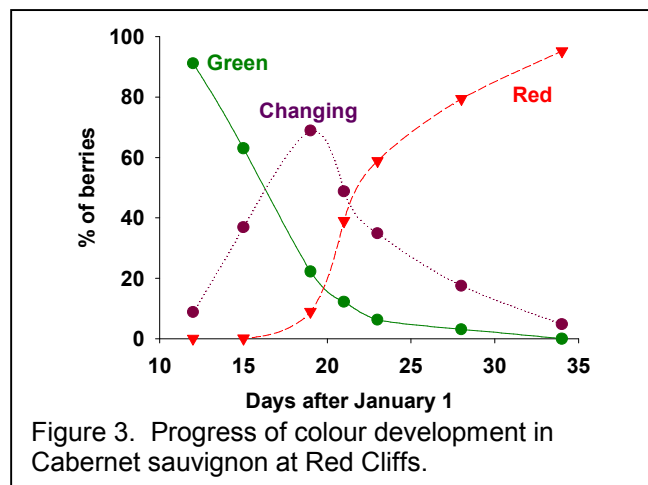


Figure 3. Progress of colour development in Cabernet sauvignon at Red Cliffs.

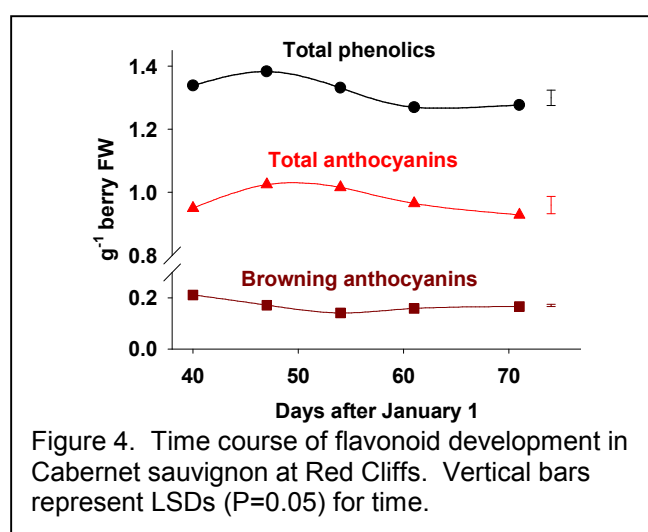


Figure 4. Time course of flavonoid development in Cabernet sauvignon at Red Cliffs. Vertical bars represent LSDs ( $P=0.05$ ) for time.

There were no significant interactions between the time (sample date) and treatment. In other words, the nature of the response to the treatments did not change as the berries developed. Effectively, this means that the response had probably occurred before sampling began and the extent of the response did not abate with time.

Plot means across sampling dates were also analysed using the concentrations of N, P, K, Ca, Mg and S in the petioles opposite the basal clusters at flowering as covariates. The inclusion of petiole % N, P or K at flowering made the effect of the mineral sprays on total phenolics per g berry weight greater. Petiole Ca, Mg and S were not significant covariates. In other words, the N, P and K status of the vines was a significant factor in the accumulation of total phenolics, but Ca, Mg and S were not. These observations highlight the need to identify ways of managing vine N, P and K supply and status to manage crop size on the one hand and potential anthocyanin accumulation on the other, particularly if other factors important in flavonoid biosynthesis are non-limiting (Kliewer, 1977)

Analyses of the finished wines made from the combined plots down each row suggested that, with respect to the chemical criteria for describing the spectral properties of young red wines (Somers and Evans, 1977), there were no differences between the wines with respect to total phenolics, total anthocyanins, ionised anthocyanins, hue, density and the indices for chemical age (Table 2).

Table 2. Wine spectral data according to the method of Somers and Evans (1977). There were no significant differences between treatment means for any parameter measured.

Spectral parameter	Control	Adjuvants	Adjuvants + minerals
Total phenolics	65	65	66
Total anthocyanins	722	717	704
Ionised anthocyanins	90	87	73
Hue	0.53	0.53	0.56
Density	10.5	10.3	9.3
Chemical age 1	0.27	0.27	0.27
Chemical age 1	0.059	0.060	0.057
Alpha 1	12.2	12.1	10.0
Alpha 2	18.1	18.2	16.8

Nearly  $\frac{2}{3}$  of the responses in the preference testing indicated a preference for the Control wines over the Adjuvants+minerals wines in all the categories assessed (colour, fruit characters, tannins and overall appeal). Analysis of the sensory data (Table 3) suggested that the basis of the preference may be due to differences in the taste of the wines:

the mean of the palate scores for the Adjuvants+minerals wines was significantly lower than the mean of the palate scores for the Control wines. The colour and clarity, bouquet and totals scores were similar.

