Reflective mulch effects on the grapevine environment, Pinot noir vine performance, and juice and wine characteristics

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Abstract

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Reflective mulches were applied in a cool climate vineyard in Canterbury, New Zealand. Materials used were waste products: mussel shells (MS) and green (GG) and clear (CG) recycled crushed glass. A control of bare soil was also included. MS and CG were light coloured while GG and CON were dark. Treatments were applied randomly within each of three replicates.

Soil parameters tested were temperature, moisture, microbial biomass carbon, dehydrogenase enzyme activity and nutrient levels. Also investigated were canopy temperature and radiation reflected from each treatment. Vine parameters included nutrient levels, photosynthesis, leaf greenness, fruit components and pruning weights. Juice and wine parameters included wine colour, phenolic concentration and acid composition. A blind tasting was held and gas chromatography-olfactory (GC-O) was used to analyse aroma profiles of wine and juice.

Mulches affected various soil parameters. MS buffered soil temperature and all mulches increased soil water retention especially MS. MS had higher microbial biomass carbon than glass. Soil pH levels increased under MS while sodium levels were highest for GG. Higher levels of vine canopy boron, copper, potassium, molybdenum, phosphorous and sulphur were found for mulches. No differences were noted for vine gas exchange although differences were found for related parameters between mulches and CON. Cluster number and fruit weight were higher for light compared to dark treatments and pruning weights were

highest for light and mulched treatments. All mulches reflected solar radiation into the canopy. No differences were found for canopy temperature at any stage during the growing season however differences for light parameters were highly significant. Part of the ultra violet spectrum (300 - 400 nm), photosynthetically active radiation (380 – 760 nm) and red to far red ratios (660:730 nm) were all higher for light compared to dark treatments. Analysis of wine attributes such as colour, phenolics, acids and aroma suggested a treatment effect on the wine. The tasting also highlighted, that reflective mulches could be used to alter wine flavour, aroma and mouthfeel. Differences in juice aromas measured by GC-O were reported slightly differently by each panellist who detected them, but significant results were found within each data set. The first panellist recorded more differences between mulch treatments and CON with higher results in most cases for CON regardless of aroma type. For the second panellist results were more varied with significant results for different comparisons and a range of aromas.

Reflective mulches directly affected environmental and vine performance parameters. They could be applied in the vineyard to optimise the distribution of radiation, improve vine health and productivity. These effects have had a subsequent impact on the fruit and wine produced. It is clear from this trial that mulches had impacted on juice and the research forms a basis for future work. Ultimately the mulches offer the possibility to manipulate aromas, which could be a useful tool for winemakers in cool climates who seek to make consistently distinctive wines in variable conditions.

Key words

Reflective mulch, Pinot noir, mussel shells, recycled glass, cool climate viticulture, vine performance, solar radiation, GC-O, aroma profiling, phenolics, anthocyanins.

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Table Of Contents

List of Tables

List of Figures

Chapter 1 - Introduction

Canterbury, New Zealand is a cool climate region for viticulture. Growing wine grapes in cool climate areas can be challenging, as variation in climatic conditions may cause subsequent variation in the development of the vine and the fruit. Winemaking is greatly facilitated when the grapes are healthy and reach optimum levels of ripeness at the time of harvest. In cool climate regions it can be difficult to consistently maintain the high quality of fruit needed to produce the best quality wine, and it is often necessary to use a variety of management practices to encourage ripening before the season ends (Jackson and Lombard 1993). In order to achieve quality, vines need adequate levels of light and temperature for all areas of development, including vegetative and reproductive growth (Buttrose 1974, Bergqvist *et al.* 2001). In order to optimise environmental radiant energy in the vineyard it is important to understand how the vines access this variable and how the vineyard situation may be improved. A number of methods have been explored, many involving the manipulation of the grapevine canopy or the modification of the vineyard environment. For example, various training solutions (Shaulis and May 1971, Smart 1988, Sommer *et al.* 2000,) pruning (Sanchez and Dokoozlian 2005), leaf defoliation (Bennett *et al.* 2005) or combinations of these (Smart 1985a, Koblet *et al.* 1996, Reynolds *et al.* 1996, Kliewer and Dokoozlian 2005) have been investigated for their abilities to open the canopy and improve the radiant environment. Various types of reflective material have also been used to reflect radiant energy from the ground up: materials include shells (Crawford 2007, Leal 2007, Sandler *et al.* 2009), white geotextile mulch (Hostetler *et al.* 2007a,b) aluminised sheeting (Reynolds *et al.* 1996, Razungles *et al.* 1997, Robin *et al.* 1997, Coventry *et al.* 2005) and white, red and silver coloured plastic foils (Todic *et al.* 2007). This study of the effect of glass as a reflective mulch is the first on this type of material.

