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Influence of natural reflective mulch on Pinot noir grape and wine quality

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Objectives

The intention of conducting this project is to investigate the potential of addressing the problem of excessive sugar (potential alcohol) levels in Pinot noir at harvest. This was attempted by modifying the ripening process through the use of a natural reflective mulch (mussel shells), which are also a by-product of the Nelson area seafood industry.

Changing the light environment around Pinot noir vines helps to advance the ripening of the phenolic compounds (tannin and colour) relative to sugar accumulation we potentially provide a means to achieve more balanced ripeness and therefore better quality wine.

At the same time, another option of a commercial solution for the resource management issues associated with the disposal of shells from the growing shellfish industries in Nelson and Marlborough is provided. Nelson and Marlborough are both regions that have important and rapidly expanding shellfish industries. A by-product of this is the large volumes of oyster, mussel and scallop shells that have to be disposed of, with limited end uses available.

As with any red wine, and specifically for this variety, colour and tannin quality are major determinants of wine quality. One way to positively influence both of these is through the use of reflective mulch on the soil surface under the vines, and this has been demonstrated with artificial woven reflective mulch (Creasy and Nicol, 2003). The mulch reflects light and heat onto the normally not-exposed parts of the clusters in the grape canopy.

Other possible advantages may be earlier ripening overall, and more importantly, earlier ripening of the phenolics compared with sugar accumulation. This would enable the production of wines from fully ripened grapes at lower sugar (potential alcohol) levels than is often encountered (and which is seen as a problem) in New Zealand Pinot noir.

Mulches can also reduce herbicide use in the vineyard, and have beneficial effects on soil health, root growth and overall vine health.

Approach

Brief outline of the methodology used

The trial was established at Neudorf Vineyards in the Upper Moutere, Nelson. An area of one hectare of Pinot noir vines (clone 5 on rootstock 101-14, planted in 2000) on a northern-facing slope and trained to a two-cane vertical shoot positioned (VSP) trellis was used for experimentation.

Mussel shells were spread out in October 2003 over approximately half of the area next to an adjacent, but contiguous, section of vineyard, identified as a control, or non-treated, area. The shell mulch was approximately one meter in width, directly under the vine row. The Control was maintained as a one-meter wide weed-free strip underneath the vines, which is current industry practice.

Experimentation, particularly in the 2004-2005 season, identified that there was significant variation up and down the slope (Crawford, 2006), so information in later seasons was taken from only a relatively small area in the middle portion of the slope (See Appendix). Data were collected to characterise vine performance through the seasons, as well as fruit maturation. Fruit for the replicated microvinifications were harvested on and processed under a rigourous regime designed to test the treatment effects on wine qualities. The fruit from the remainder of the trial area was harvested commercially and made into wines using commercial practice. Wines from the microvinifications and commercial scale were assessed by wine industry peers using a set of qualitative scales. The wines were also analysed quantitatively for colour and other phenolics using spectrophotometry and HPLC. Some preliminary data on aroma compounds as determined through GC-MS are also presented.

The trial has been run over three complete seasons and under differing crop load and environmental conditions.

Outcomes

Summary of results. Comment on extension of results, e.g., any further actions required? Any further plans for the project?

This has been a long-running trial, and this report will focus on synthesising and condensing the overall findings of the work. Detailed information about what was discovered can be found in the various reports and two theses generated by students, Michelle Crawford (Crawford, 2006) and Gerardo Leal Perez (Leal, 2007).

Though the trial was started in 2003, there was limited information that could be gathered in the 03-04 season due to the unexpected departure of the first student hired to work with the project. In addition, some important data (i.e. tasting results from the January 2004 Southern Pinot noir Workshop) were lost (though it should be noted that the wines tasted at that workshop were from the small pilot mulch trial established in 2001 (for details, see Creasy and Nicol, 2003) and not the trial reported on here).

It is worth noting that the trial design does not lend itself to statistical analysis, as there is no replication of the treatments in the trial area. This has complicated presentation of the results, leading to, in most cases, confidence intervals for means being used to indicate whether the difference between means is likely to be real or not. In other cases, however, the data collected could be analysed statistically as there was replication (*e.g.* microvinification wine lots).

The report will now summarise the findings according to rough phenological stage of development.

Budburst

Information about mulch effects on budburst were collected in 2004, 2005 and 2006, but no significant differences resulted (data not shown). This was despite some variation in budbreak date in each season, with 100% budbreak occurring on 21st September, 26th September and 9th October, respectively. It was thought that changes to soil temperature (see later section) may be enough to influence budburst, but it appears that the magnitude of the change is not enough to have a practical effect (Creasy *et al.,* 2003).

Canopy characteristics

The one part of the vine environment that was sure to change with the use of shells was the canopy microclimate. There were many measurements taken to attempt to quantify these changes, which in some cases were notable and in others, not significant.

Temperature

When the first canopy level measurements were made in the trial, it was unknown what effect the shells would have. The pilot trial indicated that shells could cause a slight increase in cluster temperature (Creasy and Nicol, 2003), but some aspects of how the data were collected in that trial were unknown.

Table 1 shows a segment of each season's temperature for the four seasons data are available. The time period would coincide with berry expansion post-fruit set. In three out of the four seasons the canopy temperature in the Shells treatment was slightly higher than in the Control during this time period. In the exception, there was considerable cooler weather and rainfall in December, which may have contributed to changing the relationship. There did seem to be an effect of Shells on the minimum temperatures reached in the trial, with the Shells having slightly lower values than the Control. This is a reflection of the night time temperatures being consistently lower in the Shells treatment (See Fig 1 for an example).

Table 1. Comparison of three seasons' data for canopy temperatures measured between December 14 and January 26 in each season.

Season	Treatment	Average	Min	Max
2003-2004	Control	16.4	1.4	32.8
	Shells	16.6	1.1	33.3
	Shells-Control	0.14	-2.9	2.8
2004-2005	Control	18.2	4.2	32.6
	Shells	17.9	3.8	32.8
	Shells-Control	-0.32	-2.4	1.8
2005-2006	Control	17.6	4.4	33.5
	Shells	17.6	3.8	34.4
	Shells-Control	0.07	-1.3	2.7
2006-2007	Control	17.6	4.4	33.5
	Shells	17.7	3.8	34.4
	Shells-Control	0.07	-0.6	0.9

Table 1 also shows that the difference between Shells and Control can be quite negative, with values approaching -3.0°C. Closer examination of the data reveal that this is not actually because the canopy in the Shells area is getting cooler, but rather that in the first hour of sunlight, the canopy in the Control tended to warm up much more quickly than in the Shells treatment. This could be due to the position of the loggers in the trial area, an effect of the mulch in resisting early morning warming or some other factor not considered.

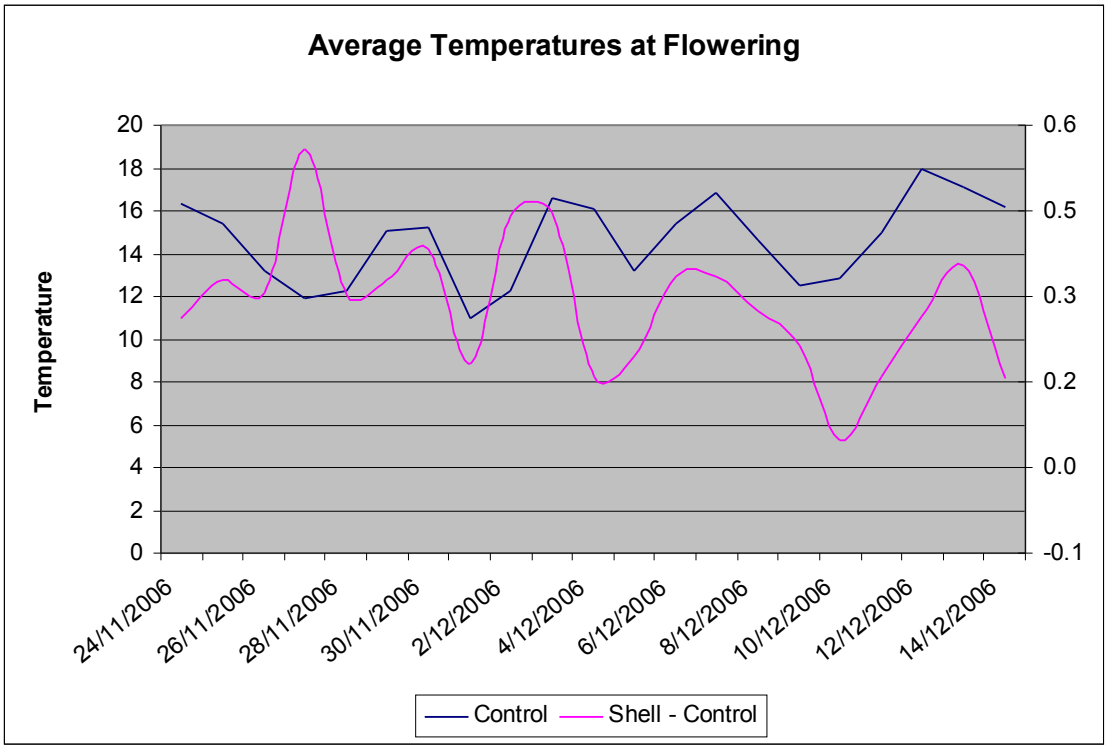

Figure 1. Average canopy temperature over an average 24-hour period during flowering 2006. Shell minus control (curved line) uses the right-hand axis.

Daytime temperatures are significantly higher in the Shells compared to the Control for most of the daylight hours (Figure 1). It is possibe that night-time temperatures are cooler because the soil temperature is lower under the shells (see soil section) and/or that the shells insulate the soil surface, meaning less heat can travel through it at night. This could be a concern during the early or late season frost season, so temperatures under those conditions were also studied. The results here are inconclusive, as the temperatures are very similar in some instances and slightly different either way in others (See Figs 2 and 3).

Whether or not the Control or Shells result in colder below-freezing temperatures may depend on the weather conditions leading up to the frost event. As this Nelson site rarely sees freezing temperatures after budbreak (where day/night temperatures can be quit different from those in mid-winter, when frosts are more common), this is a question that cannot be answered using data from this trial. Collection of data with greater frequency (*e.g.* every 10 minutes rather than 30min) may also help in more fully describing the treatment responses.

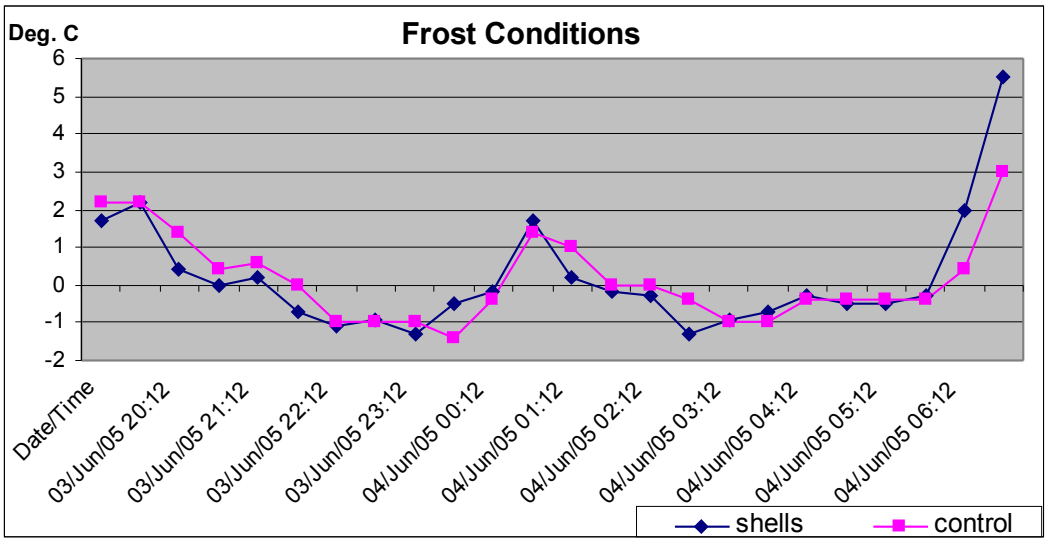

Figure 2. Canopy temperature under frost conditions, June 2005.

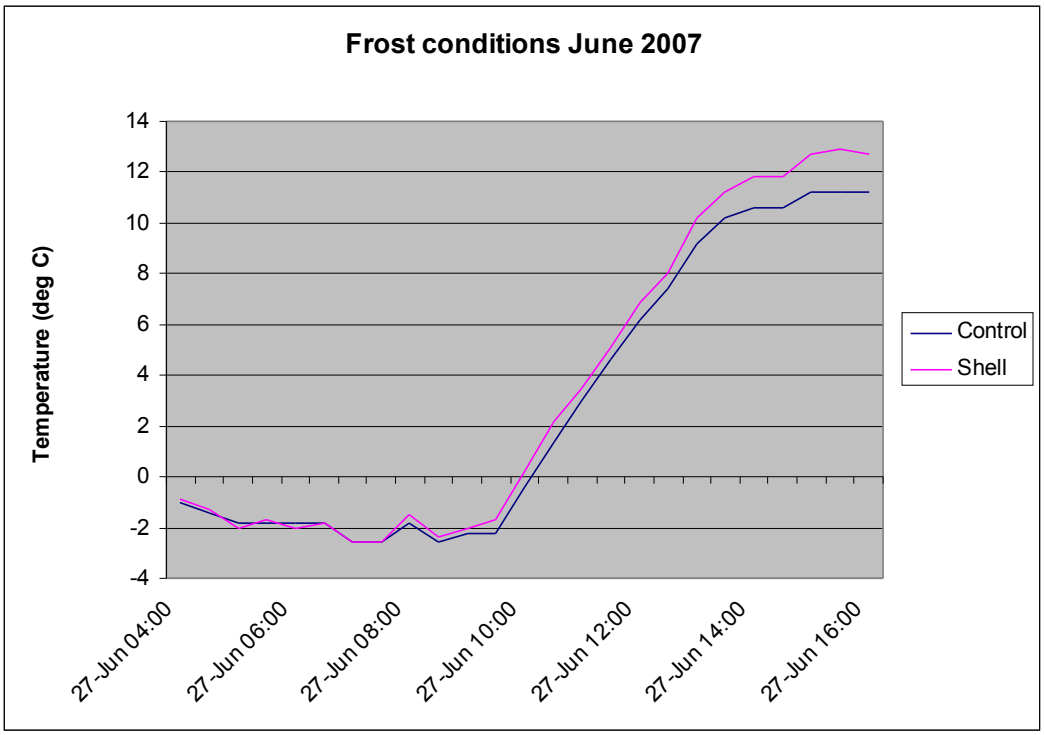

Figure 3. Canopy temperature under frost condition (26-27th June 2007).

Table 2 gives an overview of heat accumulation in the canopy for key points of the 2006-2007 season. In each stage, the temperature in the Shells area is greater than in the Control, though by relatively small, but consistent, margins (5% during flowering to harvest and veraison to harvest, and 7% during early shoot growth to harvest). It is highly probable that even this slight boost in the temperature environment results in some change in fruit development.

Table 2. Temperature accumulation in the fruiting zone, expressed as growing degree days for different periods.

	Growing degree days (°C)		
Treatment	100% flowering to harvest[1]	100% veraison to harvest[2]	5th leaves unfolded to harvest[3]
Control	934	209	1183
Shell	981	219	1262

[1] 13th December 2006 to 31st March 2007
[2] 5th to 31st March 2007
[3] 2nd November 2006 to 31st March 2007

Berry temperatures were measured in March 2004 in an attempt to look at actual fruit temperature as opposed to the temperature of the air in the fruiting zone. Small thermocouples were placed inside ripening berries, which retained turgidity for a few days after insertion. The results from these measurements are quite variable, but indicate that the shells may also be having an effect on individual berry temperature (Figure 4). Whether this is due to light reflection from the mulch or infrared radiation from the mulch is not directly known, though ground surface temperature measurements would seem to indicate the latter (see Soil section).

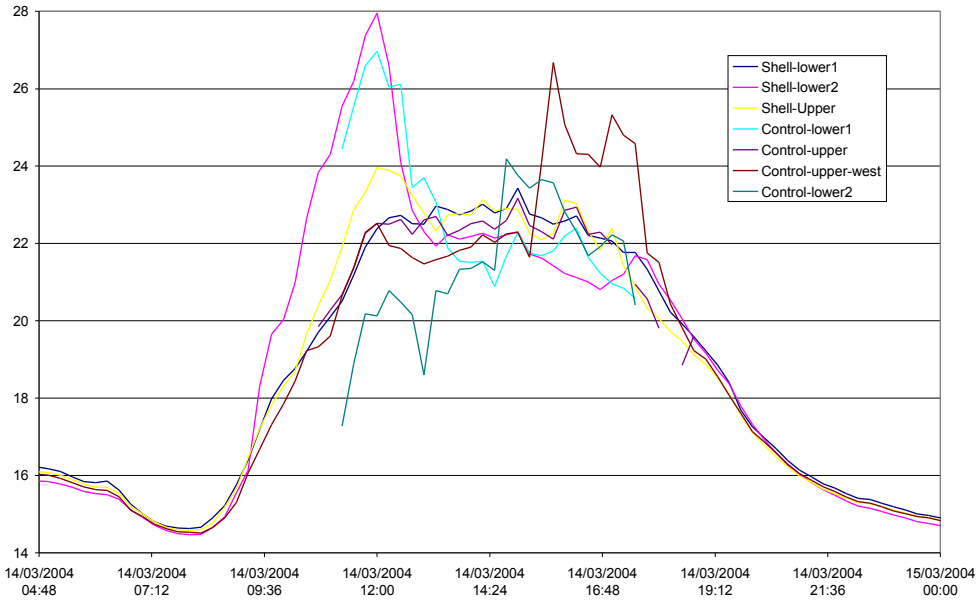

Figure 4. Examples of berry temperatures in the field under Shells and Mulch treatments on the 14th of March, 2004. Each line represents data from a single berry in a cluster. "Lower" in the legend indicates that the berry was largely facing the ground, and Upper that it was facing the sky. the Shell Lower 2 berry was catching some of the morning sun, while the Control Upper West berry was intercepting the afternoon sun. It should be noted that berry temperatures are generally higher over the shell mulch.

The overall effect of the use of shell mulch in the vineyard is a small, but consistent increase in canopy temperature for most seasons. It is likely that these changes result in alterations to fruit ripening, and are at least partly responsible for the sensory differences seen in the wines (see Sensory section).

It is suspected, but not known, that the shells create a warmer microclimate because one side of the shell is darker coloured than the other, leading to more heat absorption and then re-radiation. Visual inspection of the shells shows that they tend to bleach with time, meaning that the shells are whiter several years after they were first laid. Measurements taken in this trial are not detailed enough to pick up any differences this may have made to canopy temperature.

Point Quadrat

It was thought that use of the shell mulch would have an effect on soil moisture and then vine vigour. There is also the possibilty that the changed light environment could alter shoot growth. To this end, Point Quadrat analyses of the canopies in each treatment were completed to better characterise the canopies. Across the seasons that there are data for, there are few differences between the treatments. Leaf layer number, % interior leaves and % interior clusters are all very close, with no statistically significant differences between them. In % canopy gaps there is an inconsistency in the response with in 04-05 the Control being higher than Shells and the opposite in the other two seasons. Overall, however, there is little to distinguish between the canopies of vines in these treatments, which shows the presence of the shells has minimal impact on this important production variable.

Table 3. Comparison of Point Quadrat assessment for three seasons in the trial area. Standard errors are indicated in parentheses.

Year/Date	Treatment	Percent gaps	Leaf layer number	Percent interior leaves	Percent interior clusters
2005 Jan18	Control	3.1 (0.2)	3.1 (2.1)	40 (9.6)	86 (6.6)
	Shells	1.6 (0.2)	3.3 (2.1)	43 (9.6)	86 (6.6)
2005 Dec21	Control	17.2 (2.5)	1.1 (0.0)	4 (1.2)	9 (5.9)
	Shells	17.8 (7.6)	1.3 (0.2)	9 (1.5)	24 (11.3)
2006 Dec05	Control	7.3 (1.8)	1.9 (0.1)	23 (2.0)	34 (2.8)
	Shells	9.7 (2.7)	2.0 (0.1)	24 (2.3)	37 (8.1)

Shoot lengths

Shoot lengths were also measured, to see if there was an effect of mulch on early season vine growth. Prior research using methods of changing soil temperature found a reduction in shoot length as a result of cooling the soil (Creasy et al. 2002), which could be a disadvantage for wine production. Table 3 shows again that there are relatively small differences between the treatments. Shoots in the Shell treatment area were statistically longer than Control in 2004, but shorter in 2006. However these differences are small relative to the length of the shoot (13% and 3% in 2004 and 2006, respectively, though the measurements were made at different times in the season). Although there are not more data to confirm this, it is possible that early season shoot growth is slightly faster in the Shells treatment, but as the season progresses, the Control shoots catch up.

Table 4. Vine shoot length (cm) in the trial area for the 2004-2005 and 2006-2007 seasons.

Date	Control	Shells	Std error
2004 Nov01	41.4	46.9	1.36
2006 Nov27	89.2	86.4	0.99

The number of nodes on each shoot were recorded in 2006, but showed no differences between treatments (data not shown), which matches the results expected from the shoot length measurements.

To see the effect of shoot thinning on the canopies, shoot numbers were recorded pre and post-shoot thinning, as performed by the Neudorf vineyard staff. Importantly for the interpretation of data from the trial, thinning was applied evenly across the treatments (data not shown) and resulted in a significant decrease in shoot density (falling from approximately 27 shoots per vine pre- to 15 shoots per vine post-). This uniform application of vine management means that there should be no unequal impact of it on the treatment responses.

SPAD

SPAD is an acronym for Soil Plant Analytical Device that was coined by Minolta for use with meter that measured leaf greeness. SPAD values have been correlated with leaf chlorophyll content and photosynthetic capacity of the leaf tissue (Candolfi-Vasconcelos *et al.*, 1994). It has been measured here to see if the changed light and radiation environment that the mulch provides has any effect on potential vine productivity.

Significant differences were found in the SPAD data for most dates of measurement. Leaves in the Shell treatment were more green than in the Control in the 04-05 and 06-07seasons, but the post-harvest measurement in 2005 and the early season measurement in 2005 and 2006 were the reverse (Table 5). The latter corresponds with data collected from other trials, where leaves can be more yellow in the early season, which may be caused by UV radiation (Creasy and Bizet, unpublished data). In the case of the shells, it seems the yellowing effect is slightly increased early season, but then leaves are more green later in the season. This could be due to the increased UV radiation and light reflected back to the vines from the mulch (see Radiation and Light section).

The post harvest treatment difference, though showing the Shells as having higher SPAD values, is not significant in the 04-05 season, but this may be due to the fact that the leaves were measured more than two weeks following harvest.