The mean normalised FTIR absorbance spectra for wines from each treatment are presented in Figure 5. The major areas of difference were clearly located

Table 3. Effect of spray treatments in mean (n=4) wine sensory qualities. Asterisk indicates a significant (P<0.05) difference between means. An absence of an asterisk indicates that there was no significant difference between means (P=0.05).

Sensory parameter	Control	Adjuvants +minerals
Colour and clarity	3	3
Bouquet	4.5	4.2
Palate	5.8	5.2*
Total	13.3	12.3

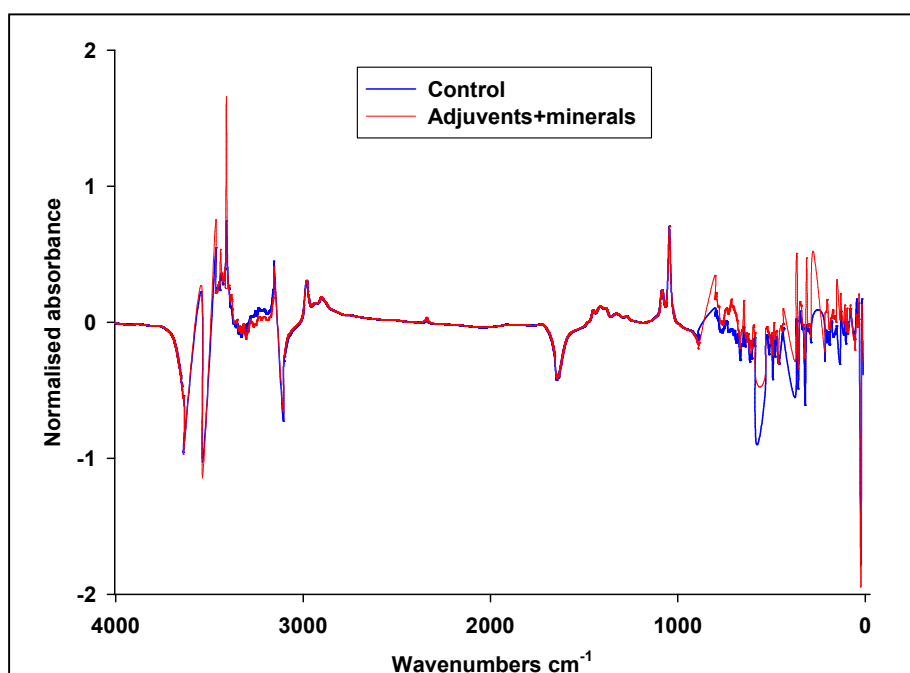
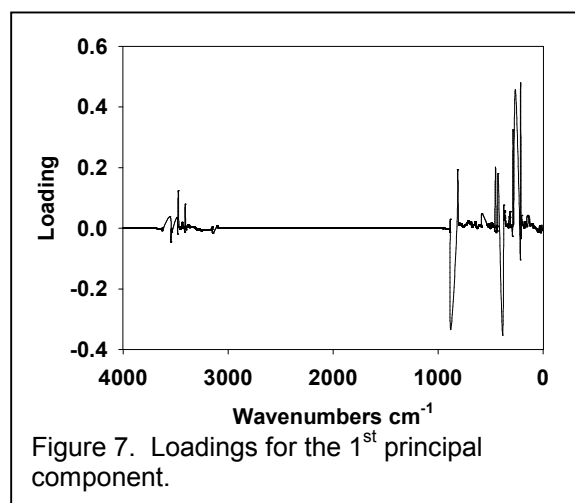
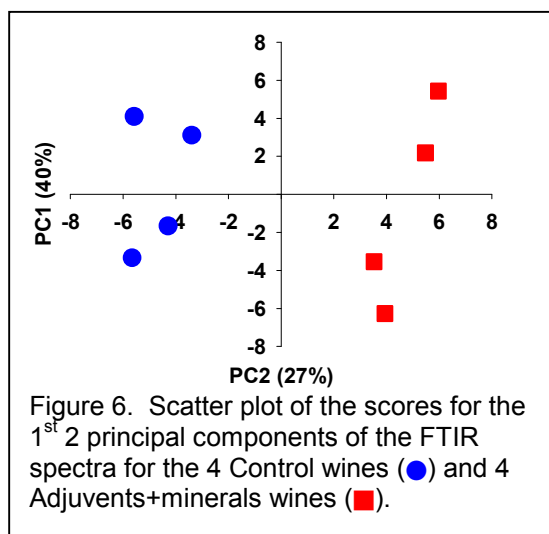