It was hypothesised in this trial that mulch made from crushed glass could be used to redirect radiant energy into the vine's canopy to enhance the microclimate environment. Mulches made from various materials are commonly used in vineyards and depending on the type of mulch the benefits can include soil moisture retention, soil temperature moderation, reduced levels of grapevine disease, increased crop loads and weed suppression (Agnew and Mundy 2002). Increasing solar radiation in the vineyard could also increase production of wine phenolics (Price *et al.* 1995, Cortell and Kennedy 2006) and improve wine quality by reducing vegetative aromas (Reynolds *et al.* 2007). The use of glass as a mulch not only offers the potential to improve the vineyard radiant environment and therefore wine quality, it additionally provides an alternative use for this material. Recycled

glass is now collected in New Zealand in such high quantities that the country has reached its processing capacity (Thomas 2005).

This trial was made up of two sections including an investigation into the effects of reflective mulch on the vine environment and on Pinot noir vine performance. The second section involved an investigation into the mulch effects on juice and wine parameters. The following objectives were examined:

Section one – Reflective mulch effects on the grapevine environment and Pinot noir vine performance

- Objective 1: to test the effects of the mulches on the soil in the under-storey area of the grapevines, including mulch effects on temperature, moisture, micro-organisms and nutrients.
- Objective 2: To investigate the effects of the mulches on the vine, including effects on vine nutrition, photosynthesis, leaf greenness, fruit parameters and pruning weights.
- Objective 3: to test the ability of the mulches to reflect and increase the total radiant energy for use by the vines, including its effects on fruiting wire height temperature, reflected photosynthetically active radiation and red to far-red ratios.

Section two – Reflective mulch effects on juice and wine characteristics

- Objective 1: To investigate the effects of the mulches on wine quality, including wine phenolics, colour and acids. A wine tasting was also carried out as well as analysis of the aroma of the wine using gas-chromatography olfactory.
- Objective 2: To examine the influence of mulches on the fruit, using gas-chromatography olfactory. To analyse the different aromas in the juice from the fruit and investigate how they had been affected by the different mulches.

The reflective mulches offer an alternative use for waste products and the opportunity to positively influence the grapevine micro-climate. They are a possible way to maximise the quantity and quality of light available in the vineyard and are a method that could potentially improve some aspects of fruit and therefore wine quality in cool climate areas.

Chapter 2 - Literature review

2.1 Radiation and its effects on vine performance and fruit production

Plants respond to changes in the radiant light environment; this is one of the most important factors affecting vegetative production (Smart 1974) and berry development (Buttrose 1974) in grapevines. Radiation from the sun affects photosynthesis, which is the conversion of light energy to chemical energy by plants. Plants use energy from the sun to convert carbon dioxide and water to oxygen and carbohydrates. In addition, photosynthesis ultimately produces fatty acids, amino acids and nitrogen bases from which all the organic constituents of the plants are derived (Jackson 2000). The part of the spectrum at which vines carry out photosynthesis occurs in the visible spectrum range between 400 and 700 nm (Smart 1974). Energy is strongly absorbed directly from the sun, while at the same time a small amount is transmitted through and reflected among the leaves (Smart and Robinson 1991).