Table 5. SPAD values for the trial at different times of the season in 2004-2005 and 2006-2007."nsd" indicates no significant difference at p=0.05.

Date	Treatment	SPAD value	se
2005 Feb19	Control	41.5	0.43
	Shells	44.9	0.32
2005 Mar23	Control	38.5	0.28
	Shells	41.0	0.31
2005Apr23	Control	27.7	0.46
	Shells	28.6	0.43
2005Nov23	Control	36.3	nsd
	Shells	35.9	
2006 Nov01	Control	24.8	0.26
	Shells	23.3	0.26
2007 Feb17	Control	38.1	0.23
	Shells	40.6	0.19
2007 Mar27	Control	34.1	0.44
	Shells	38.1	0.44
2007 Apr05	Control	27.8	0.51
	Shells	34.7	0.33

Radiation: UV and light

It is obvious on a sunny day, when in the trial area, that the shells do reflect a lot of additional light back up from the ground. This project attempted to quantify some aspects of this changed environment.

Because UV radiation can cause changes to plant growth and development (Barnes *et al.*, 1990), both visible light and UV being reflected back up off the surface of the ground were measured. Visible light was quantified using a sensor that measured photosynthetically active radiation (PAR) and UV radiation with sensors that were sensitive to UVA and UVB radiation (UVA radiation has less energy than UVB and has the potential to cause less damage to cells).

Figure 5 indicates the amount of UV-B radiation that is reflected off the ground's surface in both treatments through a daytime period. It is very clear that the shells are having a significant effect on the radiation environment in the vineyard for most of the daylight hours.

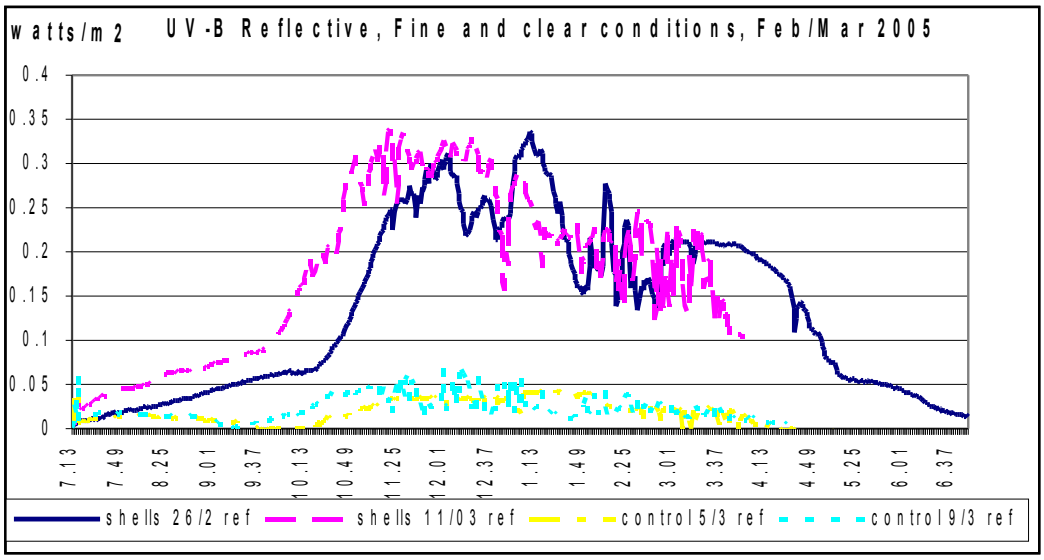

Figure 5. Reflected UVB radiation in Control and Shells treatment areas. Radiation measured at canopy height. Fine and clear conditions in February and March 2005.

The amount of reflected radiation over the incident at canopy height is changed under both sunny and cloudy conditions as well (Figure 6), with Shells redirecting a higher percentage of the incident radiation back into the canopy when it is overcast. Roughly the same amount of UVA radiation is reflected as UVB.

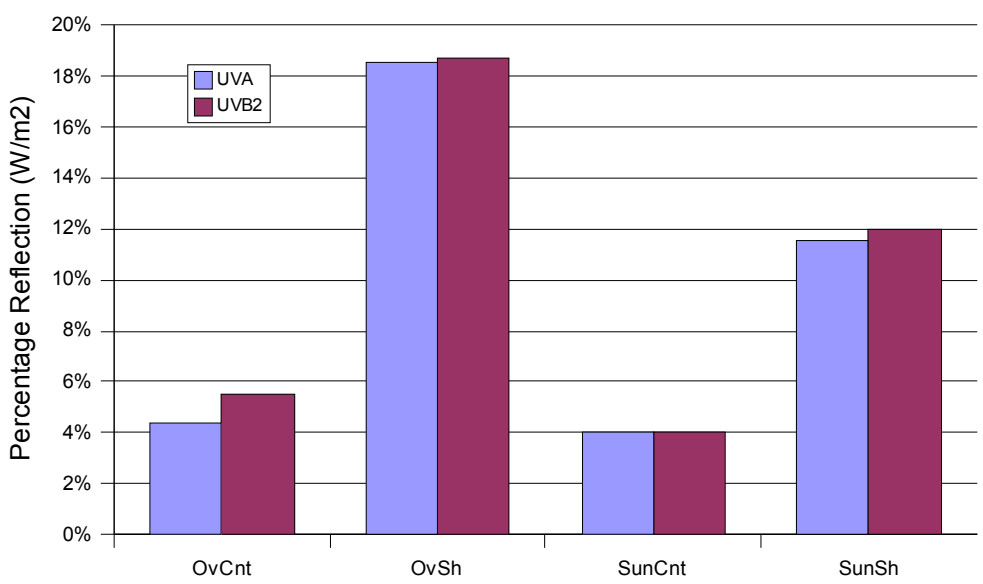

Figure 6. Percentage of UVA and UVB radiation reflected (coming back from ground versus incident) in the cluster zone under overcast (Ov) and sunny (Sun) conditions in the Control (Cnt) and Chells (Sh) treatments. Both UVA (lower energy) and UVB (higher energy) behave similarly. Data taken on January 23, 2006

In March 2007 measurements were made of the reflected energy in the trial area, this time including PAR. Though there is a small amount of radiation being scattered back from bare ground, the amount in the shells area is consistently greater, and across the spectrum of wavelengths measured (Figure 7). Higher energy wavelengths were not reflected as efficiently, shown by the lower values for UVB than PAR or UVA.

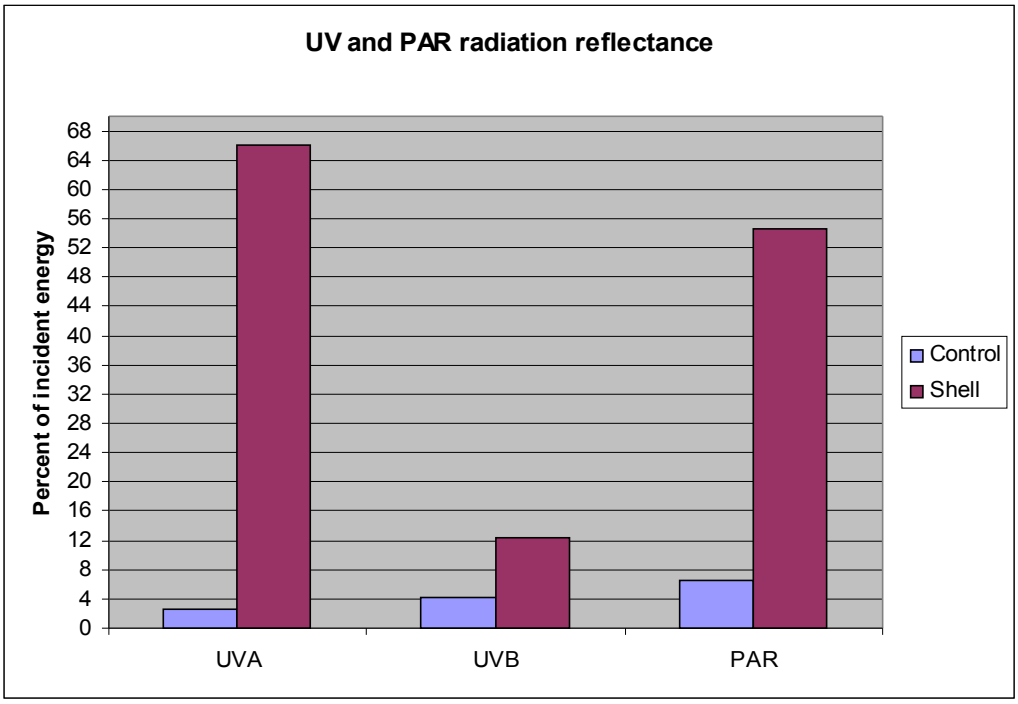

Figure 7. Percentage of reflectance of the UV and PAR radiation at ripening stage on 2007, under sunny conditions.

There is little doubt that the Shells treatment is affecting both the light and UV radiation environment for the grapevines and fruit. Increased PAR can result in greater photosynthetic efficiency for the vine under both sunny and cloudy conditions, as light is reflected back up and onto leaf surfaces not normally getting high levels of sunlight. Increased light and UV radiations can have an influence on phenolic compounds generated in the leaves and fruit, and change other fruit components, such as those responsible for flavour (Winter, 2002).

Pruning data

The vines in the trial area have maintained their pruning weights per vine during the trial (Table 6). The fruit to pruning weight ratio (Ravaz Index), however has varied through the seasons, probably due to the 2004-2005 and 2006-2007 seasons having light crops due to poor weather at fruit set. There are relatively small differences between pruning weights in the two treatments, and the Ravaz Index and number of nodes retained at pruning are also reasonably close. Overall this indicates that the Shells treatment is not having a large effect on vine growth (though perhaps a small increase in pruning weight), though when the yield component data are discussed, it does seem to have an effect on the Ravaz Index due to alterations to fruit set in 2006-2007.

Table 6. Pruning weight comparisons in the trial area.

Year	Treatment	Avg pruning wt/vine (kg)	Ravaz Index	Avg nodes retained/vine
2004	Control	1.12	2.12	17.9
	Shells	1.20	2.24	17.6
2005	Control	1.92	1.64	13.7
	Shells	2.11	1.68	13.2
2006	Control	1.13	2.74	26.3
	Shells	1.28	2.34	26.4
2007	Control	1.64	0.89	na
	Shells	1.56	0.60	na

Internode lengths and lateral shoot production

The frequency of node and lateral production on shoots may be affected by changes to the light and UV radiation environment (Barnes *et al.*, 1990). This could have follow-on effects on canopy microclimate, so in order to evaluate this possible effect, the internode lengths were measured on dormant canes in each treatment. Table 7 shows data from 2006 and 2007, which indicate that the shells were having an effect in both seasons, but in opposite directions. In 2005-2006 the Control shoots had longer internodes than in the Shell treatment shoots while the opposite was true in 2007. It is possible that there is a crop load effect on internode length, as the crop in both treatments was high in 2006, and much lower in 2007, with Shells vines having lower crops than those in the Control (see Yield Components section). The very low level of crop in 2007 may have resulted in more vine energy going into shoot growth in the Shells vines, which could be elucidated by looking at internode lengths along the canes.

Table 7. Average internode length in two seasons from 20 shoots collected randomly post pruning.

	Average internode length (cm)	
	2006	2007
Control	8.2	6.7
Shell	6.9	7.2
p-value	<0.001	0.04
sed	0.25	0.24

If a lower than Control crop load was contributing to the longer internode lengths in the Shells treatment, then it could appear as a difference in relative internode lengths along the shoot. Table 8 investigates this possibility and shows that in the 2005-2006 season the internode length up to the 10th node position was always longer in the Control shoots when compared to the Shell treatment shoots. In 2006-2007 this was true early in the season, for the first two internode positions, but then the Shells treatment shoots had longer internodes from the 3rd to the 10th positions. It is unlikely that crop is having a significant effect on internode lengths at these positions on the shoot, so there is likely some other reason for the differences in internode length between the treatments. What this is remains unknown.

Table 8. Internode length versus position along the shoot in two seasons. n=20 in 2006 and n=18 in 2007

		Internode length (cm) at node position								
Year	Treatment	1	2	3	...	7	8	9	p-value	sed
2006	Control	4	6.3	8.1		10.5	10.2	10.5	<0.001	0.61
	Shell	3.2	5.5	7.9		8.6	8.3	8		
2007	Control	3.5	5.6	7.5		7.9	7.9	7.9	0.04	0.58
	Shell	2.9	5	8.1		10.2	8.3	8.7		

Lateral production on shoots was accomplished by counting the number of developed and developing lateral shoots on the vines and then later by measuring their lengths. There were no significant differences in lateral counts between treatments in November 2004 (data not shown) or in early December 2006 (Table 9). In 2006, however, more detailed information regarding the laterals arising from each of the first node positions was recorded, and it can be seen that relatively few arise out of the most basal node on the shoots.

Table 9 Number of lateral shoots per vine arising from the basal four node positions. Data recorded pre leaf plucking in early December 2006 (n= 30; 95% confidence interval).

	Lateral shoot / vine			
	Control		Shell	
Node position	Average	Conf ±	Average	Conf ±
1	2.3	0.62	3.1	0.74
2	11.0	0.93	10.7	0.90
3	12.6	0.74	11.7	0.77
4	12.8	0.75	12.4	0.96

Lateral shoot length was also recorded in early December 2006, again showing no significant differences between treatments, but demonstrating that the further the node from the base, not only is there more likely to be an active lateral shoot, but it will also be longer (Table 10).

Table 10. Length (expressed in cm) of lateral shoots arising from the basal four node positions of shoots. Data taken pre leaf plucking from 30th November to 5th December 2006. Confidence interval at 95%.

	Average lateral shoot length (cm)			
	Control		Shell	
Node position	Average	Conf ±	Average	Conf ±-
1	0.4	0.21	1.0	0.50
2	5.0	0.95	5.9	0.99
3	8.5	1.50	9.6	1.35
4	10.4	1.70	10.2	1.21

From the data collected it appears that use of the Shell mulch does not affect these vigour-related aspects of vine growth.

Trunk circumferences

Trunk circumferences, being an integrative measure of woody plant productivity (Strong and Azarenko, 2000; Heazlewood *et al*., 2006), were measured on two dates, November 2006 and September 2007, from 90 and 30 vines per treatment respectively. It was predicted that if the mulch was encouraging vine growth through increased water availability, nutrient availability or some other means, there would develop, with time, a difference in the capacity of the vines, which could be measured through differences in the trunk circumference. However, no significant differences were found between treatments at either date (Table 11).

Because the data were taken one year apart, it may also have been possible to distinguish between the treatments in terms of the rate of increase in trunk girth. Again, though there was a slight difference in the rate of increase, it was not a statistically significant one (0.95cm for Control (sed=0.18), and 0.85cm in the Shell area (sed=0.07).

Table 11. Trunk circumference data collected in November 2006 and September 2007. 95% confidence interval indicated.

	Nov 2006		Sep 2007	
	Control	Shells	Control	Shells
Mean	10.04	10.11	11.05	10.92
Std dev	1.29	1.06	1.35	1.17
n	90	89	30	30
Conf int ±	0.27	0.22	0.48	0.42

This is more evidence that the shell mulch is not having an effect on the vegetative growth of the vine, which means there is no significant effect on vigour.

Petiole sampling

Because of the nature of the shells and their proximity to the roots, nutrient testing of the vines was performed three years after the mulch had been laid down.

Nutrient values were different in both types of leaf tissues tested: petioles and blades (Table 12). While N and S were higher in blade samples compared to petiole tissues, the levels of Mg were higher in petiole than in blade samples. Although no statistical data are available from these analyses, it is possible identify some trends. In comparing petiole samples in both treatments, N concentration was higher in the Shell treatment. The table also indicates that Ca levels were higher and Mn lower in the Shell area compared to the Control. In leaf blades, Ca, Mn and Zn were higher in the Shell treatment leaves and Cu lower. The values for Mn and Cu are quite high, and this could be due to application of fungicides that contain these metal ions, or that the time of season the blades were collected does not match up with the usual timing, which is at veraison: as a result, the levels at flowering may not be representative of those found at colour change.

Table 13. Nutrient analyses of blade and petiole samples collected from opposite the basal cluster. Ten leaves with petioles were taken per row at the end of November 2006 (during flowering) and combined before analysis, as according to Hill Laboratories guidelines. No statistical data are available. Medium range of nutrient values supplied by Hill Laboratories.

Element	Unit	Shell Blades	Control Blades	Medium Range	Shell petioles	Control petioles	Medium Range
Nitrogen	%	2.80	2.50	2.8-3.4	1.20	1.10	0.8-1.5
Phosphorus	%	0.31	0.26	0.22-0.35	0.24	0.22	0.18-0.45
Potassium	%	0.80	0.80	1.1-1.5	0.80	0.90	1.7-2.7
Sulphur	%	0.40	0.35	0.30-0.50	0.18	0.18	0.13-0.25
Calcium	%	2.19	1.58	1.2-2.0	2.17	1.45	1.3-2.1
Magnesium	%	0.29	0.30	0.2-0.4	0.53	0.55	0.30-0.60
Sodium	%	0.02	0.02	0-0.1	0.03	0.02	0-0.15
Iron	(mg/Kg)	74	81	40-150	27	26	20-50
Manganese	(mg/Kg)	750	640	40-200	140	210	25-140
Zinc	(mg/Kg)	860	610	25-80	120	140	25-60
Copper	(mg/Kg)	8	26	6-12	11	14	5-20
Boron	(mg/Kg)	126	112	28-45	57	44	28-40
Molybdenum	(mg/Kg)	0.16	0.13	0.15-0.50	0.05	0.04	
Nitrate-N	(mg/Kg)	<100	<100	500-2000	1060	270	400-1600

Leaf HPLC

Because changes to the vine environment have occurred with use of the mulch, phenolic compounds in the leaves were analysed by HPLC to determine if the constituents of the leaves had been altered. Particularly of interest was the flavonol content of the leaves, as with the increased UV radiation coming from the mulch, it was expected that the vine would increase the production of these compounds, which are thought to help the plant screen out the harmful radiation (Price *et al.*, 1995).

Leaf blade samples at flowering (7th December 2006) and post-veraison (6th March 2007) were analyzed by HPLC (Keller *et al.*, 2000). Similar concentrations of the flavonol rutin were found in Shell and Control treatments at flowering, with values of 161 and 170 mg/L respectively ($p=0.41$; sed= 9.98), with no differences found by comparing peak areas of other phenolics contained in leaves from both areas at flowering and post-veraison. However, areas obtained from leaves collected at flowering were then log transformed (base 10) prior to statistical analysis, as this compensates for the large magnitude of peak area differences in the data.

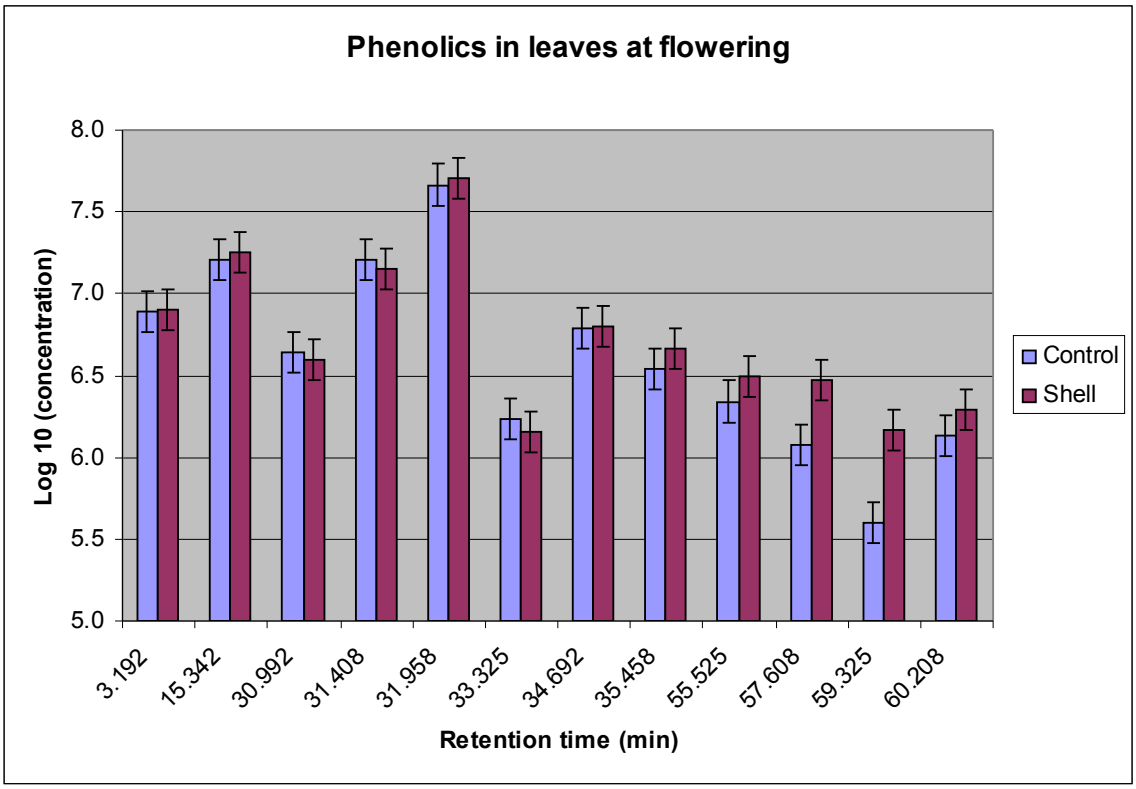

Figure 8. Phenolics in leaves at flowering (7th December 2006). Mean logs 10 of the areas are statistically significant at p<0.05 between treatments, but not at p<0.001 for all of compounds. Error bars are standard errors of treatments means (n=6).

As can be seen in Figure 8, several unknown phenolic compounds defined by their retention times varied slightly in comparing both treatments. However, the compounds with retention times of 57.6 and 59.3 minutes were the most different between treatments, both being greater in Shell wines when they are compared through the overall standard errors (sed=0.13).

These results indicate that increases of UV radiation and light early in the season modify phenolic content in leaves, though it was not possible in this study to identify the individual compounds. Ambient (versus reduced) UV increased the concentration of flavonols (quercetin-glycoside derivates) in leaves collected at mid-season, but had no effect on hydroxy-cinnamic acids (caffeyl and p-coumaryl tartaric acids) (Keller and Torrez-Martinez, 2004), using the same analysis method described by Keller *et al*. (2000). In addition, higher UV levels and low nitrogen stimulated accumulation of leaf flavonols, which are located mainly in the epidermis and cuticular wax, acting as a sunscreen for plant tissues (Jansen *et al*., 1998). Another study (Kolb *et al*., 2003) used different light regimes provided by foils exhibiting different UV transmission and found biosynthesis of flavonols in grapevine leaves was enhanced by UV-B radiation, whereas high visible radiation stimulated accumulation of hydroxy-cinnamic acids. Therefore elevated levels of both of these compounds would be expected in the Shell area.