Figure 5. Mean (n=4) normalised FTIR absorbance spectra for Control and Adjuvant+minerals wines.

in a region covering 0-900 and in a region covering 3150-3400 wavenumbers  $\text{cm}^{-1}$ . The region between 900 and 3150 wavenumbers  $\text{cm}^{-1}$  contained no differences between the 2 spectra.

The principal components analysis conducted on the entire data set confirmed that the two groups of wines had different spectral characteristics (Figure 6). Good separation between each group of 4 wines was obtained by projecting the 1<sup>st</sup> and 2<sup>nd</sup> principal components. These 2 principal components accounted for 40 and 27%, respectively, of the variability between spectra. Similar separation of the wines was obtained when plots of the 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> principal components against the 1<sup>st</sup> were prepared (not shown). The 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> principal components accounted for 27, 14, 8 and 6% of the variability.

The loadings for the 1<sup>st</sup> principal components for each wavenumber recorded confirmed that the differences between the two groups of wines were confined to the wavenumber regions mentioned above (Figure 7). The strength of the loadings (in either a positive or negative direction) indicate the degree of influence that that particular wavenumber had.



The preference testing and the sensory data suggested that the application of calcium and phosphorus was associated with a negative quality trait in the wine compared to wine made from grapes off vines that had not been sprayed. The chemical and spectrophotometric analyses of those wines indicated no differences, but the FTIR analyses suggested that there were. In the mid-IR range (400-4000 wavenumbers  $\text{cm}^{-1}$ ), there were 12 regions of the spectra differing, but there were no differences between the spectra at the wavenumbers used to estimate ethanol, glycerol, sugars, acetic acid and organic acids (*i.e.* 1045 to 1395 wavenumbers  $\text{cm}^{-1}$ ) (Vonach *et al.*, 1998; Patz *et al.*, 1999). There appeared to be 9 regions of the spectra differing between the 2 groups of wines in the far-IR range (5-400 wavenumbers  $\text{cm}^{-1}$ ), but some of these are close to each other and may represent a broad band of wavenumbers that are similar between the two groups of spectra. The far-IR region contains the absorbances for ligands featuring cations (including  $\text{NH}_4^+$ ) (Ozin *et al.*, 1989). Given the differences in the IR spectra in both the mid and far ranges, it is interesting to speculate that information about the basis for the preference of one group of wines over the other may be contained by the absorbances where the two groups of spectra differ. That aside, the

possibility exists that the preference for one group of wines over the other group may be related to vine mineral nutrition, which was disturbed in this case by application of Ca and P. The identification of the basis of that negative trait and of the circumstances leading to it, may lead to improved practices and better quality wine from the region.

### Conclusion

Spraying Cabernet sauvignon vines with calcium and phosphorus resulted in small (2-3%) but statistically significant increases in total phenolics and anthocyanins in the grapes. The extent of the response was probably limited by application of the sprays after the period of maximum expression of the genes involved in the biosynthetic pathway. Further, the P, K and, particularly, the N status of the vines were significant influences on the extent of the response.

The application of calcium and phosphorus did not result in any differences in wine composition, as indicated by the conventional measurements taken. But, a distinct preference was expressed for wines made from grapes from vines not sprayed with calcium and phosphorus, and differences in the FTIR spectra between the 2 groups of wines were noted in the far-IR and mid-IR ranges.