Apart from the visible spectrum ultraviolet (UV) is another type of solar radiation that is becoming increasingly important as the world climate changes (Shultz 2000), particularly with the depletion of the stratospheric ozone layer (Rowland 2006). Increasing levels of UV-B radiation, which occurs at wavelengths 280 to 315 nm, could affect vines in a number of ways. Shultz (2000) predicted that increased levels of UV-B would impact on the plant genes controlling the accumulation of flavonoids and anthocyanins, and also concentrations of chlorophyll and carotenoids leading to changes in aroma compounds. Price *et al.* (1995) reported increases in levels of quercetin in sun-exposed berries. Kolb *et al.* (2003) also found that higher levels of UV increased berry phenolics. In this case, concentrations of quercetin and kaempferol in berry skins were highest in grapes with the most exposure. Li *et al.* (2008) found that when berries were exposed to increasing levels of UV-B there were subsequent increases in the production of *trans*-resveratrol in their skins. Lafontaine *et al.* (2005) who investigated effects of UV on Riesling found that increased exposure to UV radiation caused increases in phenolics and reported higher levels of total bound glycosidic secondary metabolites; this led to changes in the aromatic properties of the wines produced. Reduced chlorophyll content in leaves exposed to UV radiation were also reported by Lafontaine *et al.* (2005). Increased exposure to UV has many potential outcomes for the vine and its environment. It has been shown to affect grape berries and the vine canopy. Shultz (2000) also predicted that increased levels of UV radiation could affect populations of soil microflora and fauna, which would change the availability of nutrients in the soil.

At the other end of the visible electromagnetic spectrum, into the near infra red zone the ratio that occurs between the wavelengths red (660 nm) and far red (730 nm) is used to measure the impact of radiation on vine performance. Plants use specialised photoreceptors called phytochromes to receive these wavelengths (Franklin and Whitelam 2007). Phytochromes are plant photoreceptors of red and far red light. They are photoswitchable using covalently attached bilin chromophores to enable photoconversion between the two types of light (Rockwell *et al.* 2006). The red:far red ratio is used by the plant to measure the configuration of light available (Franklin and Whitelam 2007). Phytochromes detect changes in the proportion of red to far red light and signal to the plant causing a physiological response. Phytochrome response to the red to far red ratio is thought to regulate important enzymes connected to fruit ripening (Kliewer and Smart 1989). Kliewer and Smart (1989) investigated the action of phytochrome by supplementing vines with light from the red spectrum. They specifically tested three enzymes that are known to be light dependent: nitrate reductase (NR), phenylalanine ammonium lyase (PAL) and invertase. Activity of all of these enzymes was increased by higher red light levels. This subsequently resulted in higher synthesis and metabolism of malic acid, more production of phenolics and anthocyanins as well as greater sucrose hydrolysis. Higher ratios of red to far red light were found to significantly advance the beginning of fruit ripening, enhance berry weight and increase berry sugar levels. From the results in this trial, Kliewer and Smart (1989) speculated that light from the far red spectrum was also responsible for the regulation of the reduction of nitrate and the accumulation of phosphate.

The effect of radiant energy on vine photosynthesis means that it is also a crucial factor for vine vegetative development and production. Edson *et al.* (1995) showed that leaf area was inversely related to crop load and Kliewer and Dokoozlian (2005) showed that the fruiting capacity of vines was dependent on the area of leaves exposed to full sunlight. Bennett *et al.* (2005) also reported an effect of canopy on vine carbohydrate status, showing that where leaves were removed, a subsequent reduction in stored carbohydrates was seen. This was due to the reduced ability for the vine to photosynthesise and growth was therefore restricted in the following season. In another trial that investigated the effects of defoliation on the vine, Kliewer and Fuller (1973) showed that defoliation carried out at fruit set reduced dry weights of canes, trunks and roots of potted Thompson Seedless vines. Another trial looked at the effects of different trellis systems on vine capacity of Aglianico vines (Cavallo *et al.* 2001). It was found that the bilateral free cordon vines that created the most shade in the fruiting zone had the lowest vine capacity. The upright shoot trellis system, which had the greatest exposed leaf area, had the greatest vine capacity. Where the light environment was

improved in the vineyard, and where vines had sufficient canopy to carry out photosynthesis, accumulation of dry matter was observed (Kliewer and Fuller 1973, Edson *et al.* 1995, Cavallo *et al.* 2001, Creasy *et al.* 2003a, Creasy *et al.* 2006, Todic *et al.* 2007). Vine growth and production in cool climate vineyards could therefore be improved by enhancing the vineyard light environment.