Interestingly, there was no effect of UV and light on phenolic contents when leaves were sampled after veraison. Under field conditions, flavonol compounds such as quercetin were detected in leaves of *Vitis labruscana* collected at ripening (Park and Cha, 2003). According to these results it could be assumed that UV and light had no effect on these compounds, but further research by using HPLC-MS could clarify these differences and determine if the vines in the Shell area are investing energy into the production of flavonols.

Soil

The effect of laying down the shells on soil temperature was consistent across all seasons. While soil temperature was decreased by its use, there was significantly less variation in soil temperature at 10cm depth (see Figure 9 for a representative example). This could be beneficial to root growth, as the surface roots would not be subjected to excessively high temperatures that could inhibit growth (Kuhns *et al.*, 1985).

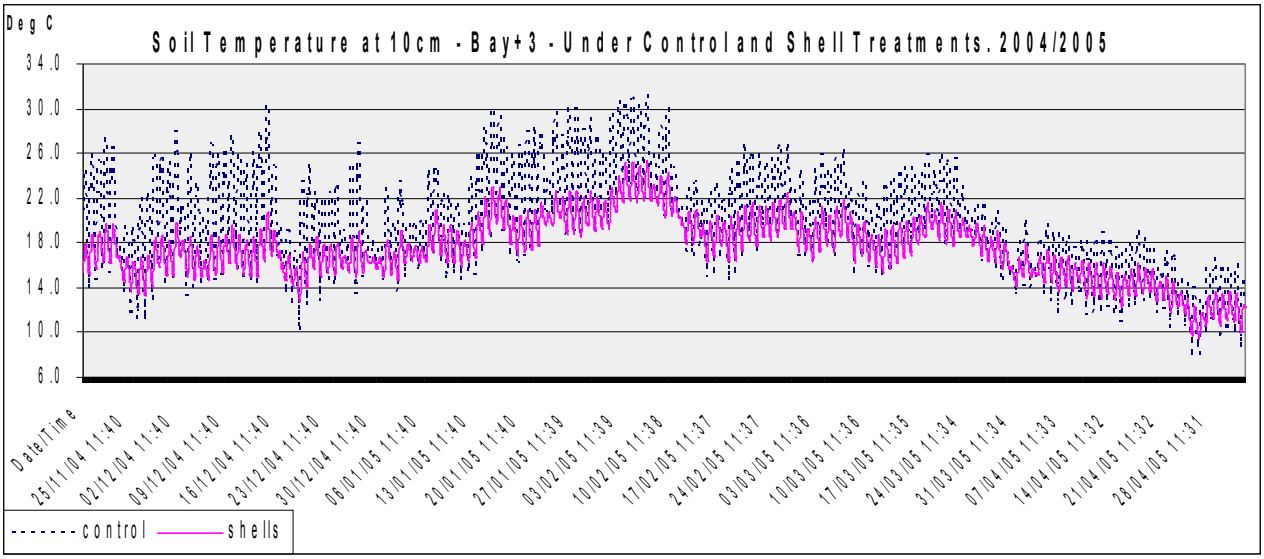

Figure 9. Absolute soil temperatures at 10cm depth in the 2004-05 season.

Because of this effect on the soil temperature, the differences between treatments were at times, quite high. Figure 10 shows that the at times the Control soil was up to 4°C warmer in the spring, which also carried on into January (Figure 11). However, as the ripening season progressed, the difference between Control and Shells decreased, becoming quite similar by harvest.

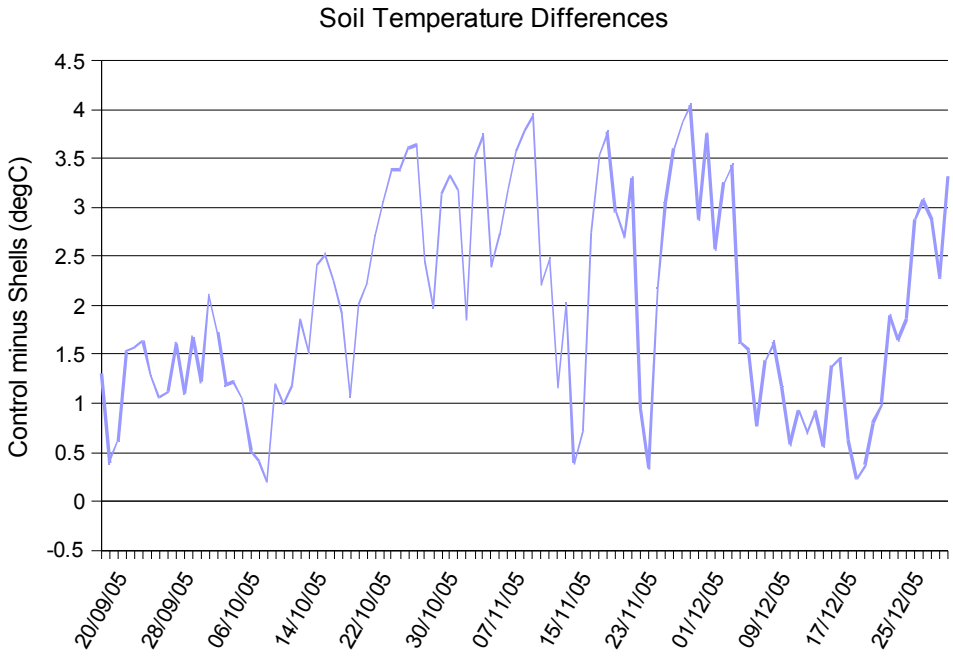

Figure 10. Differences of soil temperature (Control minus Shell values) from September to December 2005

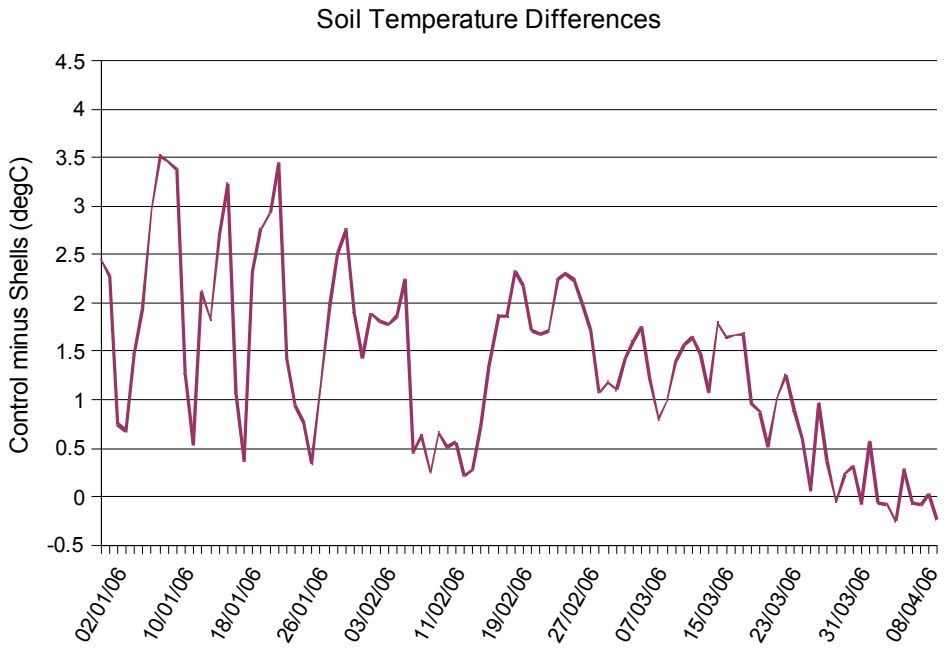

Figure 11. Differences of soil temperature (Control minus Shell values) from January to April 2006.

Looking over an average 24 hour period in the late season, 2007, it can be seen that variation in the Control soil temperature was from about 16°C to 21°C, and that soil in the Shells treatment was slightly warmer than the Control until about 1pm, and then was cooler for the rest of the day, until about 1am (Figure 12).

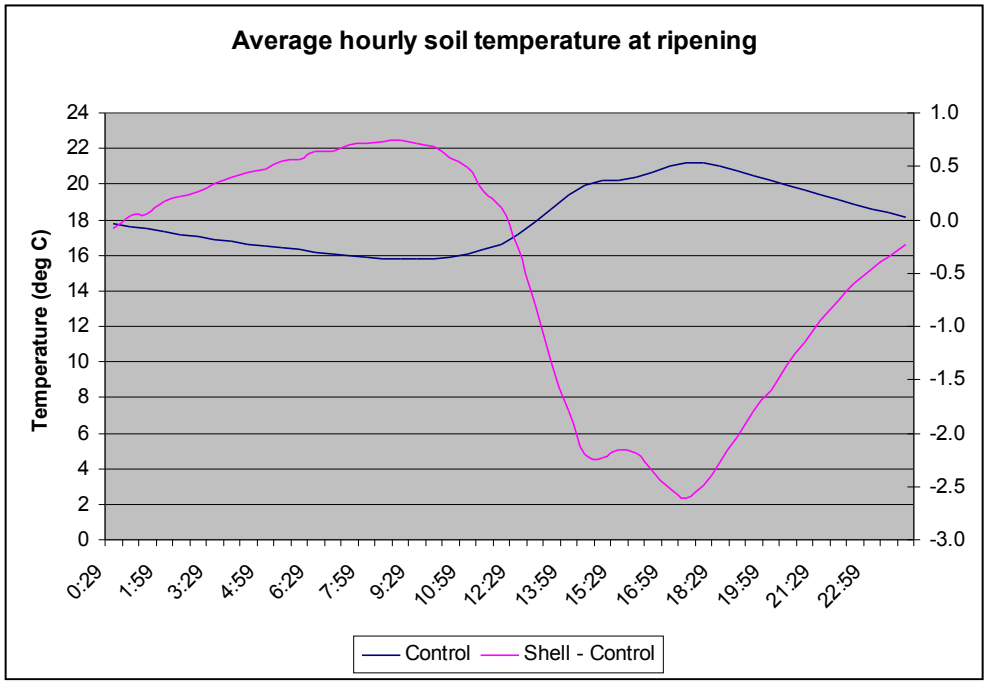

Figure 12. Average hourly soil temperature during ripening stage, from March 16 through 31, 2007.

To gauge the effect of the mulch on the surface temperature of the ground cover, an infrared thermometer was used. This indicated that at the height of summer (Jan 26, 2005), the surface of the mulch was about 8°C warmer than the surface of the bare ground (Control 24.4°C; Shells 32.5°C). This provides additional

evidence that the shells are radiating quite a bit of extra heat, which makes its way up into the canopy. This effect would be most pronounced on less windy days, as the heat would be better retained in the vineyard.

Moisture

It was expected that the mulch would help to retain soil moisture, both from the standpoint of decreasing weed development (see later section) and preventing evaporation from the soil surface. Measurements made with gypsum blocks verified this, showing that the upper profile of the soil in the Control area had increasing soil water tension as the season progressed (Figure 13). Note that these blocks were installed in late March, 2005, so measurements taken in this time period may not be entirely accurate due to incomplete contact between the soil and the gypsum blocks, even though 188mm of rain had fallen in the area shortly before they were installed. These measurements were made in the upper part of the trial block, which had less soil-available water in it than the bottom. Treatment differences were not so pronounced in the lower part of the slope, due to the greater amount of soil moisture available.

Similar measurements made in the spring of 2005 also demonstrated that the soil in the Shells area was under less water stress than the Control area (Figure 14). Despite this, there was little difference in terms of canopy density and vigour measurements in the trial (see earlier sections).

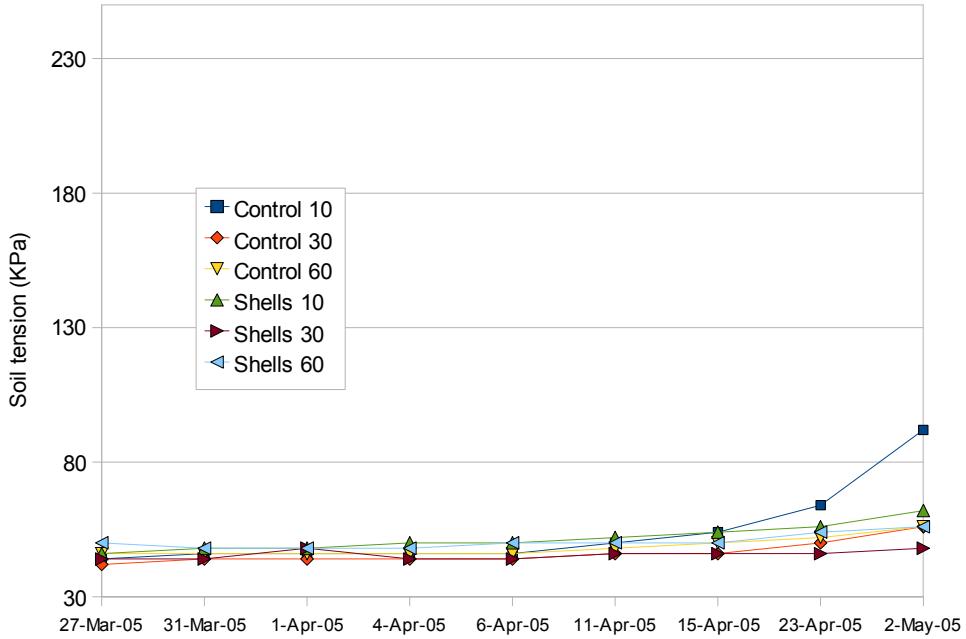

Figure 13. Soil moisture in the trial area at three depths in both treatments, 27 March 2005 to 5 May 2005

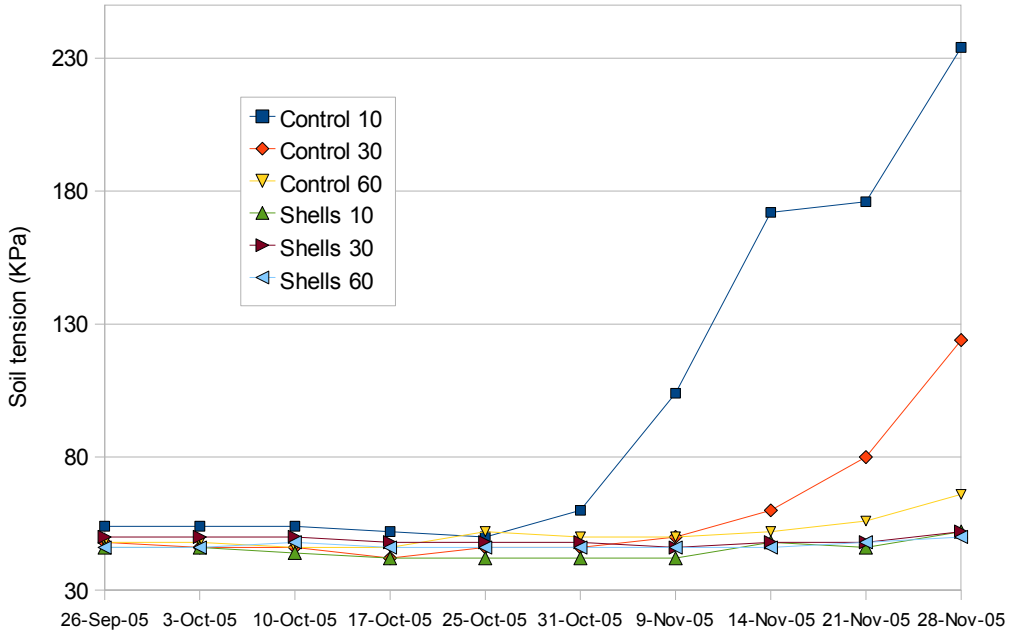

Figure 14. Soil moisture in the trial area at three depths in both treatments, September to November 2005

Given the changed soil environment, a preliminary investigation of vine roots and mycorrhizal colonisation was done. A total of one metre of 5-10 mm diameter roots were taken from 4 vines in each treatment at a depth of 30-40 cm in the soil. Table 13 shows the results of this sampling and demonstrates that the Shells treatment was resulting in more fine root growth, potential nematode damage and less mycorrhizal development than in the Control. The increased numbers of fine roots in the soil profile is beneficial for vine growth, and given the increased soil moisture in the Shells area, more shallow roots would be encouraged. While mycorrhiza are beneficial for plant growth and development, slightly lower levels of colonisation in the Shells treatment is not necessarily a bad sign. Mycorrhizal development is very much dependent on soil nutritional factors, and if the vines are not lacking them (particularly phosphorous), then mycorrhizal associations will not develop (Karagiannidis and Nikolaou, 1999). While soil phosphorous did not appear to be different between treatments (see below), other soil-related factors may influence mycorrhizal development and vine health.

Table 13 Mycorrhizal colonisation of root samples, July 2005. Data from a report to Neudorf Vineyards from Agconsult, Waihi, NZ.

Trtmt	Control	Shells
Sample wt. (g), washed Total root (% fine roots)	25 (~15%)	30 (~35%)
Root branches/g (fine roots)	~100	~160
Active white root tips % of fine roots	~5%	0
Nematode root damage	None	Very slight
% EM (ectomycorrhizal colonisation)	40% reasonably well developed	13% very thin
% VAM (vesicular Arbusclar Myc. Col)	67.00%	47.00%
Comments	Not many medium roots. Fine roots mostly emerging directly from thicker roots.	Very nice root system with good balance of medium and fine roots.

Soil nutrients

Soil samples were sent for nutritional analysis in the late winter of 2004 and 2005. At the first sampling, in 2004, the shells had been on the ground for just under a year. After this amount of time, there were some considerable differences between the treatments (Figure 15). The pH was increased slightly under the mulch, and there was an overall higher concentration of soluble salts. Nitrogen was also increased with use of Shells, which suggests that there is more microbial activity in that soil. Calcium and sodium appear higher as well, which may follow from addition of the high calcium carbonate in the shells and any salt-water residue that could have washed off of them. Given the slight increase in pH, a significant decrease in soil potassium was unexpected, however, it is possible that the increase in calcium in the soil has led to fewer sites for potassium to be associated with, leading to the decrease. The decreases in magnesium, iron, manganese, zinc and aluminium in the Shells treatment soil could be due, in part, to the slight increase in soil pH, which makes these nutrients less available.

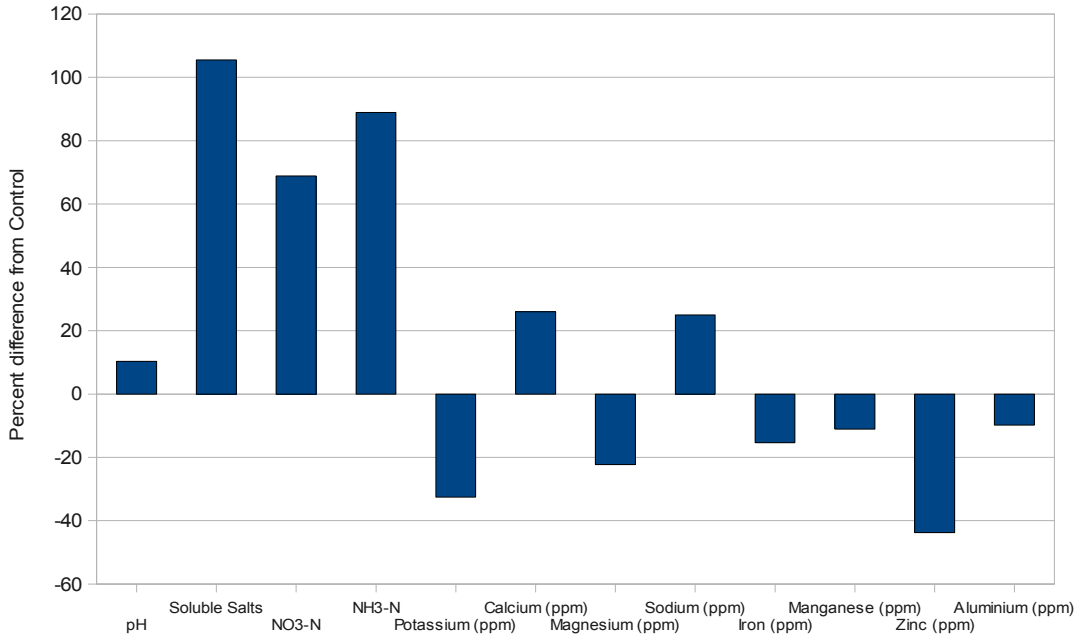

Figure 15. Soil analyses of samples taken in August, 2004. Data are shown as the percentage difference of Shells to the Control sample. Soil plugs were taken from 0-15cm into the soil, with 27 or 28 samples combined from each treatment.

For the 2005 soil test the overall picture is similar, though because the detail in procedure for collecting the soil samples was different (no surface soil was taken for this year), direct comparison from one year to the next is not advisable. pH was slightly higher in the Shells treatment area, as were concentrations of N, Ca, and Na. Magnesium was found to be slightly higher in the Shells area in this year, contrary to what was found in 2004, possibly due to the decrease in K in this part of the soil profile. Changes to Fe, Mn, Zn and Al were again possibily due to the slight increase in pH or competition from other nutrients that had been made more available.

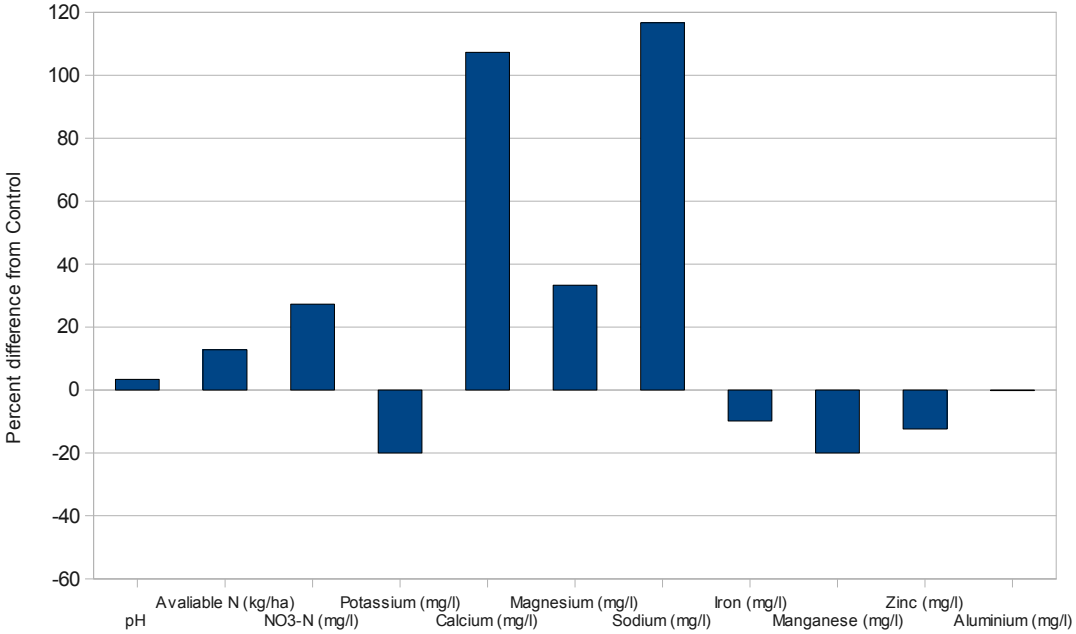

Figure 16. Soil analysis of samples taken in July, 2005. Data are shown as a percentage difference of Shells to Control. Soil taken from a 5-15 cm depth.