### Literature cited

- Anonymous. 2004. Colour concern. Color scores reveal grey area. *Murray Valley Winegrowers Grapevine* May/June, 1.
- Awad, M.A. and de Jager, A. 2002. Relationships between fruit nutrients and concentrations of flavonoids and chlorogenic acid in 'Elstar' apple skin. *Scientia Horticulturae* 92, 265-276.
- Becker, H. and Kerridge, G.H. 1972. methods of small-scale wine making for research purposes in both hot and cool regions. *Journal of the Australian Institute of Agricultural Science* 38, 3-6.
- Chervin, C.; Elkereamy, A.; Roustan, J.-P.; Faragher, J.D.; Latche, A.; Pech, J.C. and Bouzayen, M. 2001. An ethanol spray at veraison enhances colour in red wines. *Australian Journal of Grape and Wine Research* 7, 144-145.
- Downey, M.O.; Harvey, J.S. and Robinson, S.P. 2003. Synthesis of flavonols and expression of flavonol synthase genes in the developing grape berries of Shiraz and Chardonnay (*Vitis vinifera* L.). *Australian Journal of Grape and Wine Research* 9, 110-121.
- El Kereamy, A.; Chervin, C.; Souquet, J.M.; Moutounet, M.; Monje, M.C.; Nepveu, F.; Mondies, H.; Ford, C.M.; van Heeswijck, R. and Roustan, J.P. 2002. Ethanol triggers grape gene expression leading to anthocyanin accumulation during berry ripening. *Plant Science* 163, 449-454.
- Gómez-Cordovés, C.; Varela, F.; Larrigaudiere, C. and Vendrell, M. 1996. Effect of Ethephon and Seniphos treatments on the anthocyanin composition of Starkng apples. *Journal of Agricultural and Food Chemistry* 44, 3440-3452.

- Hilbert, G.; Soyer, J.P.; Molot, C.; Giraudon, J.; Milin, S. and Gaudillere, J.P. 2003. Effects of nitrogen on must quality and anthocyanin accumulation in berries of cv. Merlot. *Vitis* 42, 69-76.
- Hubble, G.D. and Crocker, R.L. 1941. *A Soil Survey of the the Red Cliffs Irrigation District, Victoria*. Bulletin No. 137. Council for Scientific and Industrial Research, Melbourne. 63 pages and map.
- Iland, P.; Ewart, A.; Sitters, J.; Markides, A. and Bruer, N. 2000. *Techniques for chemical analysis and quality monitoring during winemaking*. Patrick Iland Wine Promotions, Campbelltown, South Australia
- Keller, M and Hrazdina, G. 1998. Interaction of nitrogen availability during bloom and light intensity during veraison. II. Effects on anthocyanin and phenolic development during grape ripening. *American Journal of Enology and Viticulture* 49, 341-348.
- Kliewer, M. 1977. Influence of temperature, solar radiation and nitrogen on coloration and composition of Emperor grapes. *American Journal of Enology and Viticulture* 28, 96-103.
- Larrigaudiere, C.; Pinto, E. and Vendrell, M. 1996. Differential effects of Ethephon and Seniphos on colour development of 'Starking Delicious' apple. *Journal of the American Society for Horticultural Science* 121, 746-750.
- Li, Z.H.; Gemma, H. and Iwahori, S. 2002. Stimulation of 'Fuji' apple skin colour by ethephon and phosphorus-calcium mixed compounds in relation to flavonoid synthesis. *Scientia Horticulturae* 94, 193-199.
- Ozin, G.A.; Baker, M.D.; Godber, J. and Gil, C.J. 1989. Intrazeolite site-selective far-IR cation probe. *Journal of Physical Chemistry* 93, 2899-2908.
- Patz, C.-D.; David, A.; Thente, K.; Kürbel, P. and Dietrich, H. 1999. Wine analysis with FTIR spectrometry. *Viticultural and Enological Science* 54, 80-87.
- Robinson, J.B.; Treeby, M.T. and Stephenson, R.A. Fruits, Nuts and Vines. In Reuther, D.J. and Robinson, J.B. (Eds.) *Plant Analysis: An Interpretation Manual*. 1997. CSIRO Publishing. pp 349-382.
- Rohlf, F.J. and Sokal, R.R. *Statistical Tables*. 2<sup>nd</sup> Edition. 1981. W.H. Freeman and Company, San Francisco.
- Sommers, T.C. and Evans M.E. (1977) Spectral evaluation of young red wines: anthocyanin equilibria, total phenolics, free and molecular SO<sub>2</sub>, "chemical age". *Journal of the Science of Food and Agriculture* 28, 279-287
- Vonach, R.; Lendl, B and Kellner, R. 10998. High-performance liquid chromatography with real-time Fourier-transform infrared detection for the determination of carbohydrates, alcohols and organic acids in wines. *Journal of Chromatography* 824, 159-167.