The effects of light on grapevine development are closely related to how fruitful the vine becomes. Many trials have shown that an increase in leaf area will result in a subsequent increase in yield (Shaulis *et al.* 1966, Kliewer and Lider 1968, Hopping 1977, Kliewer and Smart 1989, Sommer *et al.* 2000). In the season preceding flowering and fruit-set, weather conditions during a period of three weeks in spring are highly influential on bud fruitfulness (Baldwin 1964). It is during this time that the anlagen form in the apex of a latent bud. The anlage may differentiate into a number of different structures, including tendrils, inflorescences, shoots or transitional forms of each of those (Dunn 2005). While tendrils are connected with vegetative growth, inflorescences are involved in the reproductive activity of the vine as they first develop into flowers and subsequently into berries. Differentiation of the anlagen into a shoot is rarely seen (Dunn 2005). Srinivasan and Mullins (1981) showed that greater light intensity and warmer temperatures during this time period caused increased branching, leading to the formation of inflorescences as opposed to tendrils. By contrast, direct shading of the buds during the period at which differentiation takes place has been found to reduce the formation of inflorescences (May and Antcliff 1963). Shoot crowding has also been found to reduce bud fruitfulness as it causes shading within the canopy (Shaulis and May 1971). May *et al.* (1976) additionally found that the choice of a training system could influence bud fruitfulness. It was discovered that where canes were allowed access to full light exposure, this increased the productivity of those canes.

Light and also temperature are two of the main variables influencing vine performance throughout the season. Light affects vine fruitfulness in the subsequent seasons and vegetative growth is affected through the accumulation of dry weight or storage of reserves shortly before the vine enters dormancy (Howell *et al.* 1994). Light also has a major influence during berry ripening and affects the development of phenolic compounds in the fruit (Keller and Hrazdina 1998, Price *et al.* 1995, Haselgrove *et al.* 2000, Kolb *et al.* 2003). These compounds are important for wine quality parameters and contribute to wine aroma and flavour (Jackson and Lombard 1993). They also affect mouthfeel, colour and the ageing potential of the wine (Jackson and Lombard 1993). Temperature affects vines throughout the season and in general a warm environment is important for differentiation (Dunn 2005), fertilisation (Staudt 1982), flowering (Krstic *et al.* 2005), fruitset, berry development and ripening (Srinivasan and Mullins 1981).

The radiant spectrum can be measured using a spectroradiometer. Spectroradiometers allow an accurate measurement of a wide range of the radiant spectrum from the ultraviolet through to the infrared. This type of equipment was chosen in this trial because it was able to detect the quality of light being reflected from the mulches. Spectroradiometers work by taking spot measurements of light. A beam of light that had reflected off the mulch passed through the screen, it then passed the diffraction grating where it was split into each of its component colours. Finally each of the individual light components were analysed by the photodetector to give a reading of their intensity.

Cool climate vineyards operate on the margins of wine production and are exposed to higher levels of climatic variation, but they have the potential to produce highly distinctive wines (Bulleid 2000). Variations in light and temperature, especially in cool seasons, can cause variation in vine health, yield and fruit quality. It is therefore important especially in cool climate growing areas, to understand how it might be possible to manipulate the vineyard environment towards increasingly favourable conditions. With this it will be possible to gain greater consistency in the vineyard, to guard quality parameters and to maintain healthier vines from season to season.

2.2 Mulch application in the vineyard

Mulch is a type of ground cover that may be made from various materials and is used in vineyards for reasons that vary from soil amelioration to improving canopy microclimate. One of the greatest challenges faced by viticulturists involves maintaining a healthy and productive soil. In cool climates, viticulturists are also faced with the challenge of gaining fruit that is consistently ripe enough to make top quality wine. Depending on the situation, mulch can be a viable option towards amending these issues.