Leaf blade and petiole nutrient content could be related to soil nutrients. Nitrate N tended to be higher in leaf petioles from the Shells area, which mirris what was found in the soil. Leaf blade Ca, Mn and Zn concentrations were higher in the Shell area whereas in the soil Mn and Zn tended to be lower. In the petioles the findings for Ca were the same, but those for Mn and Zn were the opposite to leaf blades, the latter matching findings for the soil, where the Shells resulted in a decrease in their concentrations.

There is little doubt that the use of shells is resulting in changes to soil nutrients, but because the effects of the mulch may take many years to be seen these data should only be interpreted as preliminary. Further samples should be taken and analysed in the future.

Weed development

Another benefit of the use of some mulches is reduced weed growth. Therefore, weeds were assessed between 16th and 23rd November 2006 taking into consideration the numbers and area (expressed in cm^2) covered for each weed type. Overall, the mean total area covered per bay was higher in the Control than Shell treatment (36,432 cm^2 and 31,026 cm^2, respectively). However, the mean weed number per bay was higher in the Shell than Control treatment (146 and 114, respectively). It was also noted that clover coming from the inter-row area encroached more in the Control than Shells treatment. Thus, weeds were classified according to their growing class in annual, perennial and those that do not fit logically into either category.

Table 14. Total weed number according to classification.

	Weed number			
	Annual	A-P	Perennial	Total
Control	511	80	92	683
Shell	654	50	172	876
Total	1165	130	264	

The majority of weeds were classified as annuals in both areas, but the number was higher in the Shell treatment (Table 14). On the other hand, the total area covered for annual weeds was similar between treatments and perennial weeds in Shell treatment covered the double area compared to the Control (Table 15).

Table 15. Total area covered by weeds according to their class. Clover is not included.

	Area covered (cm²)			
	Annual	A-P	Perennial	Total
Control	47820	3742	11105	62667
Shell	48988	1981	20084	71053
Total	96808	5724	31188	

However, when clover was included in the analysis it was shown that weeds covered a higher area in the Control treatment (218,591 cm² versus 186,157 cm² in Shell area). This indicates that Shell mulch was effective in keeping clover from growing in the area under vines.

Analysing some weeds individually, clover was the more common specie present in both treatments, being higher in the Control area. *Malva* sp. (Mallow) and *Anagallis arvensis* (Scarlet pimpernel), both classified as broad leaf annual weeds, were also found more frequently in the Control area. On the other hand, *Capsella bursa - pastoris* (Shepherd's purse) and *Veronica persica* (Scrambling speedwell) were recorded in greater numbers in the Shell treatment. These results agree with another report where Mallow was ranked as the most frequent weed found in vineyards in Nelson and Marlborough, followed by Fathen, clovers, Redroot and grasses (Dastgheib and Frampton, 2000).

Some weed species were localised only in a particular area (Table 16). Of these, Storksbill (*Erodium cicutarium*) and Annual poa (*Poa annua*) were only found in the Shell area, whereas Narrow-leaved plantain (*Plantago lanceolata*) and Groundsel (*Senecio vulgaris*) were found in the Control treatment.

Table 16. Weeds species localised only in Shell or Control area according to their growing class.

Annual	Perennial	A-P
Shell		
Wild radish	Creeping butter cup	
Storksbill	Indian doab	
Annual poa	Turf speedwell	
Clammy goosefoot	Prostrate amaranth	
Bitter cress		
Control		
Bristle grass	Cat sear	Narrow-leaved plantain
Scotch thistle	Parsley dropwort	Onehunga weed
Bromus		
Annual mouse-ear chickweed		

These results indicate a shift in weed species between treatments, which could have implications on the management of weeds in the longer term. Different weed control methods such as herbicides, cultivation and mulches have been considered as management tools for weeds (Pool *et al*., 1990). Herbicide accumulation in the soil, which could damage vine roots, contaminate irrigation dams and leach into the ground water have been reported as possible long-term problems through the use of herbicides (Lennartz *et al*., 1997). While chemical weed control has showed a trend toward less dependence on residual herbicides such as simazine since 1994 for Nelson area, glyphosate continues as the most common herbicide used in vineyards, followed by amitrole, glufosinate ammonium and paraquat/diquat (Dastgheib and Frampton, 2000).

The chemical weed management at Neudorf Vineyards consisted of glyphosate applied in the middle of September and December depending on the season. Buster (glufosinate ammonium) is sprayed in January. Clovers and Mallow are tolerant to glyphosate and its repeated use has caused an increase of these weeds on the ground (Dastgheib and Frampton, 2000): these two plant species were found in greater amounts in the Control (bare soil) versus the Shell areas.

A proper rotation of herbicides with different modes of action is a key to avoiding a shift in weed composition

or the evolution of resistant weeds. Although no case of resistance has been reported in New Zealand, some species are becoming more tolerant to the most-used herbicides (Dastgheib and Frampton, 2000). In Australia, *Lolium rigidum* (Annual ryegrass) has been reported as being resistant to glyphosate (Powles *et al*., 1998).

However, appropriate ground management practices are essential to develop a sustainable production system (Pimentel *et al*., 1992). Non-chemical weed management practices such as cover crops between rows and even under vines (Tesic *et al.*, 2007; Hostetler *et al.*, 2006), winter grazing and cultivation to increase vineyard soil temperature and reduce the risk of frost were described in this study (Dastgheib and Frampton, 2000). The use of mulching also has contributed to the control of weeds in several studies, demonstrating that its use has other advantages at the same time such as increased soil organic matter, increased earthworm activity, and increased water holding capacity.

In practice, the Neudorf Vineyard staff have found that perhaps one to two fewer herbicide sprays could be used in the Shell block of this trial. Observations were that as the shells aged, there was more weed growth in the trial, so periodic replenishment of the shells may help to keep weed populations down and require less use of chemical controls, though the effect of this practice has not been formally tested.

Yield Components

Use of shells did not show an effect on the observed fruitfulness expressed in the 2006-2007 season as the number of inflorescences or clusters per vine after budbreak, which were around 46 (Table 17). This is contrary to what could be expected due to initiation and differentiation happening in the previous season under a greater light environment, but a consistent finding throughout the study (Crawford, 2006). Flower cluster numbers were similar between treatments after shoot thinning as well (Table 17), and so would not have a direct influence on final yield.

Table 17. Flower cluster per vine in the trial area, November 2006. Confidence interval at 95%.

	Flower cluster			
	Pre shoot thinning		Post shoot thinning	
	Control	Shells	Control	Shells
Overall mean	46.3	46.0	28.2	26.6
Std dev	8.69	9.21	4.94	5.48
n	90	89	90	89
Conf int ±	1.80	1.91	1.02	1.14

The progression of flowering was followed over the trial as well, and in each season the response was the same: a slight advancement of flowering in the Shells area. A graph of this for the 2006-2007 season is shown in Figure 17.

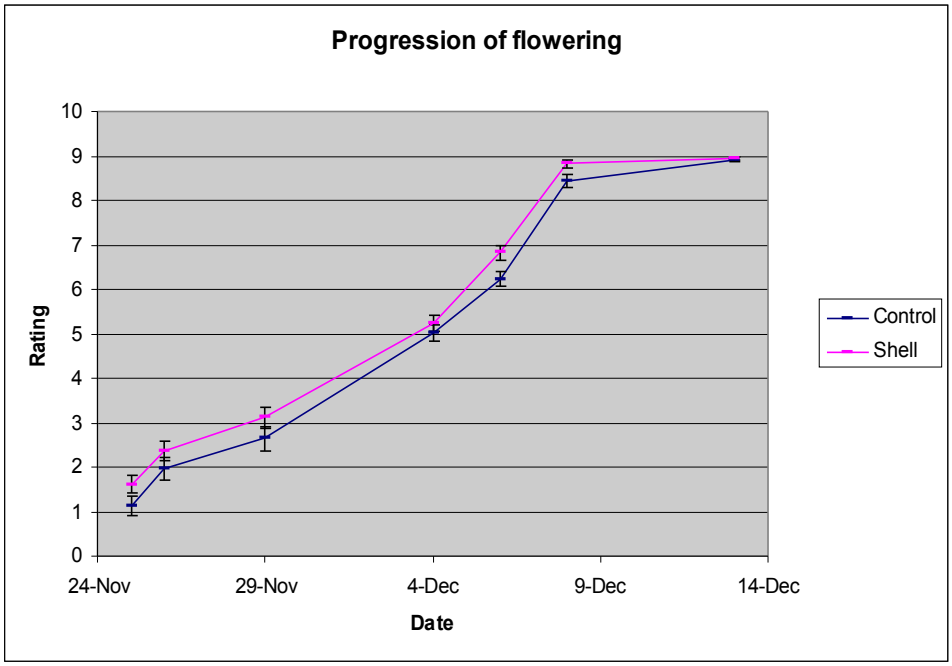

Figure 17. Progression of flowering during 2006/2007 season. Bars indicate standard errors of treatment means (n=90, Control; n=89, Shell).

Prior to bloom, a number of inflorescences were enclosed in a net bag to retain all of the flower caps (a process which has been found not to influence fruit set in grapes (May, 2000), though New Zealand experience may differ (see below)). The bags and contents were removed two weeks before full veraison and frozen at -20ºC. Samples were analysed later, drying bunches, counting shed caps and classifying individual berries according to their sizes.

Table 18 shows that the number of berries per bunch was statistically different between treatments and greater in the shell area. However, the overall percentage fruit set was not different between Shell and Control.

Table 18. Number of flower and berries per bunch, and rate of fruit set in Pinot noir from 72 samples in the 2006/2007 season. Confidence interval at 95%.

	Mean		Conf int ±	
	Control	Shell	Control	Shell
Number of caps	318	361	34.2	28.9
Number of berries	130	180	13.6	26.9
Fruit set (%)	42.6	50.9	4.67	6.75

To better explore the effect of the mulch, the types of berries were also analysed (Table 19). Although the number of larger seedless berries (Seedless A) and seeded berries were similar between treatments, the smaller seedless berries (Seedless B) were different between treatments. The proportion of flowers that developed into smaller seedless berries was greater in bunches coming from the Shell area, accompanied by a reduction in the proportion of flowers that formed seeded berries. This fall would be based in higher number of total berries recorded (Table 18).

Seedless berries were identified through two diameters, A and B (4 to 5mm, and ≤ 3mm, respectively), due to browning during the drying process, which meant they could not be classified by colour. However, as few green berries were seen at veraison assessment and harvest, it was assumed that all of berries smaller than 3mm were coloured and in consequence classified as seedless berries. Hence, fruit set was affected negatively by the reflective mulch due to a lower percentage of seeded berries being set.

Table 19. Number of different type of berries classified during fruit set assessment. Confidence interval at 95%.

		Mean		Conf int ±	
Type of berry	Diameter (mm)	Control	Shell	Control	Shell
Seeded	≥ 6	45.5	38.3	5.3	6.9
Seedless A	4-5	44.6	56.2	6.9	17.7
Seedless B	≤ 3	39.4	85.4	8.9	15.7

Fruit set is strongly influenced by supply of assimilates to the inflorescence during and after anthesis (Coombe, 1973), where sufficient leaf area provide assimilates for fruit set (Keller and Koblet, 1994). This trial did not show any petiole nutrient concentrations outside of the normal ranges at flowering in either treatment (except potassium, which was lower than normal levels in the shell area, but May (2004) cites that there are no studies showing that K has an effect on fruit set). Roubelakis and Kliewer (1976) found that higher light intensity produced a greater proportion of seedless berries in grapes, which could explain the Shell response found in this trial. However, the impact of this on vineyard yields and wine qualities needs to be kept in mind.

The progression of veraison was assessed between 19th February and 7th March during the 2006/2007 season using the visual scoring system (Figure 18). Although the change of colour was monitored starting from about 70% completion according to the rating, grapes in the Shell area were slightly advanced compared to grapes in the Control. The only statistically significant differences in the 2006-2007 season appeared on the three samplings from 26th February. This advance of colour change is a response seen in multiple years of the trial (Crawford, 2006).

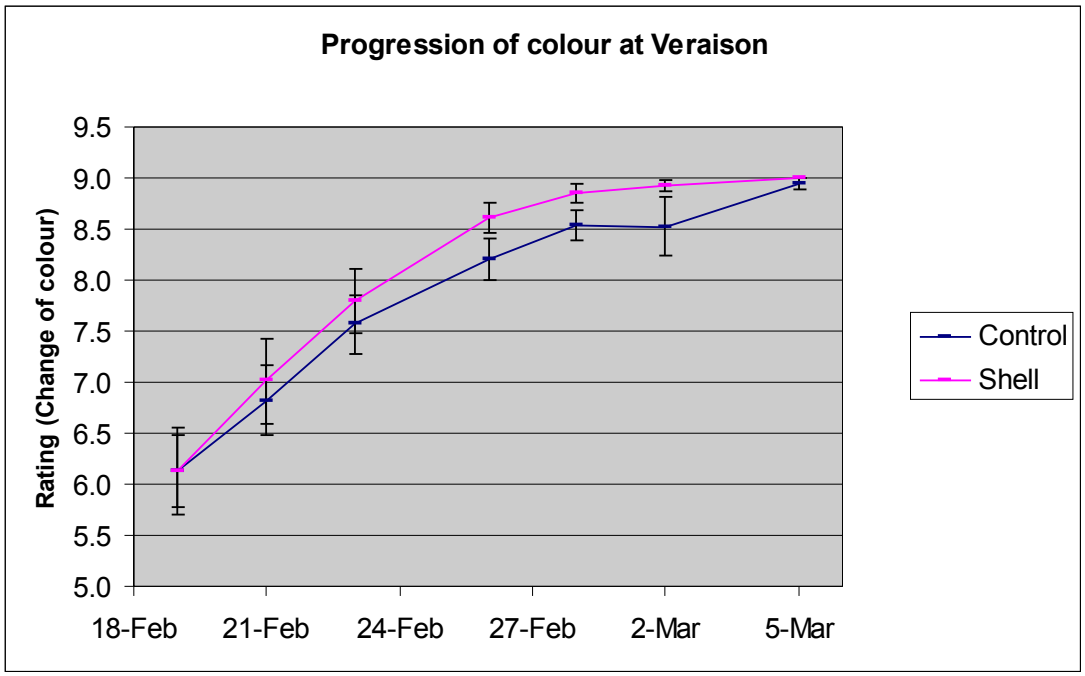

Figure 18. Progression of colour change at veraison in 2007 evaluated with a visual rating from 1 to 9. Bars indicate standard errors of treatment means (n=90, Control; n=89, Shell).

Colour variability within clusters was assessed at veraison in February 2007, visually classifying individual berries into four different degrees of colour: green, pink, red and blue. Figure 19 shows these data and indicates that the majority of berries were classified under the blue colour in both treatments, but there were no statistically significant differences between Shell and Control treatments. These results are in agreement with the values scored in both areas on 21st February when veraison was visually ranked (Figure 18), where no statistically significant differences between treatments were observed. However, it does appear that grapes in the Shell area are developmentally more advanced than in the Control area.

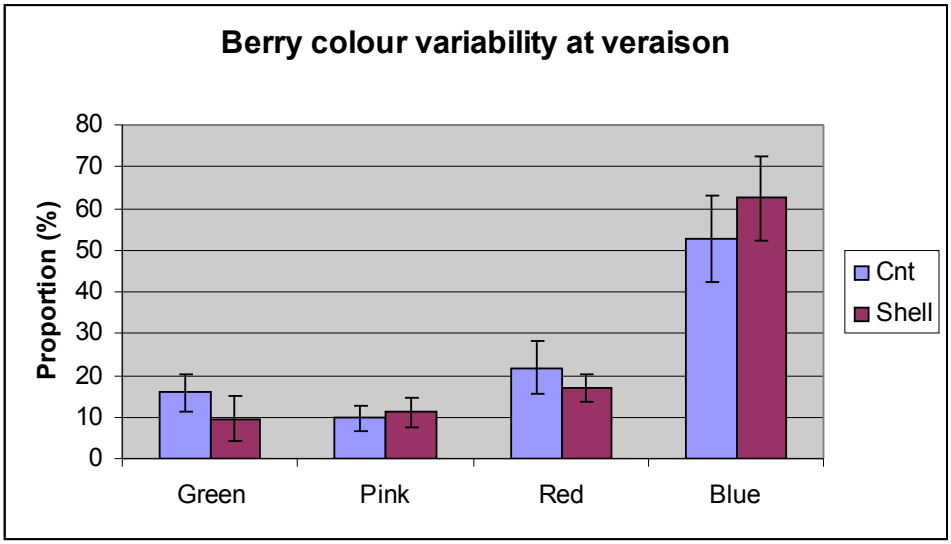

Figure 19. Proportion of berries classified under four categories of colour: green, pink, red and blue at veraison, February 2007.

Crop estimations were performed in the 2004-2005 and 2006-2007 seasons, both of which had relatively low crop loads. In 2005, the forecast for cluster weight at harvest was greater for the Shells area due to slightly higher numbers of clusters per vine and a slightly higher cluster weight (Table 20).

Table 20. Crop estimations for 2005 vintage. Samples of 25 clusters per treatment were taken on 25 February 2005 with harvest on 5 April, 2005. ± indicates 95% confidence interval.

Treatment	Est. No. clusters/ vine	Cluster weight[1] (g)	Est. cluster wt at harvest[2] (g)	Est. Kg/vine
Control	26.3 ± 6.4	63.6 ± 13.4	82.7	2.2
Shells	28.7 ± 4.1	67.3 ± 20.3	87.5	2.5

[1]Subsample of 10 bunches/treatment at veraison
[2]Veraison bunch weight multiplied by a factor of 1.3

In 2007 the Control vines had higher cluster weights than Shells, but fewer berries per cluster (Table 21). This is explained by the fruit set data, which showed that set of seeded (and thus larger) berries was lower in the Shells area (Table 19). Berry numbers in this calculation were slightly different to those measured in the fruit set calculations (Table 18), which may be due to the different populations of clusters selected, different definitions of what a berry was, and/or an effect of the bags themselves. Despite the literature suggesting that use of net bags for determining fruit set does not affect the measurement (May, 2000), it is possible that their use does affect fruit set. One industry person in New Zealand has found that the use of bags may increase fruit set (Larry Morgan, personal communication).

Table 21. Yield components taken at 90% veraison completed (22nd February 2007) from 12 bunches in each treatment. 95% confidence intervals are also indicated.

Treatment		Cluster weight (g)	Berry number
Control	Mean	102.9	91.9
	Confidence Interval ±	10.4	15.8
Shell	Mean	66.2	159.4
	Confidence Interval ±	7	13.9

The 2007 estimate of harvest cluster weight is shown in Table 22, predicting the reverse of what was predicted in 2005: Control vines looked to be carrying a heavier crop than Shell vines.

Table 22. Yield estimation from the trial site, February 2007. Estimate of harvest cluster weight obtained by multiplying the veraison cluster weight by 1.3.

	Est. clusters/vine	Sample cluster wt (g)	Est. harvested cluster wt (g)	Est. Kg/vine
Control	28.2	102.9	133.8	3.8
Shell	26.6	66.2	86.1	2.3

Limited information about harvest 2004 is available. In this vintage cluster weights in the Shell area were higher than in the Control, though no statistics are available from this season.

Table 23. Cluster weights near harvest, 2004.

Treatment	05/Apr/2004[1] (g)
Control	110
Shells	123

[1]Sample size unknown
[2] Shell treatment harvest 13/04/04, Control treatment 14/04/04

When it came to harvest for the microvinifications in 2005, the cluster weight for Shells (Table 24) was much lower than predicted (Table 20). Therefore, the weight per vine was down 0.4kg from the earlier prediction, while that for the Control was slightly higher. This loss of cluster weight appears to be genuine, and not something to do with sampling error or variability within the block. Table 25 shows that the average berry weight in the Shells area decreased by 25% from March 10 whereas in the Control area the berries decreased by 14% from their maximum. A significant amount of berry shrivelling in the Shells area was noted in vintage 2005 (Crawford, 2006).

Table 24. Microvinification bays cluster weights and yield, 5th April 2005. 95% confidence interval indicated by ±.

	mean cluster weight (g)[1]	Crop (kg/vine)[2]
Control	85.9 ± 3.3	2.32 ± 0.16
Shells	72.8 ± 6.3	2.07 ± 0.20

[1]Subsample of 10 bunches/treatment at harvest
[2]Mean per vine from total harvest weight

Table 25. Mean berry weights from cluster samples taken for maturity monitoring in 2005. 95% confidence interval indicated by ±.

	Control	Shells
3/Mar/2005	0.76 ± 0.5	0.81 ± 0.6
10/Mar/2005	0.84 ± 0.6	0.93 ± 0.5
17/Mar/2005	0.95 ± 0.6	0.90 ± 0.5
24/Mar/2005	0.89 ± 0.6	0.81 ± 0.8
4/Apr/2005	0.82 ± 0.6	0.70 ± 0.6

A very detailed analysis of frozen clusters from the 2006 vintage revealed a slight shift in the berry populations for berry weight and Brix, with the Shells seeming to result in more berries in the large classes and more berries with higher Brix (Figure 20 and 21).

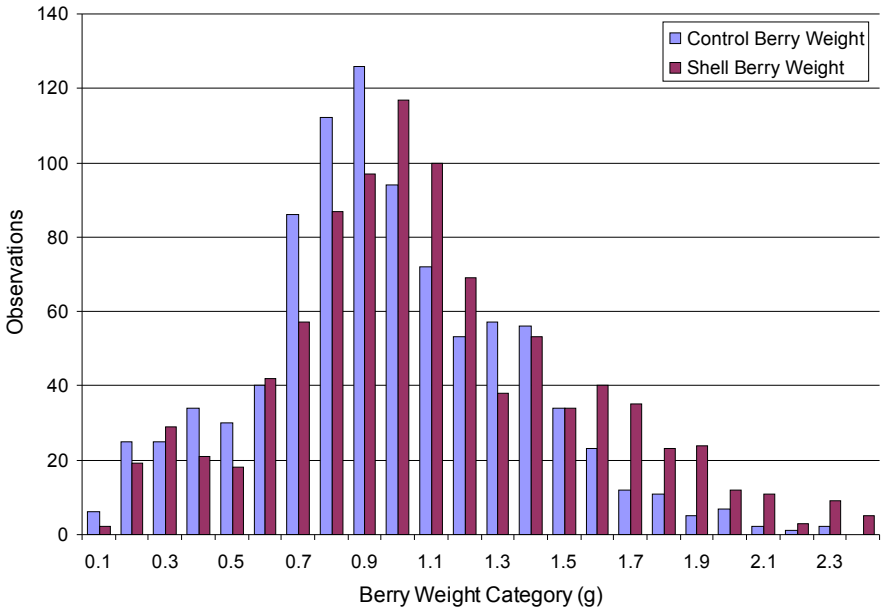

Figure 20. Histograms of berry populations for weight classes in both treatments. 2005-2006 season fruit.