Three main types of mulch exist. Organic mulches are made from materials such as grape marc, straw, compost, vine prunings, green waste, animal manure, mussel shells or combinations of the above. Living mulches are grown in the vineyard most commonly between the vines, but sometimes also beneath them. Finally mulches may also be made from inorganic materials such as plastic, stones or glass.

Organic mulches are often applied to retain soil moisture, release nutrients, suppress weeds and increase yeast available nitrogen in grape juice (Agnew and Mundy 2002). Mundy and Agnew (2001) also reported an increase in total soil fungal colonies where organic mulch was applied. Watson (2006) found that where compost and straw were used as mulch in dry vineyards in Australia, water retention increased and crop loads were up to 40% higher. Soil

biodiversity also improved and potassium deficiency was reduced. Paper, another organic material, used as mulch was found to have the benefit of strengthening grape berry skins to help prevent diseases such as *Botrytis cinerea* (Jacometti *et al.* 2007).

Living mulches are also applied in the vineyard to amend soil nutrient and moisture levels. They compete with weeds, prevent erosion (Hartwig and Ammon 2002) and may also increase vineyard biodiversity, which can in turn encourage beneficial insects to prevent disease (Nicholls *et al.* 2004).

Inorganic materials have a more physical impact on the soil and microclimate and may be used to alter aspects such as light and heat. One trial in Switzerland that investigated the application of gravel mulch found that where the mulch was used soil temperatures were higher and vines received additional radiation, associated with increased crop loads of higher quality fruit (Nachtergaele *et al.* 1998). A number of trials have investigated the effects caused by mulches made from synthetic products such as white plastic woven sheeting (Hostetler *et al.* 2007a,b, Sandler *et al.* 2009) and aluminised sheeting (Robin *et al.* 1997, Razungles *et al.* 1997, Coventry *et al.* 2005, Todic *et al.* 2007). These mulches have been shown to increase photosynthetically active radiation (Coventry *et al.* 2005, Sandler *et al.* 2009) and temperature in the fruiting zone (Robin *et al.* 1997). Canopy density was found to increase over light coloured mulch (Sandler *et al.* 2009) and increases in crop load have been reported (Robin *et al.* 1997, Hostetler *et al.* 2007a,b).

Wine quality has also been altered by the application of reflective mulches in the vineyard. Changes have been found in wine aroma (Razungles *et al.* 1997, Reynolds *et al.* 2007) and tannin concentration (Robin *et al.* 1997, Coventry *et al.* 2005). By contrast, where dark mulches were tested, Hostetler *et al.* (2007a) found that they absorbed radiation as opposed to reflecting it. Black geotextile mulch used in this trial also reduced weeds, increased soil moisture retention and increased vine growth (Hostetler *et al.* 2007a). Colours of mulch other than black and white have also been trialled. Kasperbauer (2000) investigated the affects of red plastic mulch beneath strawberries. The mulch altered the red:far red radiation ratio, and, via phytochrome, caused the plant to produce higher levels of photosynthate. This aided in the development of larger berries and resulted in a higher yield. This trial also found berries with red mulch had higher sugar to organic acid ratios and emitted higher concentrations of flavour and aroma compounds (Kasperbauer 2000, Kasperbauer *et al.* 2001).

There are potentially many positive outcomes from the application of mulch in the vineyard, but some negative aspects however, also exist. Mulches can be expensive, messy and may break down quickly limiting their usefulness. They may also obstruct mechanisation of the vineyard. It is therefore important to choose the right type of mulch and to match it carefully

to the situation and towards the desired outcome. Mundy and Agnew (2001) advise consideration of the availability of resources including space, time, staff, equipment and expertise when using mulch.

2.3 Mulch and the soil

Mulch can impact on the soil in a variety of ways. As it is a product that is placed on the soil surface it can have a large impact, especially in the soils' upper layers. Aspects that may be affected include soil temperature, moisture levels, nutrition and the presence and activity of microbes. The impact on soil temperature by mulch depends on the material the mulch is made from and its colour. Agnew and Mundy (2002) tested soil temperature to 10 cm depth under bare earth that had organic mulch applied to it and found that where organic mulches were used, these could buffer soil temperatures. During summer they found that treatments with bare earth could fluctuate by up to 13°C, while mulched soils only changed by up to 1.5°C. Hostetler *et al.* (2007a) investigated black and white geotextile mulches, bark and bare earth and found that the bark was cooler in the early growing season and warmer later in the season, compared to other treatments. At the end of the growing season, treatments that had been mulched had soils that were several degrees warmer than the bare earth treatment (Hostetler *et al.* 2007a). Creasy *et al.* (2003b) investigated the effect of different mulches on soil temperature in the Canterbury area. In that trial, clear and white plastic mulches were tested alongside white polystyrene mulch covered with white plastic. A control of bare earth was also included in the trial. The clear plastic was found to increase the soil temperature compared to the un-mulched control, while the polystyrene mulch reduced the soil temperature (Creasy *et al.* 2003b). Soil beneath the white plastic mulch experienced similar temperatures to the control. In the first year the clear and white mulches were found to have increased the number of caps per bunch compared to the polystyrene mulch. Percent fruitset was also higher for these treatments compared to polystyrene. The clear plastic mulch was additionally found to have higher fruitset than the control. These results suggested that increasing soil temperature could have a positive influence on fruit set.

Agnew and Mundy (2002) and Watson (2006) observed that soil moisture retention especially in the upper soil layers was increased by mulch. They also observed that, as moisture was lost from the soil through evaporation, the loss was delayed by up to six weeks in the mulched treatments. Mulches normally increase the moisture levels in the upper regions of the soil as the cover from the mulch prevents evaporation from this area (Agnew and Mundy 2002). As a result of moisture retention, the use of irrigation can be delayed, thus saving water and its application costs (Agnew and Mundy 2002, Watson 2006). Increased

soil moisture content may additionally reduce erosion and improve the structure of the soil (Agnew and Mundy 2002).

Mulches can also alter soil nutrient levels and this is especially true for organic (Agnew and Mundy 2002) and living mulches (Hartwig and Ammon 2002). Inorganic mulches are less likely to change nutrient levels initially though changes might be seen over time, especially if these mulches prevent the growth of weeds, allowing nutrients to become available to the vines (Hostetler *et al*. 2007a). The availability of nutrients is dependent on a number of variables, which include temperature and moisture levels (Paul 2007), but also soil pH (Lanyon *et al.* 2004) and the presence and activity of soil micro-organisms (Paul 2007). Lanyon *et al.* (2004) reported that tillage could reduce the number of microbes present in the soil due to the disruption of the habitat of these beneficial organisms. The use of mulch offers one method to amend soil health without disturbing the soil environment.

The soil is influenced by, and functions as part of, a larger ecosystem due to diverse and complex communities of micro-organisms that live within the soil. Communities of microbes are continually in the process of recreating, enhancing and transforming the soil environment. The soil provides a substrate through which essential nutrients, minerals and gases must pass and micro-organisms are required for the successful completion of processes such as decomposition, humification and mineralization (Paul 2007). Micro-organisms therefore help control the availability of vital nutrients and carbon and are centrally important to the sustainability of the plants and animals that live within and around the soil environment (Bardgett 2005). Ultimately micro-organisms determine the productivity of natural and managed ecosystems and are fundamental in sustaining life on earth.

Two experiments were carried out in this thesis work, one to estimate the presence of microbes in the soil and another to investigate their activity. Microbial biomass carbon is an important indicator of soil fertility (Beck *et al.* 1997). Determination of biomass carbon allows an understanding of the size of the microbial population. It is based on the idea that there is a correlation between carbon extracted with potassium sulphate, then liberated by fumigation with chloroform and the microbial biomass carbon in the soil (Vance *et al.* 1987). The experiment is carried out twice, once with a fumigation step and once without, the difference between the two gives the microbial biomass carbon.