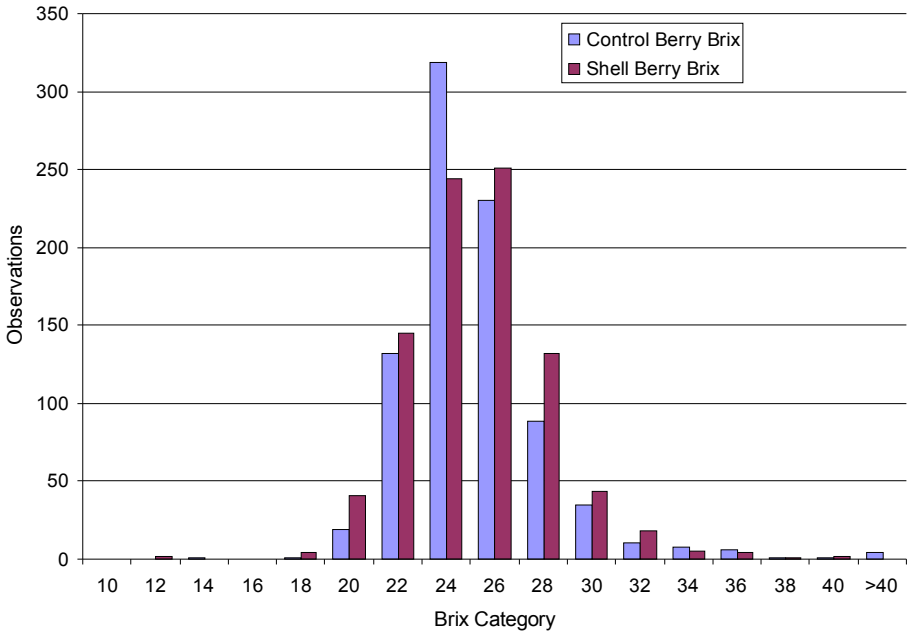

Figure 21. Histograms of berry populations for brix classes in both treatments. 2005-2006 season fruit.

For that 2006 vintage fruit, there was a significant difference in cluster weight between the treatments, with the Shells treatment, as for the 2004 vintage, having higher values than the Control (Table 26). Berry number per cluster was also different between the treatments, but the sample size was not large enough to say that it was statistically different. However, the slightly higher berry number, combined with the significantly larger berry weight contributed to the difference in cluster weights.

Table 26. Cluster weight, berries per cluster and berry weight from the 2006 vintage. Data obtained from 10 clusters taken from each taken from the trial at harvest.

Treatment	Cluster wt (g)	Berry No/ clst	Berry wt (g)
Control	97.2	85.5	0.97
Shells	134.2	96.1	1.10
p-value	0.022	0.230	<0.001

In 2007 yields were the lowest of any season monitored, the Shells clusters getting down to 36g each and Control clusters not being much higher than that (Table 27): much lower than predicted (Table 22). As was found for vintage 2005, the Shells clusters were smaller than those in the Control, the reasons for which has already been discussed in the fruit set section. Again, it appears that there was a loss of berries or shrivelling of berries particularly in the Shells area (Leal, 2007).

Table 27. Yield components gathered from the fruit used for 2007 microvinifications.

	2007	
Average	Control	Shells
[1]Wt/vine (kg)	1.48	0.94
[2]Cluster weight (g)	53	36

[1] Values from average Kg/bay divided by 5 vines
[2] Values from average Kg/vine divided by average cluster #/vine

Considering the trial across the years monitored, it appears that in low yielding years, Shells cause a decrease in harvest cluster weights, while in higher cropping years the opposite is true. Why this would be is not clear, although other seasonal changes (*e.g.* rainfall, wind, specific periods of heat etc.) could be responsible for the effect. At this point, there is insufficient data to say which.

Fruit composition

Fruit composition during ripening was monitored in the 2003-2004, 2004-2005 and 2006-2007 seasons. In 2004 Shells appeared to ripen more slowly than grapes in the Control, perhaps due to their increased crop load (Table 28). However, titratable acidity tended to be lower and pH slightly higher. This could be attributed to the different microclimate in the Shells area.

Table 28. Pinot noir fruit sampling in 2004.

		05/Apr/2004	10/Apr/2004	18/Apr/2004
Control	Brix	23.9	23.8	26.5
	TA	8.7	na	8.5
	pH	3.18	3.09	3.52
Shells	Brix	23.1	23.4	26.0
	TA	7.0	na	8.0
	pH	3.14	3.21	3.61

In 2005, where the crop load was lower the Brix were very similar between treatments, and though there appeared to be some differences during the ripening period, by the time the grapes were harvested, Brix and TA were identical (Table 29). pH in the Shells grapes was just slightly higher than in the Control.

Table 29. Pinot noir fruit sampling in 2005.

		3-Mar	10-Mar	17-Mar	21-Mar	24-Mar	29-Mar	31-Mar	4-Apr
Control	Brix	16.0	19.4	21.3	22.7	22.4	23.1	22.6	24.1
	TA	15.4	12.4	9.1	9.2	8.5	7.9	7.2	8.2
	pH	2.83	2.94	3.26	3.10	3.24	3.35	3.27	3.38
Shells	Brix	16.4	19.4	21.7	22.7	23.0	23.7	23.0	24.1
	TA	13.8	11.2	8.8	9.5	8.5	8.4	7.5	8.2
	pH	2.88	2.99	3.35	3.09	3.24	3.37	3.32	3.50

In 2007, the situation was similar to that in 2005, but there were more distinct difference in the grape samples at harvest, with Brix and TA being slightly higher than the Control (Table 30). pH was essential the same between treatments.

Table 30. Pinot noir fruit sampling in 2007.

		5-Mar	12-Mar	18-Mar	24-Mar
Control	Brix	19.5	20.9	21.7	23.5
	TA	10.1	8.9	8.4	8.3
	pH	2.89	2.99	3.08	3.11
Shells	Brix	19.7	21.4	22.3	23.8
	TA	10.1	8.4	8.7	8.9
	pH	2.93	3.03	3.02	3.09

In 2005 grape samples from during the ripening period were sent to ETS Labs in California for phenolic profiling analysis. Three samples were sent to represent the ripening phase (3-Mar, 17-Mar and 4-Apr). Figure 22 shows the development of the phenolic profile through those dates with the Shell treatment as a percentage of the Control treatment. It is apparent that the Shell treatment has an effect of the proportion of catechin in the grape samples. This phenomenon is in the order of -8.4% relative to the Control at the beginning of the ripening period to 31.5% at the end of the period. This was also observed with epicatechin (-18.1% on Mar 03 to 21.1% on Apr 04).

Catechin and epicatechin are flavan-3-ols and are found primarily in the seeds and stems of grape clusters. Studies have shown that the amount of these polyphenols extracted declined with berry maturity and that these less mature seeds have both higher catechin levels and that the catechin is more easily extracted when compared to more mature seeds (Czochanska *et al*., 1979; Romeyer *et al*., 1986). This has significance for the resulting wines as the concentration and amount of the bitter and astringent tannin may be increased. Interestingly, the percentage in the wines is lower in the Shell treatment (-6.1% catechin, -14.8% epicatechin). This is possibly similar to the 2004 wines, in which the Shell treatment wine was tasted and determined to have less bitterness than the Control wine (See Sensory section). Fermentation practices can affect the amount of catechin extracted, but as the micro-vinification wines were treated the same this cannot be considered an influencing factor in this situation. These results indicate that the Shell treatment is not having a positive effect on the synthesis of catechin and epicatechin in the fruit.

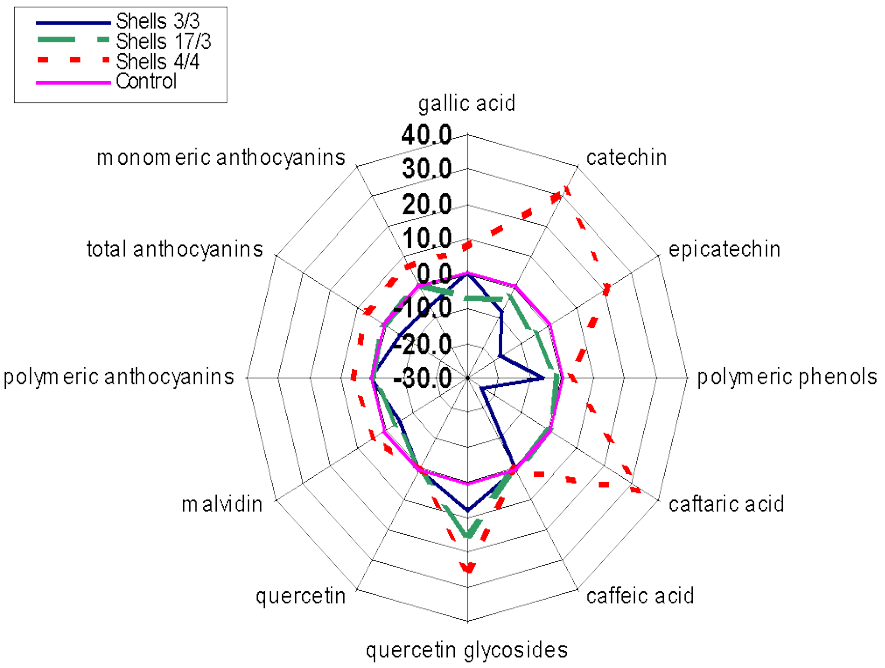

Figure 22. Phenolic profile of the Shell treatment fruit at three dates, shown as a percentage of the Control. Data from analyses done at ETS Laboratories, USA.

Harvest data

Harvest data for the vintages 2004 through 2007 are shown in Table 31. There appears to be little in the way of consistent differences between treatments over this four year period, which has also followed for other compositional parameters measured (see below). Note that the pH and TA values are high and low, respectively, due to frozen samples being analysed.

Table 31. Summary of harvest data for the trial period. Note that data for 2006 vintage were taken from frozen cluster samples, which is most noticeable through the low values for TA and high values for pH.

		18/Apr/2004	04/Apr/2005	14/Mar/2006	24/Mar/2007
Control	Brix	26.5	24.0	24.0	23.5
	TA	8.5	7.9	3.7	8.3
	pH	3.52	3.40	4.67	3.11
Shells	Brix	26.0	23.6	23.9	23.8
	TA	8.0	8.4	3.7	8.9
	pH	3.61	3.41	4.58	3.09

The frozen clusters from vintage 2006 were processed for phenolic determination through spectrophotometry (Iland *et al.,* 2000, Figure 23). Again, there are few differences between mulch and Control. Anthocyanin on a concentration basis was slightly lower in the Shells clusters, which could have been due to the slightly greater berry size which alters the skin area to juice ratio (Table 26). A similar story could explain the slightly lower Total Phenols values for Shell clusters on a concentration basis.

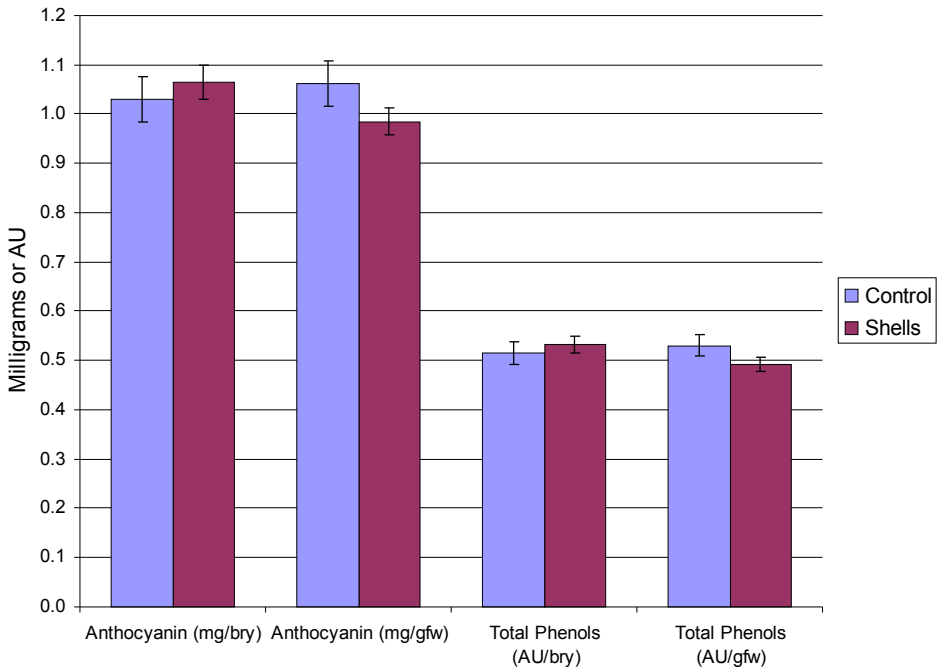

Figure 23. Spectrophotometric evaluation of phenolic extracts from whole cluster samples collected at harvest 2006. Berries were processed according to Iland et al. (2000). Bars indicate standard errors of treatment means (n=10)

All of the berries from the 2006 frozen clusters were examined, leading to a population of 1700+ where individual measurements were made and very high precision in being able to discern a difference. This study found that Brix was not significantly different between the treatments, but malate and anthocyanins were (Table 32). Though only slightly, malic acid concentration was significantly higher in the Shells treatment, as was anthocyanins per berry. The latter was despite a significant difference in anthocyanins per unit skin

area, where the Shells berries had a lower concentration than Control ones. Again, the likely reason for this is the slightly larger berries in the Shell treatment.

Table 32. Analyses of frozen fruit samples from 2005-2006 season - individual berry measurements. n=1745 in total.

	Control	Shells	p-value
°Brix	24.2	24.3	0.645
Malate (g/L)	0.34	0.40	0.042
Anthocyanins (mg/berry)	1.73	1.87	0.051
Anthocyanins (mg/cm2)	0.621	0.600	0.004

Also as part of this detailed examination of berries, it was possible to calculate the contribution of each class of berry weight to the total sugar available in the must. This showed that seedless berries (classified as those less than 0.5g in weight), though having high sugar concentration (28°Brix, data not shown), contributed only 3.5% of the total sugar in Control and only 1.2 % in Shell grapes (Figure 24). On the other hand, large berries (classified as those greater than 2g in weight) contributed 1.1% of the sugar in Control and 6.1 % in Shells.

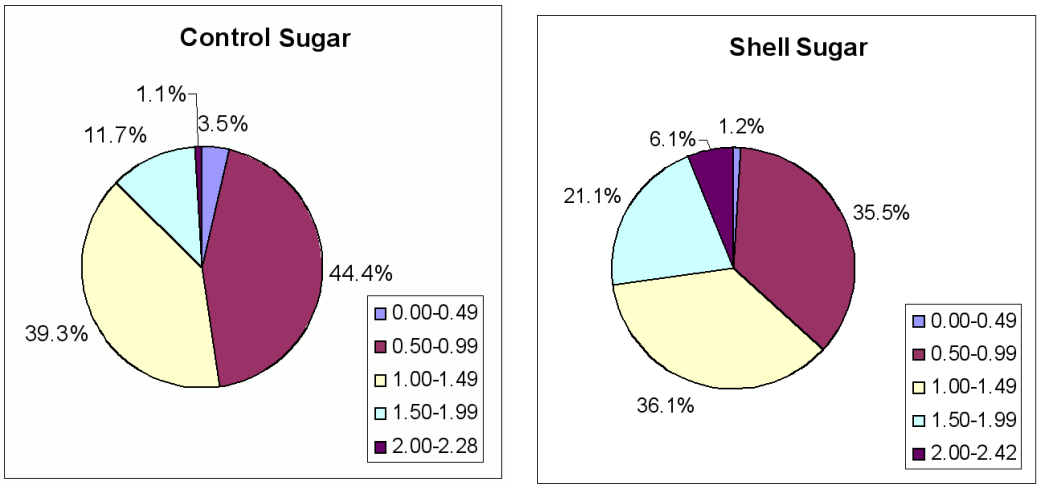

Figure 24. Contribution of each class of berry weight to the total sugar available in the must. 2006 vintage fruit

However, while seedless berries made up 10.7% of the anthocyanins (expressed in mg) for Control and 5.6% for Shell must, big berries contributed 4.4% and 19.6% of the total pigments, respectively (Figure 25).

Smallest and largest berries combined contribute one-quarter of the grape colour in Shell must, being greater than Control and demonstrating the relatively greater impact they have on potential wine colour. Overall, the largest contribution of sugar and anthocyanins came from berries with weights between 0.50 and 0.99g, but the impact of the smallest and largest berries cannot be discounted, considering there appears to be much more anthocyanins in large berries in the Shell treatment compared to the Control.

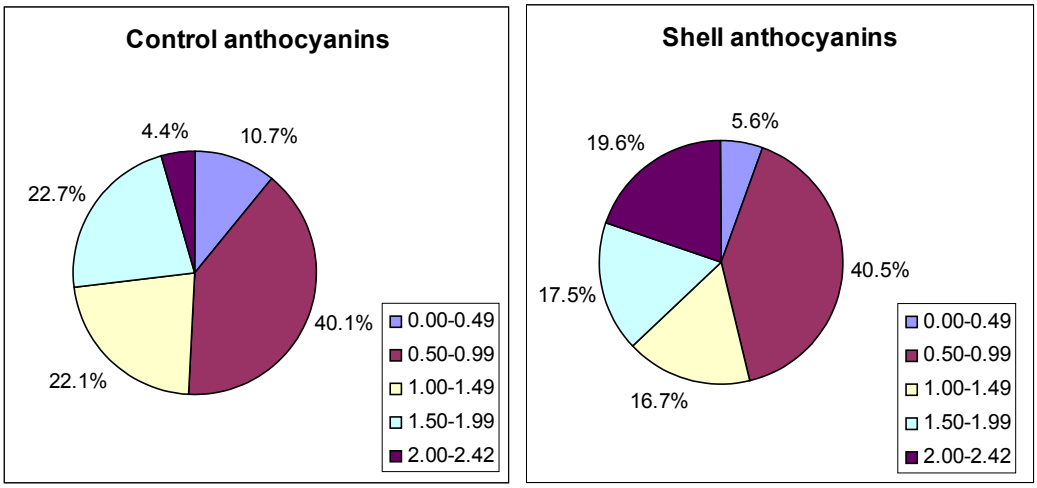

Figure 25. Contribution of each class of berry weight to the total pigments available in the must. 2006 vintage fruit

As another indicator of fruit ripening, the degree of peduncle browning (due to lignification of the tissue) was rated at two dates in the 2007 ripening season. The simple 5-point rating system from 1 (all green) to 5 (brown from shoot to first branch of cluster) was able to distinguish between treatments, with the degree of browning less in the Shells treatment vines than in the Control both at veraison and harvest (Figure 26). This was somewhat unexpected, as it was thought that the increased light environment might have encouraged more rapid maturation of the shoots and cluster stems, but it is possible that the reverse has happened, resulting in a slowing of the process.

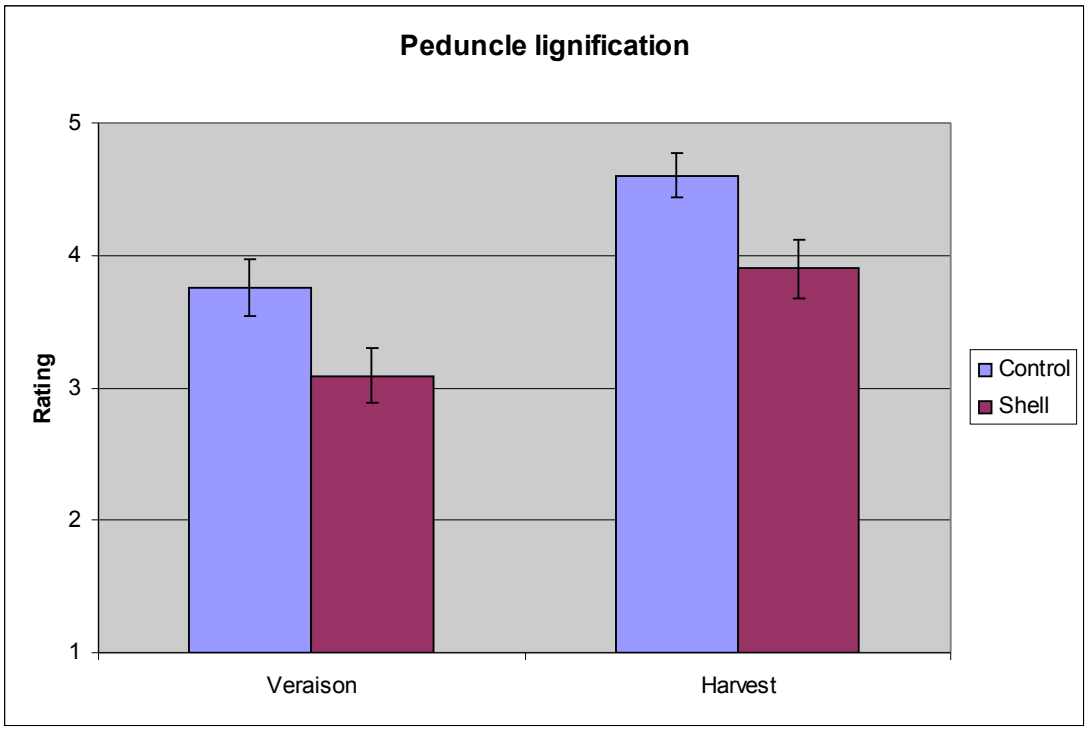

Figure 26. Peduncle lignification progression according to ranking (1, Green; 3, half green/half dark; 5, dark). Bars indicate standard errors of treatment means (n=90, Control; n=89, Shell).

A summary of harvest dates for the trial are presented in Table 33. For the most part, the commercial harvests of the trial have happened on similar dates. However, as confidence grows with working with the mulch, it is expected to be harvested earlier than the Control, despite there being inconsistent differences in Brix, TA or pH.

Table 33. Summary of harvest dates for microvins and commercial wines from the trial area.

Season	Microvins	Commercial Control	Commercial Shells
2003-2004	Apr14	Apr14	Apr13
2004-2005	Apr05	Apr05	Apr05
2005-2006	Mar14	Mar20 (2/3) Mar30 (1/3)	Mar21
2006-2007	Mar27	Mar31	Mar27

Must composition records for the microvins are available for the 2005 and on vintages (Table 34). The largest difference in treatment Brix occurred in the latest season, with Shells being 1.5°Brix higher than the Control. This could be due to the much lower crop loads in the Shell treatment compared to the Control (Table 27). Other than this, there are no notable differences between treatments, with the possible exception of must YAN, but the two years of data for that parameter do not show a trend.

Table 34. Summary of one-day soak measurements for trial musts.

	2005		2006		2007	
	Control	Shells	Control	Shells	Control	Shells
Harvest °Brix	25.4	25.4	23.6	23.9	23.9	25.4
Harvest TA (g/l)	8.0	8.0	8.4	8.0	8.1	8.4
Harvest pH	3.37	3.37	3.18	3.28	3.23	3.23
Harvest malate (g/l)	2.7	2.7	na	na	2.4	2.4
Harvest YAN (ppm)	286	337	na	na	318	281

Wine

Wine pre-bottling analyses indicate, similar to grape analyses, few differences between treatments, and if there are differences, there appear not be any discernible trends one way or the other. It would appear that season has a much larger effect on the wine composition than the treatments.

Table 35. Pre-bottling wine analyses for microvinification wines. Alcohol percentage is on a volume basis. Values for a 95% confidence interval are given where available.

Vintage	Treatment	pH	Conf. Interval	TA (g/L)	Conf. Interval	Alcohol (%)	Conf. Interval
2005	Control	3.17	na	7.95	na	12.2	na
	Shells	3.21	na	7.65	na	12.4	na
2006	Control	3.46	0.12	5.80	0.23	13.5	0.11
	Shell	3.64	0.13	5.50	0.07	13.4	0.11
2007	Control	3.52	0.06	6.29	0.24	13.9	0.07
	Shell	3.46	0.05	6.63	0.10	14.6	0.20

Spectrophotometric

One way of trying to quantify phenolics in wines is through the use of spectrophotometry, and since it was thought the changed growing environment would have an effect on grape phenolics, these measurements were done on several of the wines made. Table 36 shows these data for the 2004 vintage wines, with the analyses done in December of that year. Again there are only quite small differences in the wines both at

native and standardised pH.

Table 36. Spectrophotometric data for 2004 microvinification wines. Wine analyses completed December 2004.

Native pH						
Tom's Block	Density	Hue	Degree red pigment colouration	SO_2 resistant pigment	Total red pigment	Total phenolics
Control	3.05	0.86	16.85	0.75	9.76	25.56
Shells	2.98	0.85	16.15	0.73	9.97	25.32
Adjusted pH						
Tom's Block	Density	Hue	Degree red pigment colouration	SO_2 resistant pigment		
Control	4.35	0.62	27.56	0.78		
Shells	4.19	0.61	26.03	0.74		

An analysis of these same wines one year later show that Colour Density has increased, there are slight increases in the Hue value, the Degree of Red Pigment Colouration has increased dramatically as has the SO_2 resistant pigment (Table 37). The Total Red Pigment has decreased and the Total Phenolics has doubled. Within the limitations of using the spectrophotometric methods to quantify phenolics, this is what would be expected of ageing wines, where there are more polymeric pigments and fewer monomeric pigments left. Adjusted wine pH values have also changed with the Colour Density falling in the Control, the Hue and SO_2 Resistant Pigment increasing and the Degree of Red Pigment Colouration falling considerably.

Treatment differences become more evident with the older wine, with the Shells wines at native pH showing lower values for Colour Density, SO_2 Resistant Pigments, Total Red Pigments and Total Phenolics. From these measurements it is not possible to explain why the Shells wines have behaved this way, which had led investigations to include other methods of measuring phenolics (see later sections).

Table 37. Spectrophotometric data for 2004 commercial wines. Wine analyses completed December 2005.

Tom's Block	Density	Hue	Degree red pigment colouration	SO_2 resistant pigment	Total red pigment	Total phenolics
Control	4.58	0.89	45.3	1.69	5.35	50.14
Shells	3.54	0.93	43.1	1.27	4.24	37.61
Adjusted pH						
Tom's Block	Density	Hue	Degree red pigment colouration	SO_2 resistant pigment		
Control	3.72	0.88	12.1	1.21		
Shells	4.30	0.86	13.0	1.47		

For the 2005 microvinifications tested in December 2005, again there are few differences between the treatments (Table 38). It appears that young wines do not show treatment effects, though as they age, the effects may become more evident.

Table 38. Spectrophotometric data for 2005 microvinification wines. Wine analyses completed December 2005.

Tom's Block	Density	Hue	Degree red pigment colouration	SO_2 resistant pigment	Tot red pigment	Tot phenolics
Control	3.27	0.92	29.5	0.95	5.76	27.82
Shells	3.23	0.91	32.7	0.90	5.15	25.09
Adjusted pH						
Tom's Block	Density	Hue	Degree red pigment colouration	SO_2 resistant pigment		
Control	3.31	0.99	8.60	0.88		
Shells	3.10	0.90	8.70	0.78		

The story is similar for the spectrophotometric analysis of the young 2006 vintage wines (Table 39): there do not appear to be any significant differences between the treatments. The absolute values for Total Pigments and Total Phenolics are quite high in this vintage, possibly due to a combination of the season and winemaking techniques used for the microvinifications.

Table 39. Wine spectrophotometric measurements of the 2006 microvinification wines. Wine analyses completed Nov 2006. Confidence intervals at 95% from 3 replicates.

	Control	Conf. Interval	Shells	Conf. Interval
pH	3.54	0.04	3.60	0.02
Wine Colour Density	8.38	0.37	8.19	0.84
Wine Colour Hue	0.64	0.08	0.65	0.03
Degr. Pigment Colour	0.11	0.01	0.11	0.02
SO_2 Resistant Pigm.	1.44	0.09	1.44	0.08
Total Pigments	46	2.9	47	2.1
Total Phenolics	126	10.8	127	4.3

HPLC

High performance liquid chromatography (HPLC) enables the separation and quantification of many phenolic compounds in grape extracts and wines. There are several important compounds in wine that contribute to sensory characteristics or our ability to infer later changes the wines may go through.

ETS Laboratories (St. Helena, California) provide a phenolic profiling service to industry and samples from the 2005 microvinifications were sent there for analysis in December 2005. The Shell wine had slightly higher caffeic acid than the Control, but most of the differences were pointing the other way, with epicatechin, quercetin glycosides, malvidin and total and monomeric anthocyanins being lower by at least 15% in Shells (Figure 27).

Epicatechin (and catechin, which was also slightly lower in Shell wine than Control wine) is a building block of tannins, which are responsible for astringency and bitterness. As free monomers (that is, not tied up into the polymeric tannins) (epi)catechin lends bitterness to wines, so on that basis one would expect that the Shell wines would appear less bitter than the Control wines.

The amount of quercetin was very similar in the two wines, but there was quite a bit less quercetin glycoside in the Shell wine. Normally, there isn't so much of the glycoside of quercetin in wines, as the winemaking process tends to remove the glycoside and leave the aglycone quercetin. There is no ready explanation for this difference, especially as the grape samples from the Shells area showed to have higher quercetin glycoside than in the Control area (Crawford, 2006). Cluster exposure is the primary factor determining quercetin levels in grapes and wine (Price et al., 1995) with greater exposure to solar radiation increasing

concentration (Price et al., 1995; Spayd et al., 2002). With the change of the light and radiation environment caused by the mulch, we would have expected elevated quercetin levels in the Shell wine, which was not the case. More quercetin is generally regarded as beneficial for wine, as it is a copigment for anthocyanins, leading to more colour and colour stability from whatever anthocyanins are in the wine.

Indeed, in these wines, it appeared that the use of the mulch decreased coloured compounds, with lower malvidin (the principal pigment in winegrapes) and total and monomeric anthocyanins. However, polymeric anthocyanins were slightly increased, suggesting that in this treatment there was more and faster association of tannin and anthocyanin monomers, which should lead to more stable colour and potentially more elegant tannins on the palate (Cheynier *et al.*, 2006).

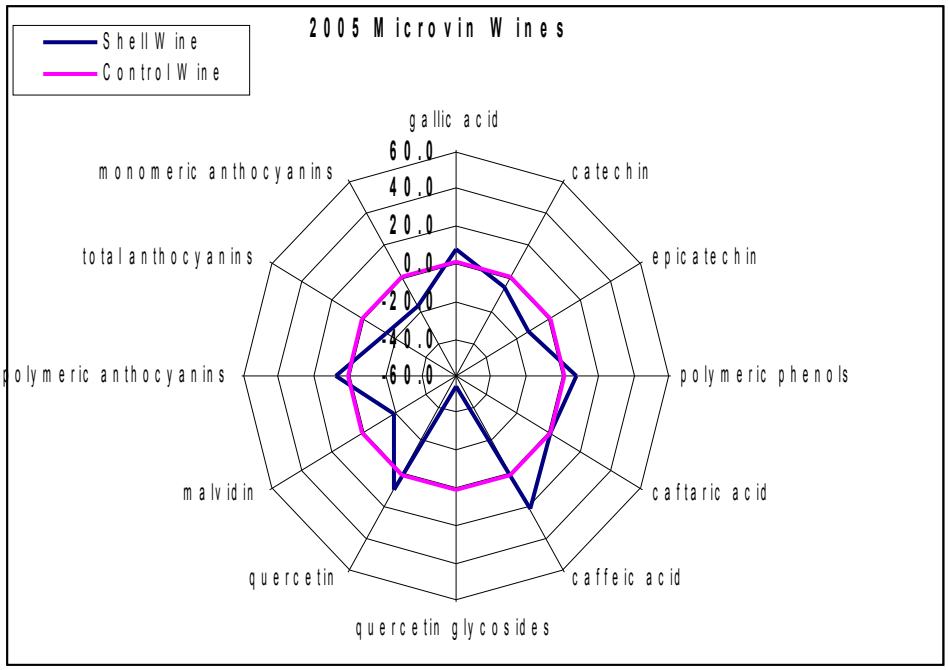

Figure 27. Phenolic profile of the 2005 microvinification wines in December 2005, with the results for the Shell wine presented relative to the Control. Analysis by ETS Laboratories, USA.

With HPLC analyses at Lincoln University a greater number of flavonol compounds could be separated, though not each identified (Figure 28). In addtion, wines from the 2004 and 2005 microvinification vintages were able to be measured. The results show a decrease for some compounds and an increase in others, with the trend being for less flavonol in the 2004 vintage Shell wines and more in the 2005.

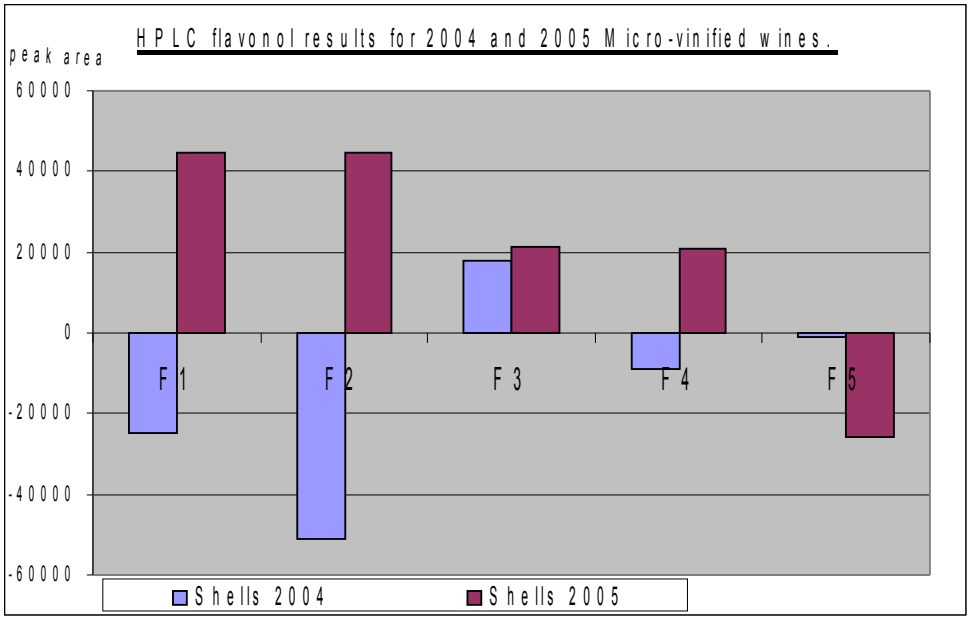

Figure 28. HPLC analyses of 2004 and 2005 microvinification wines showing Shell wine flavonol compound peak areas relative to the Control. Injection volume was the same for each sample so peak areas are directly comparable.

The main anthocyanin peaks were also examined in these HPLC runs, and the peaks were similar in terms of a percentage difference from Control, though that for peak A5 was quite a bit lower for the 2004 wine (Figure 29). This particular peak, however, is minor in size compared to the other peaks, and so there is little practical outcome from this change.

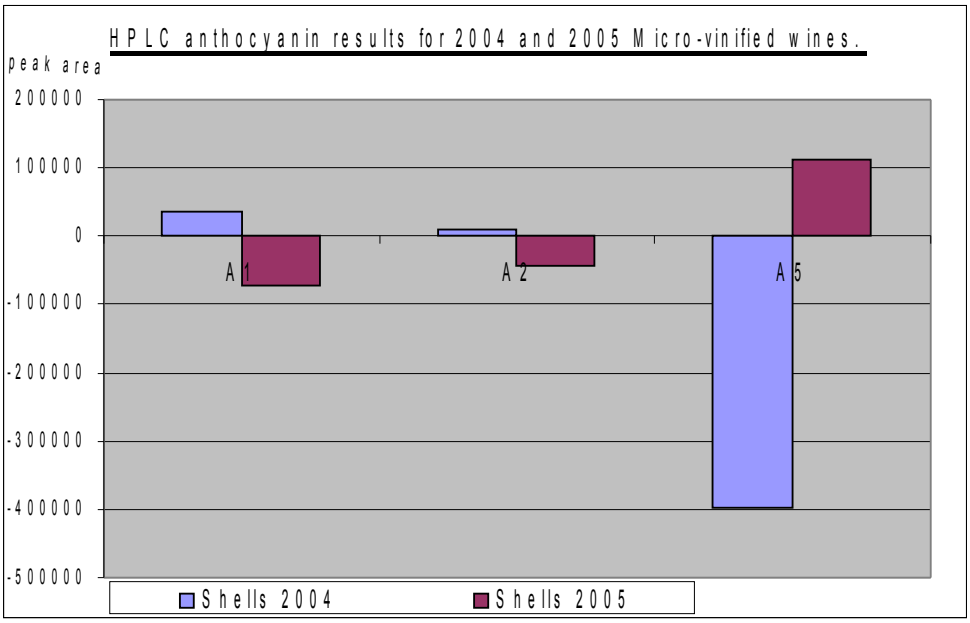

Figure 29. HPLC analyses of 2004 and 2005 microvinification wines showing the three major Shell wine anthocyanin peak areas relative to the Control. Injection volume was the same for each sample so peak areas are directly comparable.

Even with the additional resolution afforded through HPLC analysis there was little difference found between the wines in the 2004 and 2005 vintages. The major difference appeared to be with quercetin glycoside, but there is no rational explanation for this response, although replication of the analysis would confirm if the

difference were real or not.

HPLC analysis of the microvin and commercial wines from vintage 2007 was also done. There were some differences between the treatments in the microvinification wines, with the biggest being for quercetin and resveratrol (Figure 30). Compounds such as caffeic acid, gallic acid, p-coumaric acid and rutin were similar between treatments. Concentrations of epicatechin and catechin were not statistically different between treatments, though the values were slightly greater in Control wines.

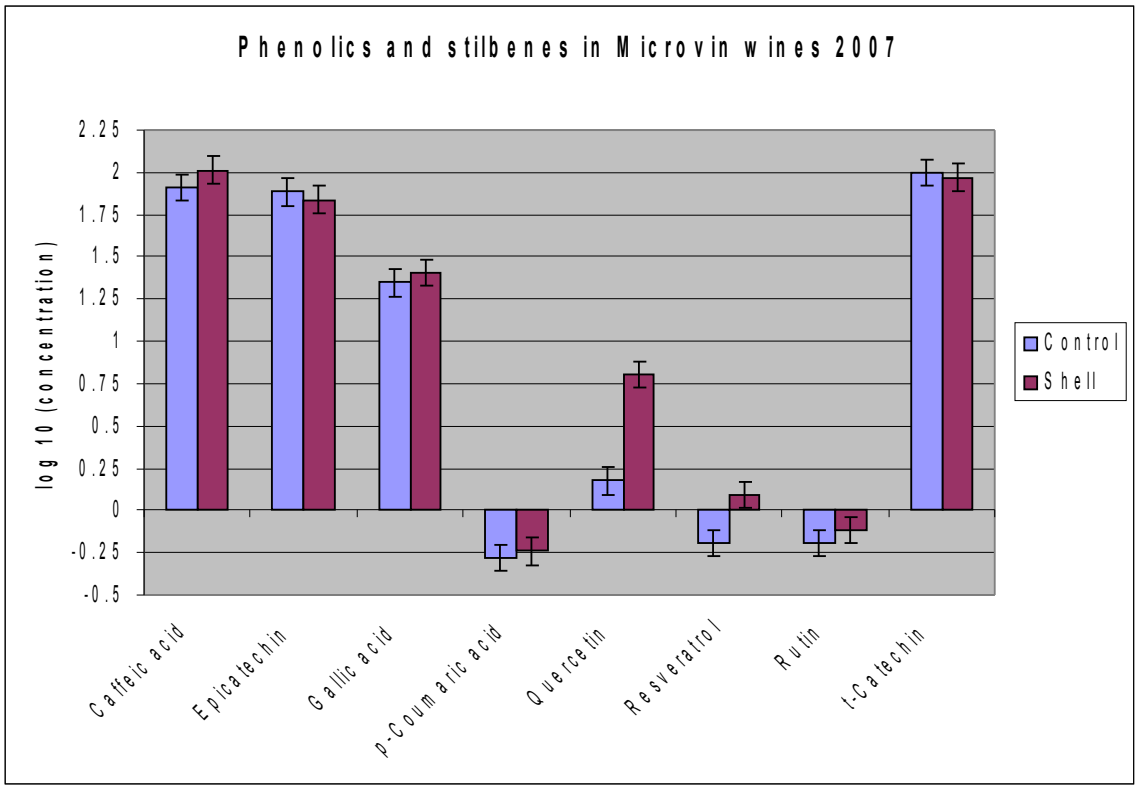

Figure 30. Phenolics and stilbenes in microvin wines, vintage 2007. Mean log base 10 of the concentration shows a statistically significant difference at p<0.001 between treatments. Bars indicate standard errors of treatment means (n=3).

In the Commercial wines the concentrations of epicatechin and catechin were statistically different, with the Control wines having slight higher values for both than the Shell wines (Figure 31). There were also differences for gallic acid and resveratrol, again with the Control having higher values than the Shell (Figure 32).

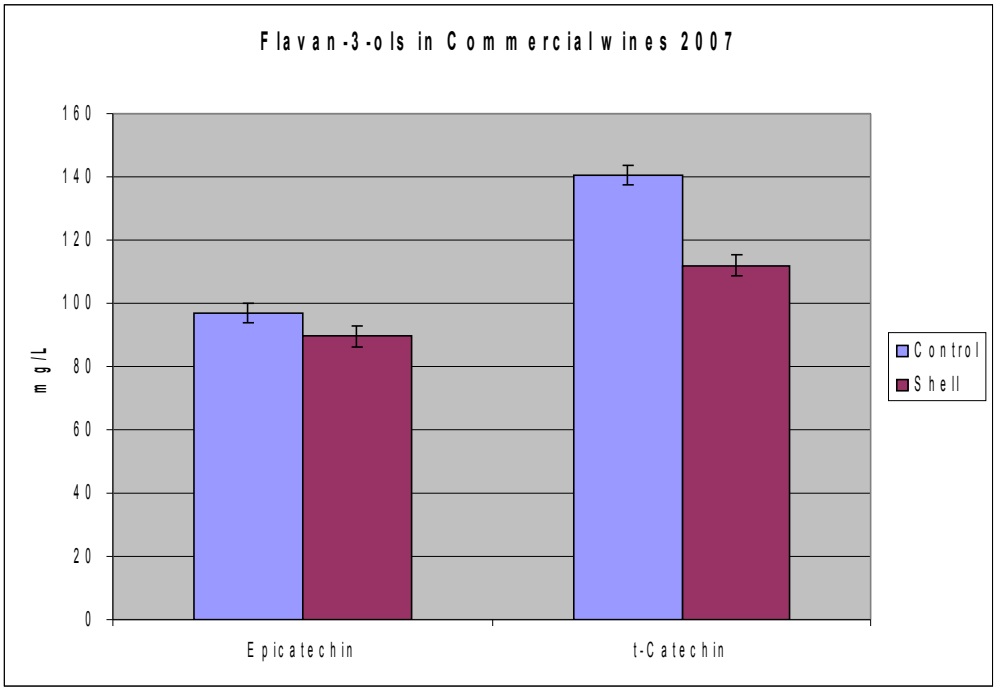

Figure 31. Flavan-3-ols in the commercial wines from vintage 2007. The difference in mean concentration expressed in mg/L is statistically significant at p<0.001 for both compounds. Bars indicate standard errors of treatment means (n=3). Concentration (mg/L) is in t-*catechin equivalents.*

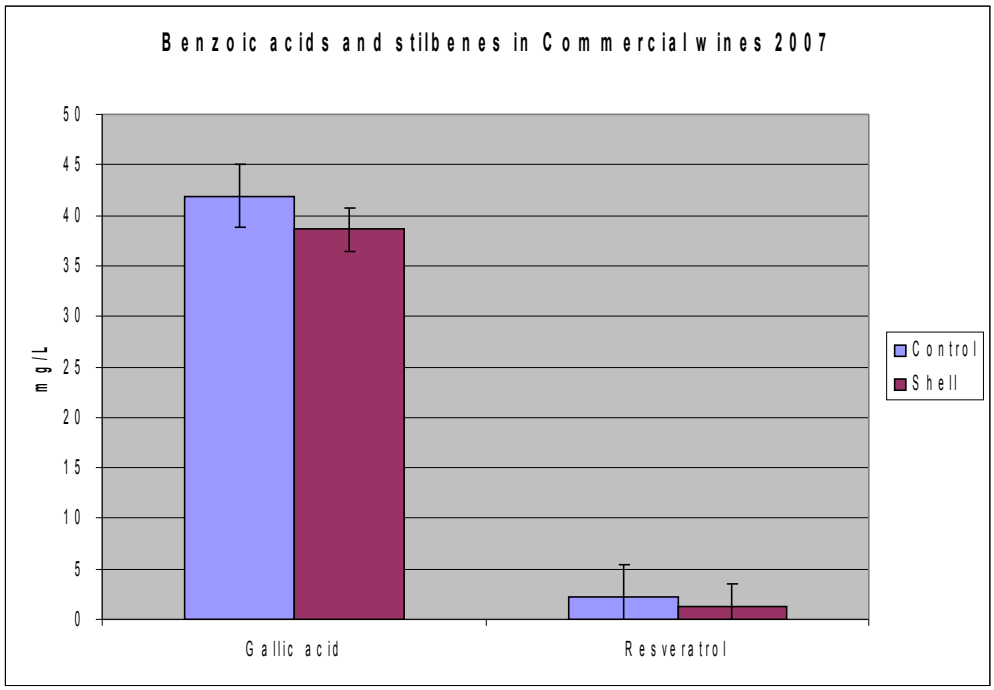

Figure 32. Gallic acid (a benzoic acid) and resveratrol (a stilbene) concentrations in commercial wines from vintage 2007. The difference between mean concentrations expressed in mg/L is statistically significant at p<0.001 for both compounds. Bars indicate standard errors of treatment means (n=3).