Dehydrogenase enzyme activity is an assay used to determine how active the microbes in the soil are. Oxidative energy transfer between microbial cells involves dehydrogenases (Friedel *et al.* 1994). During microbial metabolism, oxidative activity in the soil is related to dehydrogenase activity (Friedel *et al.* 1994). By measuring this, it is possible to determine the rate of metabolism of the microbial biota and this gives an idea of how active the

microbes are (Friedel *et al.* 1994). In the dehydrogenase enzyme activity assay, an alternative electron acceptor (water soluble tetrazolium salts) are added to the soil sample (Friedel *et al.* 1994). Almost all micro-organisms are able to reduce these salts to triphenyl formazan (Alef 1995). The water insoluble formazans are chosen because they are coloured and the rate of reduction can therefore be measured using spectrophotometry (Alef 1995).

Soil measurements are of primary importance as mulches are applied directly onto the soil surface. Mulch therefore has a direct impact on the soil and this interaction will potentially affect soil temperature, moisture, nutrients and micro-organisms. Each of these parameters subsequently effect and are important for vine performance.

2.4 Reflective mulch

The other direct impact of mulch on the vine environment, especially from reflective mulch, is related to the radiant light environment. As has already been discussed, sunlight and warmth are both essential factors in the development of ripe fruit. Warmer temperatures and sunlight generally produce fruit with balanced levels of acidity and sugar, and wines with higher levels of colour, flavour and tannin (Jackson and Lombard 1993). A number of trials have investigated the effects of reflective mulches with mixed results. Extenday® is made from white reflective woven material. Where this mulch was used on Clara Frijs pear trees, it was found that mulched trees had twice as many flower buds as control trees (Bertelsen 2005). Tests using the same product with kiwifruit showed higher levels of photosynthesis in treated plants and a higher yield and average fruit weight (Costa *et al.* 2003). Grout *et al.* (2004) found that Extenday® positively affected growth of apples by increasing fruit number and harvest weight, although no significant effects were recorded for apple size or colour. Reflectance off the mulch was found to have higher levels of photosynthetically active radiation and had a higher red to far red ratio compared to non-mulched plots (Grout *et al.* 2004). Mulch treatment rows had increased numbers of flower buds, which accounted for higher yields from these trees (Grout *et al.* 2004).

When tested on grapes growing in New England, Extenday® was found to increase levels of photosynthetically active radiation and canopy density, however no significant effects were observed on fruit composition (Sandler *et al.* 2009). In that trial it was also reported that the mulch soiled easily and its reflectance was reduced during the season (Sandler *et al.* 2009). Hostetler *et al.* (2007a,b) found that woven white geotextile mulch reflected more light into the canopy than control treatments, but not as much as aluminised mulch that had been tested in other trials. The geotextile mulch suppressed weed growth, which was thought to have led to higher levels of calcium and nitrogen in leaf petioles of these vines. Berry set

increased in mulched vines resulting in significantly higher yields. No significant differences were found however, between control and mulch treatments for timing of veraison, levels of soluble solids, pH or titratable acidity. Similarly in the berries, must and wine, no significant differences were noted for anthocyanins or total phenolics. Todic *et al.* (2007), who also investigated the effects of plastic foil, examined the effects of silver, white and red coloured mulch. Cabernet Sauvignon vines were tested in that trial, where it was found that the white mulch significantly increased levels of anthocyanins in grape skins and berry soluble solids. No effects were found for berry phenolics, though wine was not tested in that trial.

Other trials that looked at reflective mulches tested the effects from aluminised sheeting. One trial carried out by Robin *et al.* (1997) tested a product called Vitexsol® in France. This was found to increase incident solar radiation in the fruiting zone by at least 20%. It was reported that surface berry temperatures were on average 1.5 – 2°C higher than control berries during the ripening period. The mulch also increased crop load as its effect at bloom increased the number of berries per cluster and the weight of individual berries. Treated vines had higher pruning weights indicating a higher level of reserves for these vines. The increase in light additionally reduced the number of berries affected with *Botrytis cinerea* (Robin *et al.* 1997). Berry sugar levels were found to be higher in vines treated with the reflective mulch, as were total polyphenols and free amino acids. Wines made from berries that had been exposed to the mulch treatment were found to have improved characteristics for appearance, colour, typicity and sweetness; only acidity was not significantly affected. Razungles *et al.* (1997) also carried out a trial on the effects of aluminised sheeting on grapevines. It was discovered that Syrah berries from vines treated with the mulch had higher levels of carotenoids at veraison. These decreased most in the lead-up to harvest, causing a larger production of C13-norisoprenoids. The authors speculated that these compounds were accountable for the higher quality and intensity of aroma in the wines made from mulched grapes.