A similar trend was seen in the flavan-3-ols of microvin versus commercially vinified wines (Figures 30 and 31), but treatment effects for gallic acid and stilbenes were not, with the values for Control being lower than

that in Shells for the microvin wines (Figure 30), but higher in the commercial wines (Figure 32). The latter difference could very well be due to winemaking practices, as those used for the commercial wines was quite different to that used in the microvins (the microvins are treated exactly the same, use cultured yeasts, have no oak *etc.*). Greater extract from skins is associated with higher resveratrol in the resulting wines (Threllfall *et al.*, 1999) and fermentation organisms can also affect its level (Vacca *et al.*, 1997). Resveratrol is a phenolic compound associated with the "French Paradox," as it has beneficial effects on the human cardiovascular system (Siemann and Creasy, 1992), and is also a natural antifungal agent, particularly against Botrytis (Langcake, 1981). Its production in grape tissues is also known to be stimulated by short wave UVB radiation (Langcake and Pryce, 1977), and so its concentration might be expected to be higher in the Shells treatment. Price *et al*. (1995) reported similar results regarding resveratrol in exposed grape skin, though levels of stilbenes were not affected by increasing light through a reflective groundcover (Spratt *et al*., 2007).

Price *et al*. (1995) found greater concentrations of catechin and epicatechin in wine from shaded clusters, possibly originating in the grape seeds, though seed and skin catechin levels were not measured in their study. No effect of increases of light on flavan-3-ols was found in wines and grapes by using reflective groundcover (Spratt *et al*., 2007).

It is possible that the slightly greater flavan-3-ol concentrations obtained in the commercial scale compared to microvinification wines (approximately 25g/L lower in the microvins) are related to the differences in the winemaking conditions developed in both cases. Commercial fermentation conditions for both Shell and Control treatments were different to those used in microvinifications. Wild yeast and additions of pectolytic enzymes after pressing were utilized in the commercial fermentation. Sacchi *et al*. (2005) mentioned an increase of total phenolics and tannins by using pectinases, but no consistent results about the effect of yeast selection on the phenolic profile in red wines were reported. In the present study different times of maceration were applied prior to fermentation (4 days for Control and 1 day for Shell wines), suggesting that flavanols of seeds (Sun *et al*., 1999) or from skin (Watson *et al*., 1995) could contribute to the proanthocyanidin concentration into the wines. Further research in seed flavanol content would reveal additional information about this.

Commercial-scale wines were also made under higher temperatures than microvins, and consequently a greater phenolic extraction could be expected (Sacchi *et al*., 2005). Additionally, commercial wine samples were analysed at about 2 months in the barrels. It is well known that proanthocyanidin concentration in wines is determined by grape proanthocyanidin content, mainly in skins, seeds and stems, and by winemaking techniques and aging conditions (Ricardo da Silva *et al*., 1992; Gómez-Cordovés and González-San José, 1995; Fuleki and Ricardo da Silva, 1997). However, it was reported that while monomeric flavanols such as catechin and epicatechin decreased, trimeric and tetrameric derivatives increased during aging for 18 months (12 months in barrel and 6 months in bottle), demonstrating a greater polymerization and condensation of phenolic compounds (Pérez-Magariño and González-San José, 2004). In consequence, it seems that there is more of an effect of fermentation conditions rather than aging in the commercial wines because more monomeric flavanols were found in these wines

Sensory

The most important aspect of winegrape growing for premium products is actually best determined in the glass. As more data were gathered from this trial, it became evident that our ability to distinguish between the treatments analytically did not match our ability to discern a difference in the glass as we tasted the wines. This has meant that the sensory analysis of the wines became a very important part of trying to describe the effects that the mulch had on the wines, and also to lead us to new methods of analysing the wines.

The participants of the Southern Pinot noir Workshop have helped to characterise the wines from two vintages of the trial over two years. A evaluation sheet was given to the attendees that included a picture of the Mouthfeel Wheel (Gawel *et al.*, 2000) and a line where each person could specify the degree to which the wine rated for a characteristic and also how the various wine samples were perceived in relation to each other (see Appendix for an example of the sheet). The identity of the wine they were evaluating was kept secret. Responses were quantified by measuring the distance from the origin of the line to each wine's mark.

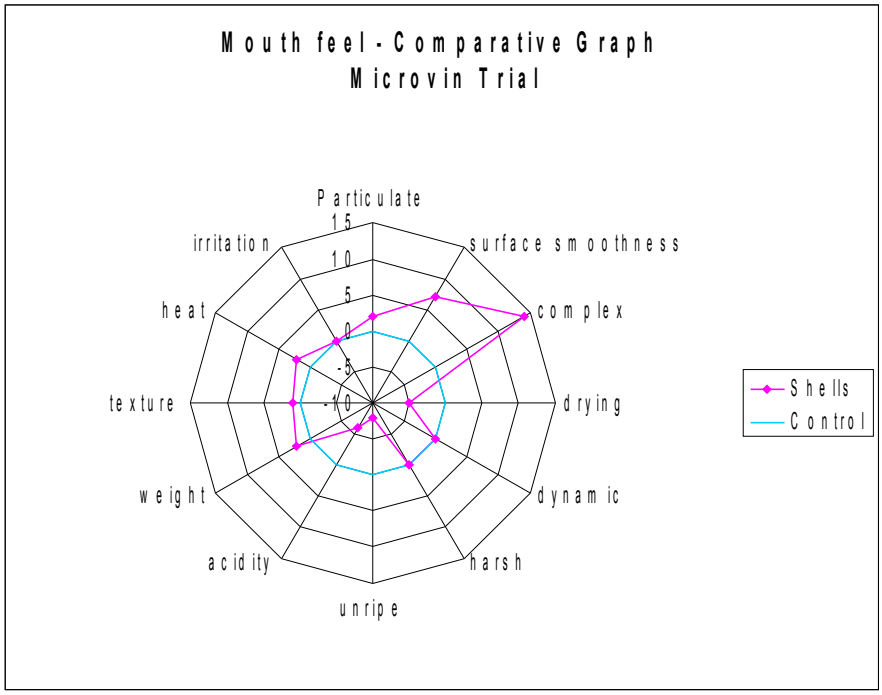

Figure 33. Mouthfeel attributes for the vintage 2004 microvin wines evaluated at the Southern Pinot noir Workshop, January 2005. Data are expressed as Shell wine values relative to the Control (n=39).

Figure 33 shows clearly that the Shells microvin wine was perceived to have greater surface smoothness, complexity and with less acidity and unripe and drying tannins: other factors were not perceived to be significantly different. Both wines received tartaric acid adjustment during fermentation in this vintage, however at different rates (the Shell wine received 0.7 g/L and the Control 0.4g/L). The overall preference was weighted heavily towards the Shells wine with 74% of participants preferring it over the Control.

These trends were reversed in the commercial wine (Figure 34) with 66% of participants preferring the Control wine. The Shells wine was perceived to have less complexity and dynamicism on the palate, but more unripe characteristics and acidity, which runs contrary to what was found in the microvin wines. There is little doubt that winemaking differences between the microvinifications and the commercial wines is contributing to the change in treatment effects, but it also demonstrates the complexity of the wine system.

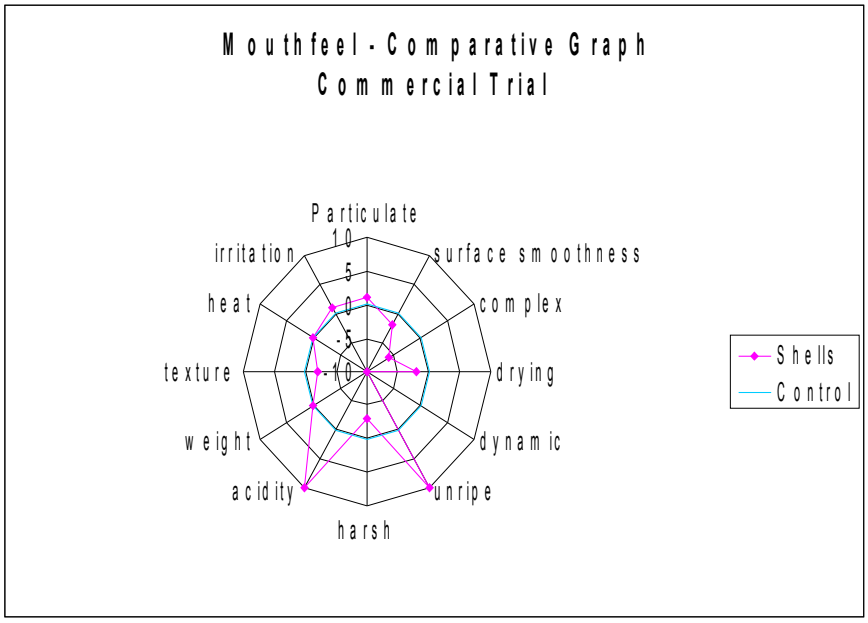

Figure 34. Mouthfeel attributes for the vintage 2004 commercial wines evaluated at the Southern Pinot noir Workshop, January 2005. Data are expressed as Shell wine values relative to the Control (n=38).

Other aspects that were rated by the delegates ended up being different between treatments as well. Colour (Figure 35), aroma intensity (Figure 36) and overall balance (Figure 37) for the microvinification wines show little perceived difference in colour or aroma intensity, but perhaps a slightly greater overall balance for the Control versus the Shell wines.

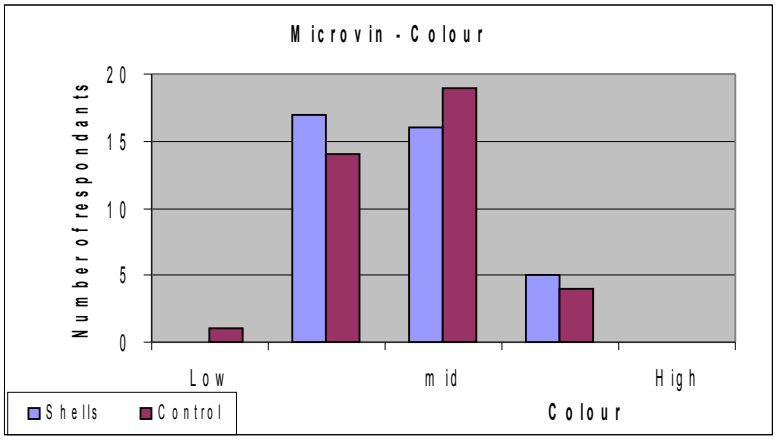

Figure 35. The colour intensity attribute for the 2004 vintage microvinification wine as evaluated at the Southern Pinot noir Workshop Tasting, January, 2005.

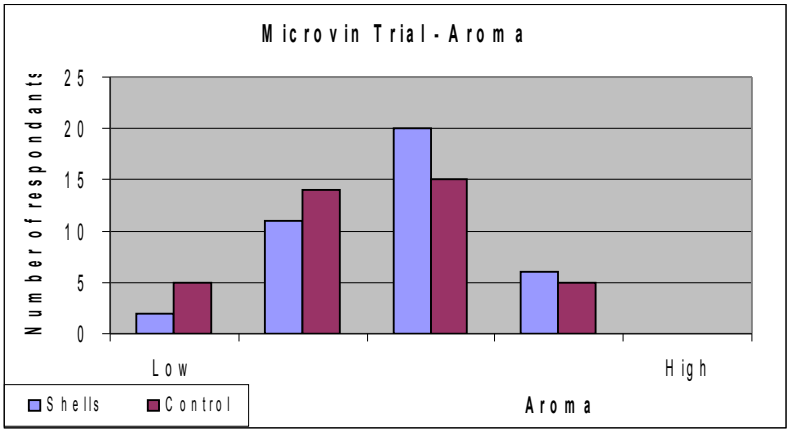

Figure 36. The aroma intensity attribute for the 2004 vintage microvinification wine as evaluated at the Southern Pinot noir Workshop Tasting, January 2005.

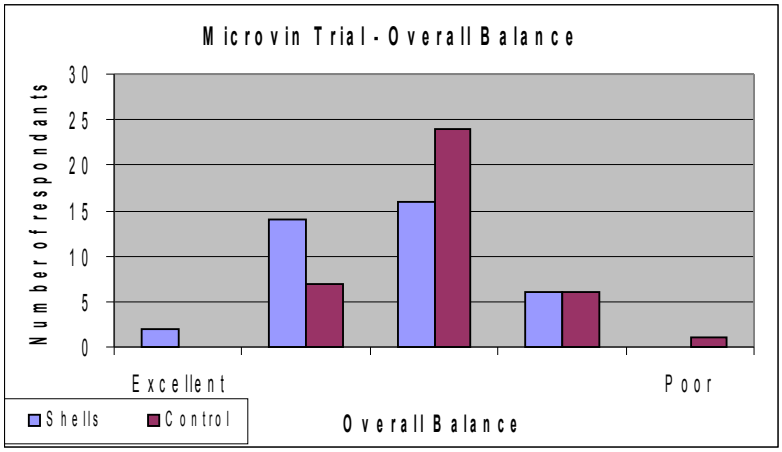

Figure 37. The overall balance for the 2004 vintage microvinification wine as evaluated at the Southern Pinot noir Workshop Tasting, January 2005.

The situation was changed when looking at the commercial wine colour (Figure 38) and aroma intensity (Figure 39). The delegates thought that the Control wines had greater amounts of both of these, although the overall balance was described as being similar (Figure 40).

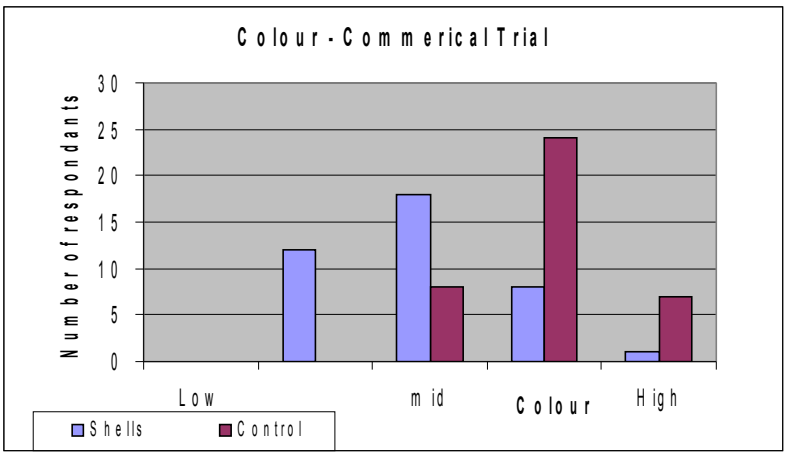

Figure 38. The colour intensity attribute for the 2004 vintage commercial wine as evaluated at the Southern Pinot noir Workshop Tasting, January 2005.

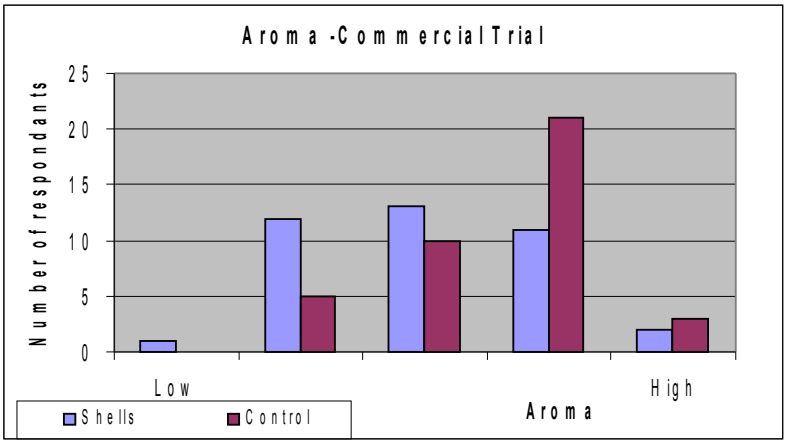

Figure 39. The aroma intensity attribute for the 2004 vintage commercial wine as evaluated at the Southern Pinot noir Workshop Tasting, January 2005.

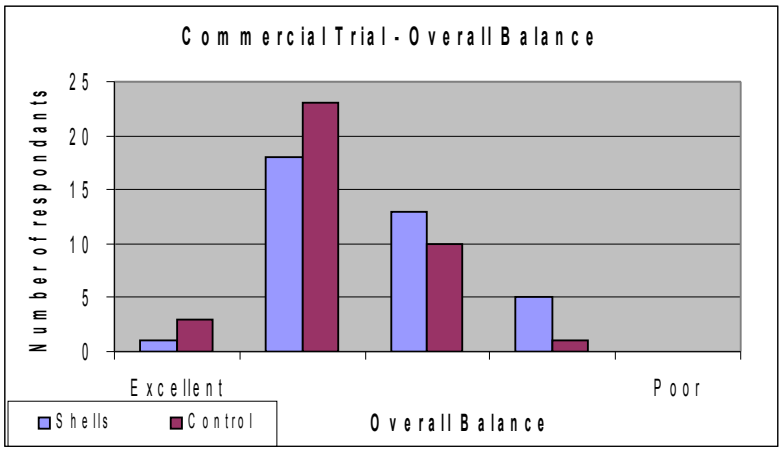

Figure 40. The overall balance for the 2004 vintage commercial wine as evaluated at the Southern Pinot noir Workshop Tasting, January 2005.

Microvin wines from the 2005 vintage and the commercial wines from the 2004 vintage were taken to the Southern Pinot noir Workshop in January of 2006 and evaluated using a similar tasting sheet. For the microvin wines vintage 2005 the values for total phenolics and phenolic ripeness were higher in the Shell microvin, as were those for hue and colour density (Figure 41). There were no real differences between the other characteristics evaluated.

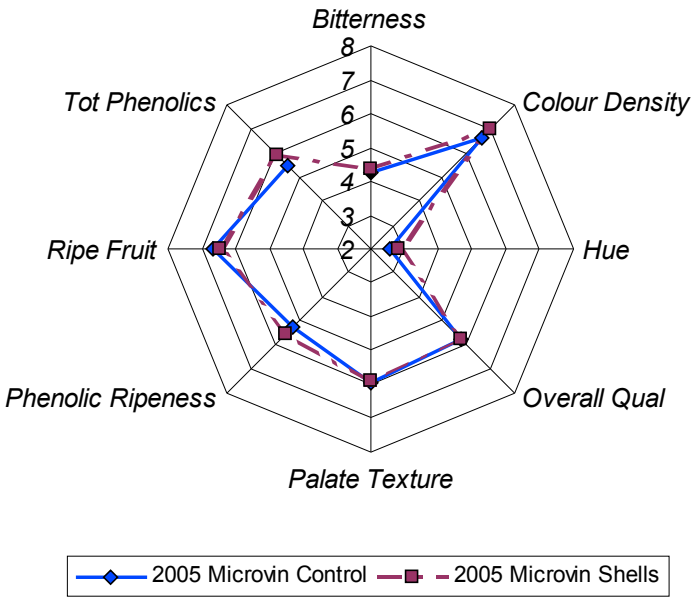

Figure 41. Mean sensory ratings for microvin wines (2005 vintage) as evaluated at the Southern Pinot noir Workshop Tasting, January 2005. Least significant differences (LSD) at p<0.001 for all of parameters. Standard error of treatment means = 0.394 (n=41).

For the same 2004 commercial wines had been assessed in January 2005 by the delegation to the Southern Pinot noir Workshop conference, the additional year of ageing proved to change the wines. The Shells wine was perceived as having greater colour density, bitterness, palate texture and overall quality, and phenolic ripeness was similar or greater than the Control. No characteristic evaluated in this year was lower than the score for the Control wines, which was also quite different than the evaluation done in 2005, in which the Shell wine was rated as having less colour than the control, but more unripe tannins. Clearly, with ageing the perception of the wine changed.

The differences found in the same wine during two followed years could be associated to changes in phenolic and aroma composition that wine goes through during the ageing process. Several reactions such as polymerisation and condensation of phenolic compounds, in conjunction with the decrease of free anthocyanins and increase of anthocyanin derivatives, were reported in wines aged for 12 months in barrels and 6 months in bottles (Pérez-Magariño and González-San José, 2004). These reactions in wines would depend on the initial anthocyanin to tannin ratio and could influence astringency changes during ageing (Fulcrand et al., 1996). Changes to flavour tend to be relatively limited during aging, meaning that a wine which tastes hard and astringent at the time of bottling, usualy retains that character even after several years (Ribéreau-Gayon et al., 2006). Further testing of these wines as they age may help to establish a trend for the effects of time on the treatment wines.

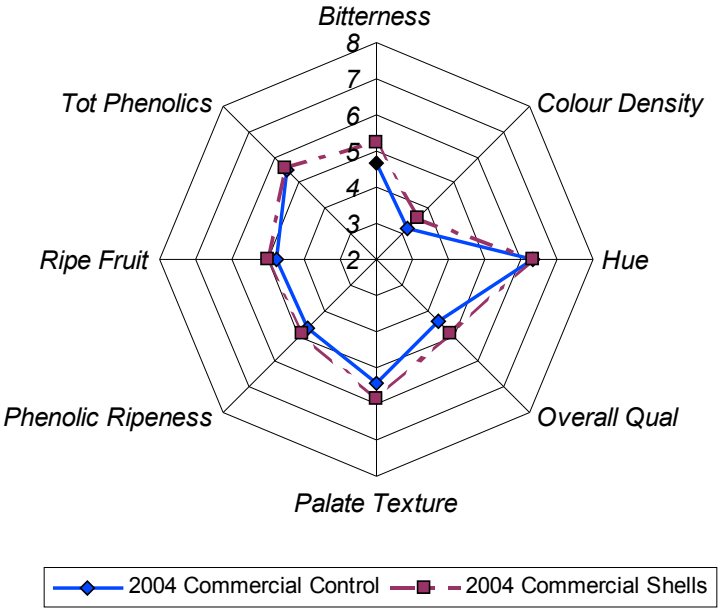

Figure 42. Mean sensory ratings for commercial wines (2004 vintage) as evaluated at the Southern Pinot noir Workshop Tasting, January 2005. Least significant differences (LSD) at p<0.001 for all of parameters. Standard error = 0.39 of treatments means (n=41).

Informal testing of these wines have indicated that there is a trend of slightly riper fruit characters and greater elegance in the Shells treatment wines in comparison with the Control during past vintages (Crawford, 2006). The majority of participants had preferred the Shell microvin wine from 2004, perceiving it as being greater in surface smoothness, complexity, texture and heat (Creasy *et al.*, 2006). This microvin wine was also perceived to be harsher than Control (Crawford, 2006). This agrees with the findings of Price *et al.* (1995), who reported harsher wines and higher levels of flavonols from sun-exposed clusters, but it was not investigated if flavonols were mainly responsible for this impression. It seems that greater grape maturity in the Shell area is affecting positively the flavour and sensory profile of the wine, not only in mouth feel characteristics, but also in aroma compounds.

Gas chromatography-mass spectrometry analyses

Sensory analysis has revealed that considerable differences exist between wines made from the two treatments. Being able to better characterise and quantify these differences was the next step in the project. However, because of the expense and complexity of the analytical process for this, a detailed analysis of the wines is beyond this project's scope. Presented here are some preliminary results, which will point the way for future research.

A powerful and relatively new tool for the evaluation of aroma compounds is gas chromatography combined with mass spectrometry (GC-MS). GC-MS determines the chemical structure of volatile compounds and has been used successfully to identify and quantify grape aroma compounds (Cabrita *et al.*, 2006). Lincoln University has developed protocols for the analysis of aroma compounds that are important to Sauvignon blanc, and has moved to using this technique for Pinot noir. Investigations into the latter is more complex than for Sauvignon blanc as there are no dominant aroma compounds.

It has been cited that Pinot noir aroma is a complex of different compounds rather than a single compound responsible for the characteristic aroma (Fang and Qian, 2006). A preliminary report done at the University of California, Davis concluded that a red and dark fruit sensory profile, slightly sweet and high alcohol content predominate in a high quality Pinot noir wine (Guinard and Tsay, 2007).

Two microvin wine samples from the 2007 vintage (Control and Shell) without replicates were analysed using GC-MS by Simpson (Leal, 2007) using the method of Sherlock (personal communication). Although no statistical comparison was possible as only one of each wine was able to be analysed, some ideas about

aroma compounds in Pinot noir wines can be generated by comparing them. Although the chromatograms (Figure 43) generated revealed that the number of volatile components and their combined area was greater in the Control sample, the distribution of aroma compounds was different between treatments. Hence, while the number of identified aldehydes and esters wa greater in the Control wine, other compounds such as acids, alcohols and hydrocarbons were higher in Shell wine. The number of lactones and ketones were similar for both samples.

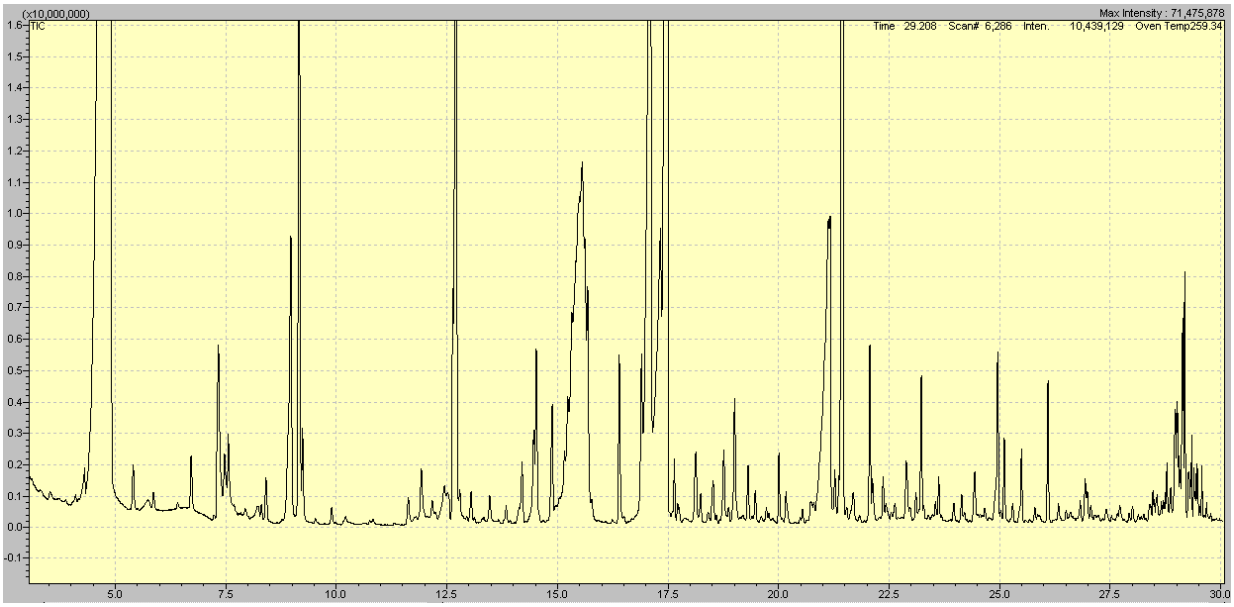

Figure 43. Example of a chromatogram generated by GC-MS analysis of a Pinot noir wine from the trial.

However, the relative area of compound classes such as acids, esters, hydrocarbons, ketones and lactones was greater in Shell wine, whereas alcohols, aldehydes and undetermined compounds were higher in Control wine.

By looking at specific odor-active compounds (Leal, 2007), the peak area of geraniol, an important monoterpene alcohol that contributes floral and cherry flavours to Pinot noir wines (Fang and Qian, 2005), was greater in Shell wine. Terpene alcohols increased in Pinot noir wines when grapes were harvested at higher sugar content (over 25° brix), lending more floral aromas in the wines (Fang and Qian, 2006), which maybe related to the effects that Shells have on the grapes. However, 1-hexanol, which contributes fruity aromas related to grape juice (Fang and Qian, 2005), was lower in Shell wine, so it is clear that this is a complex system.

Peak areas obtained for fatty acid esters such as ethyl butanoate, ethyl hexanoate, ethyl decanoate, and ethyl octanoate, were higher in the Control than Shell wine. Esters are considered secondary aromas which supply fruity odours, and though they are affected by yeast strain, temperature, oxygen and nitrogen levels during fermentation (Clarke and Bakker, 2004; Beltran et al., 2005), their concentration decreased with grape maturity (Fang and Qian, 2006). However, their contribution to wine aroma could be restricted due to high detection thresholds (Fang and Qian, 2005). Another ester also contributing to fruity aromas, but reported as less important in Burgundian Pinot noir (Moio and Etievant, 1995), was ethyl dihydrocinnamate, which was identified as being greater in Shell than Control wine.

With regard to acids, butanoic acid, which imparts sweaty odours, was not found in Control wine. Propanoic acid, which is related to spicy aromas, was higher in Control wine, and both hexanoic and octanoic acids were greater in Shell wine, possibly contributing sweaty and goaty rancid cheese characteristics (Fang and Qian, 2005).

The concentration of aroma compounds and their balance within the wine affects the quality of Pinot noir wines, and it seems that aroma active compounds increase along with grape ripening, a stage where the Shell mulch is modifying the light environment (PAR and UV radiation) and the canopy temperature. Use of white geotextile reflective mulches has been shown to improve sunlight exposure and reduce herbaceous

aromas in Cabernet franc (Hostetler *et al*., 2006). However, further research needs to be done to understand the effect of light and temperature on grape maturity and aroma composition at this level of detail. These preliminary results, though, agree with a study that identified aroma compounds in Pinot noir wine from Oregon (Fang and Qian, 2005), where a wine described as spicy, vegetative and floral was greater in compounds such as propanoic acids and aldehydes, and a fruity wine was related to higher quantities of esters, similar to was found in the Shell wine components.

There is little doubt that further investigation into characterising New Zealand Pinot noir wine aroma compounds and the effects that Shells have on them would be beneficial to fine-tuning production methods.

Conclusions

The trial was run over four seasons to evaluate the effect of using mussel shells as reflective mulch on vine performance and fruit and wine quality. Clearly, the shell mulch had several effects on phenological growth stages in grapes and also on sensory perception of the wine due to modification of phenolic and aroma compounds. The findings may be divided into several categories:

Vine environment

- Soil under mulch was cooler compared to the un-mulched Control for much of the day, but the shells buffered extremes in temperatures. This does not appear to have a significant effect on the growth, development and performance of the vines.
- Soil moisture was increased under the shells, particularly in the shallower part of the profile.
- Soil nutrients in the top 15cm of the soil profile were altered through the use of the mulch, increasing pH, Ca and Na, but had inconclusive effects on other nutrients. Further investigation into the effect on soil nutrients would be advised to track longer-term changes to the soil and on vine performance.
- Fruiting zone temperature over Shells was slightly higher during the day and cooler at night, leading to a slightly more continental environment.
- Shell mulch reduced weed growth compared to control, but over time shifted the types of species growing in it. The frequency with which shells need to be re-applied is one aspect of their use that has not yet been investigated.
- Shell mulch reflected greater amounts of UVA, UVB and PAR radiation into the fruiting zone, which could have multiple effects on vine growth and fruit composition.

Vine performance

- Dates of flowering and veraison appeared to be slightly advanced over shells. Budburst does not seem to be affected by the mulch and there was not a clear effect on advancement of harvest date. The influence of seasonal weather conditions seems larger than the effect of treatment.
- Vine growth was not affected by Shells in terms of the number of nodes laid at pruning, flower cluster and shoot number pre shoot thinning, and so was not a factor in changes to yield. This result is beneficial to adoption of shells as a mulch, as the vegetative growth appears not affected, despite increased moisture available in the soil.
- Early shoot growth, shoot lengths, pruning weights, trunk circumferences and canopy density were similar between Shell and Control. Differences in internode lengths in dormant cane material occurred, but did not seem to be related to treatment, but rather more associated to the seasonal weather conditions. Vigour, as measured through these variables, did not increase with use of shells.
- Nutritional status of the vines was not overtly affected. Leaf petiole samples showed higher nitrate-N and calcium and lower Mn in the Shell area compared to Control area. In leaf blades Ca, Mn, and Zn increased in Shells compared to Control samples and Cu decreased
- Leaf SPAD (greeness) values were higher in the Shell treatment during veraison, pre- and post-harvest, but lower shortly after budburst. The latter response may be due to early season UV radiation effects on leaf chlorophyll, which the vines grow out of.
- Leaf phenolic composition was also different between treatments, demonstrating a possible effect of increased UV radiation and PAR. However, identification of the individual compounds affected was not within the scope of this study.
- Fruit set was similar between treatments, but was considered poorer in Shell bunches due to large population of seedless berries during this 2006-2007 season. These berries impacted

negatively on vine yield, and further research into the treatment effects on fruit set should be done, particularly with relation to seasons with differing weather and crop loads.
- Yield components were affected by the Shell mulch, but varied with seasonal conditions. It appears there may be and interaction between mulch effects and season, with high yielding seasons resulting in higher cluster weights in Shells and the opposite happening in low yielding years. The effect of this on wine qualities has not yet been well characterised.
- Peduncle lignification was delayed at veraison as well as at harvest time in the Shell mulch area. Given the effects of the mulch on wine characteristics, this demonstrates that there is not necessarily a direct link between peduncle browning and fruit ripeness.

Juice and wine

- There were slight differences between treatments in fruit and wine composition of variables such as °Brix, TA, pH, alcohol. Over the length of the trial, it would be difficult to say that mulch had an effect on typical berry compositional measurements.
- Because of the different profiles of berry size classes between treatments (at least in vintage 2006), the contribution of the differentclasses to sugar and colour changed between treatments. Though there were fewer seeded berries in the Shells treatment, it appeared that the largest contributed more colour than the largest Control berries.
- HPLC analyses of commercial and microvin wines showed differences in the flavonoid profiles. While Shell microvin wines showed greater flavonol and resveratrol concentrations than Control, commercial Shell wines were lower in epicatechin, gallic acid, resveratrol, and t-catechin than Control. However, no consistent differences in total anthocyanins and total phenolics between treatments were found by spectrophotometer.
- Sensory analyses of microvin and commercial wines showed consistent differences between treatments, exhibiting lower levels of green and unripe tannins, and greater smoothness and complexity as well. This pattern existed across all vintages, including high and low yielding ones.

Despite the fact that past research using reflective mulches has shown some effects on viticultural aspects and grape and wine composition, only a few related these changes to light. Most studies have assumed the influences of light and canopy temperature on vine performance and wine quality. However, this research has contributed a greater understanding of which variables are influencing changes in grape development and wine composition. The project has shown that the use of a reflective mulch composed of a waste product, changes the temperature, light (PAR and UV radiation) and other environmental variables of the vines and fruit. This carried through to the wines, resulting in improved in sensory characteristics.

Though there are viticultural considerations in the use of a reflective mulch (*e.g.* that the mulch must be kept clear of plant debris), the benefits in terms of wine flavour and mouthfeel characteristics appear to be worth the extra effort. The commercial wine from the Shells area has consistently been regarded as being higher quality than that from the Control area. Now that the funded aspect of the trial has ended, shells are being placed in the Control area of the experimental block, as well as in other blocks where there are difficulties in getting rid of that last bit of green character in the grapes. Wine quality has improved through the use of the shells.

Research into the effects and benefits of using reflective mulches continues, with a trial investigating the use of crushed glass having been established in Canterbury. This trial includes mussel shells as a treatment as well, so that the results found here can be tested in another, cooler, growing region.

Financial

Income and expenditure statement for the project.

Information Dissemination

See Appendix for copies of these items, if available.

- April 2004 -No. 8 Wired TV programme reporters to visit Neudorf Vineyards to video a story regarding the project. The programme aired later in the year
- January 2005 -Wines from 2004 vintage tasted at the Southern Pinot noir Workshop held in Hanmer Springs.
- August 2005 -Article in the Southland Times, Viticulture Special Feature section
- October 2005 -Mention of the trial in the Cambria House B&B Newsletter
- January 2006 -Wines from 2004 (commercial) and 2005 (microvin) vintage tasted at the Southern Pinot noir Workshop held in Hanmer Springs
- January 2006 -The trial was mentioned in an article published in the Journal of Wine Research by John Salvi ("Report on official visit to New Zealand" 17(1):63-66). Article is attached to this report.
- February 2006 -Lincoln University Newsletter Infolinc mentions the project
- March 2006 -The trial is mentioned in a TV and web article about the crushed glass trial in Canterbury
- June 2006 -Article published in the Australian and New Zealand Grapegrower and Winemaker 2006 34th Annual Technical issue detailing results from the trial (Issue 509a, page 12).
- July 2006 -An enquiry from Te Awa Winery about the trial and its findings was fielded by Glen Creasy
- December 2006 -Dr Richard Smart visited Lincoln University Dec 1-4 and was given a report on this trial, as well as a tasting of the 2004 commercial wines and the 2005 microvinification wines. The response was very favourable, and Dr Smart has commencing trials with mussel shells in Tasmania, the first vintage of wines from the trial being made in 2007.
- July 2007 -Rural Delivery programme on Sustainable Winegrowing mentions project
- March 2007 -Terranova newsletter mentions project
- August 2007 -Gerardo Leal presents invited talk at the Romeo Bragato Meeting in Auckland regarding data collected from the trial
- August 2007 -Prime Minister Helen Clark mentions the trial in her opening speech for the Romeo Bragato Meeting in Auckland
- October 2007 -Marlborough Express mentions trial in article
- Throughout project - the Lincoln University Centre for V&O Research page has listed this project

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Appendices

I. Trial map

Trial site located at Neudorf Vineyard. The shells have been spread under rows 61-68 (S) and the Control rows are 69-76 (C). The cells with an "x" in them represent the areas used for data collection in 2004 through 2007

	Top of slope, South end																			
Treatment:	C	C	C	C	C	C	C	C	C	C	S	S	S	S	S	S	S	S	S	S
Row No.	Vine#	77	76	75	74	73	72	71	70	69	68	67	66	65	64	63	62	61	60	59
Bay +15	5																			
Bay +14	5																			
Bay +13	5																			
Bay +12	5																			
Bay +11	5																			
Bay +10	5																			
Bay +9	5																			
Bay +8	5																			
Bay +7	5																			
Bay +6	5																			
Bay +5	5			x	x	x	x	x	x			x	x	x	x	x	x			
Bay +4	5			x	x	x	x	x	x			x	x	x	x	x	x			
Bay +3	5			x	x	x	x	x	x			x	x	x	x	x	x			
Bay +2	5																			
Bay +1	5																			

Central Thoroughfare

Bay -1	1																		
Bay -2	5																		
Bay -3	5																		
Bay -4	5																		
Bay -5	5																		
Bay -6	5																		
Bay -7	5																		
Bay -8	5																		
Bay -9	5																		
Bay -10	5																		
Bay -11	5																		
Bay -12	5																		
Bay -13	5																		
Bay -14	5																		

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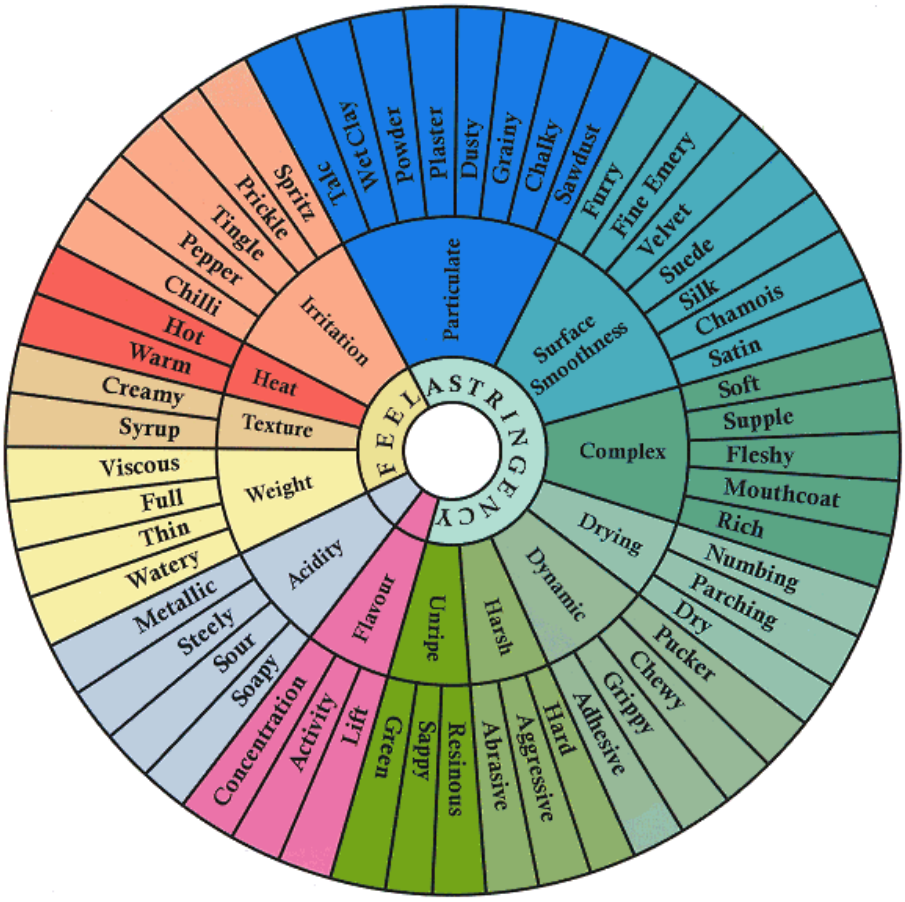
II. Tasting sheet used for sensory analyses



The Mouthfeel Wheel

Gawel, R. Oberholster, A. and Francis, I.L (2000) A 'Mouth-feel wheel': terminology for communicating the mouth-feel characteristics of red wine. Australian Journal of Grape and Wine Research, 6, 203-207

The Process

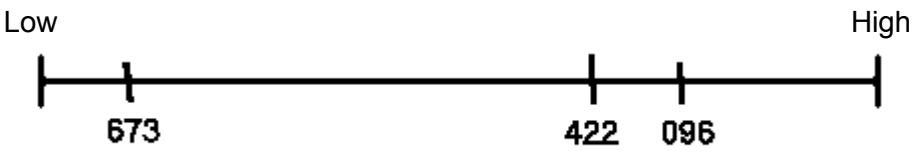
You will be presented with three wines, each with a number assigned to it.

Please fill out following page while tasting the wines. For the Appearance, Aroma, Mouthfeel and Overall Balance categories, please answer by drawing a line where you percieve to sit on the relative scale, and then mark that line with the wine's code. Please ask if you are uncertain as to what to do.

Your responses will be collated and a summary returned to Nelson Grapegrowers and Winemakers.

Example

Expected suitability for Robert Parker



Appearance

Low High

Color

Purple Red Brown

Hue

Aroma

Low High

Ripe fruit character

Mouthfeel

Short Long

Palate length

Low High

Bitterness

Tannins

For this section, pick the 5 best descriptors for the tannins in each wine, using the Mouthfeel Wheel on the previous page:

Wine 1:

Wine 2:

Wine 3:

Wine 4:

Overall Balance

Poor Excellent

III. Media mentions for project

- August 2005 -Article in the Southland Times, Viticulture Special Feature section
- October 2005 -Mention of the trial in the Cambria House B&B Newsletter
- January 2006 -The trial was mentioned in an article published in the Journal of Wine Research by John Salvi ("Report on official visit to New Zealand" 17(1):63-66). Article is attached to this report.
- February 2006 -Lincoln University Newsletter Infolinc mentions the project
- March 2006 -The trial is mentioned in a TV and web article about the crushed glass trial in Canterbury
- June 2006 -Article published in the Australian and New Zealand Grapegrower and Winemaker 2006 34th Annual Technical issue detailing results from the trial (Issue 509a, page 12).
- July 2007 -Rural Delivery programme on Sustainable Winegrowing mentions project
- March 2007 -Terranova newsletter mentions project
- August 2007 -Gerardo Leal presents invited talk at the Romeo Bragato Meeting in Auckland regarding data collected from the trial
- August 2007 -Prime Minister Helen Clark mentions the trial in her opening speech for the Romeo Bragato Meeting in Auckland
- October 2007 -Marlborough Express mentions trial in article
- Throughout project - the Lincoln University Centre for V&O Research page has listed this project