Reynolds *et al.* (2007) who also tested the effects of aluminised mulch on grapevines, found little effect on red varieties. In a tasting, the red wines were found to have slightly reduced vegetal sensory aspects. The only white variety tested, Riesling, was found to have significantly higher levels of monoterpenes in a quantitative analysis of the fruit. Sandler *et al.* (2009) also tested aluminised mulch on grapevines but found the product to have no significant effects on canopy density, yield or fruit composition. The mulch also deteriorated during winter, tore easily and was susceptible to oxidation. This resulted in the reduced efficacy of the reflective surface of the mulch by the end of the trial. Coventry *et al.* (2005) who investigated the effects of aluminised polyethylene sheeting on Cabernet franc found similar results, whereby the product deteriorated during the season, reflecting 40%

photosynthetically active radiation at the outset of the project, but only 10% at the end. The mulch, however, was found to significantly advance veraison in these grapes and increase levels of total phenolics, flavonols and anthocyanins.

Another product that has been subject to testing for its reflective properties in the vineyard is mussel shells. Shells were applied for trial in Nelson, New Zealand in 2003 (Creasy *et al.* 2007). Crawford (2007) found soil temperatures at 10 cm beneath the mulch to be cooler and that moisture retention was increased by the shell mulch. Nutrients were tested in the upper 15 cm of the soil, and calcium levels were found to be higher in mulched vines as were soil pH and sodium levels. The mussel shells were found to significantly improve shoot growth and canopy density, and pruning weights were slightly higher in treated vines. The shells significantly improved maximum canopy temperatures, while minimum temperatures over all treatments were the same. The mulch was found to advance flowering and veraison and crop loads in the shell treatment were lower with lower berry weights recorded. Levels of UV A (315 – 400 nm) and UV B (280 – 315 nm) were significantly higher in mulch treatment areas and SPAD measurements showed that chlorophyll levels in treated vines were also significantly higher at the end of the season. At a blind tasting of the wines produced from that trial, Crawford (2007) reported that 74% of tasters preferred the shell microvinification wines. These wines were rated as smoother and more complex with greater texture, heat and less perceived acidity. The wines also had less drying and unripe tannins. When commercial shell wines were tasted however, 66% of participants preferred the control wines. It was felt that the acidity in the commercial shell wines was the outstanding feature (Crawford 2007). Commercial shell wines were reported to be less drying and harsh, but were also found to be less dynamic with less texture (Crawford 2007). The difference in preference may have been caused by the different wine making techniques in each case. Control and shell microvin wines were inoculated with the same strain of yeast and had the same additions of tartaric acid, DAP and superfood nutrient. Microvin wines were also inoculated for malolactic fermentation (Crawford 2007). Commercial wines by contrast were treated as per company policy with a wild fermentation allowed to take place. Natural malolactic fermentation also occurred in these wines, and the wines were treated differently during the winemaking process (Crawford 2007).

Leal (2007) carried on research in the same trial that Crawford (2007) used. In this season it was found that the mulch had a cooling effect on the soil beneath the vines, buffering extremes of temperature. The mulch also reduced the number of weeds in the under vine area. Canopy temperatures increased during the day compared to control vines and it was cooler during the night. No differences were noted in canopy vigour. Calcium in a leaf petiole test was found to be higher in treated vines. SPAD values were lower for the shell vines at

budburst but higher in vines mulched with shells at veraison, pre- and post harvest. Crawford (2007) did not test SPAD values of vines at budburst but found similar results to Leal (2007) were the SPAD values were higher at veraison and pre harvest for the shell vines. Following harvest, there were no significant differences between control and shell vines, possibly due to a late season outbreak of powdery mildew. Levels of UV A, UV B and photosynthetically active radiation increased with use of shell mulch. Budburst was not advanced by the treatment, however, flowering and veraison were. Berry and bunch weight and also vine yield were lower in shell treated vines, but larger numbers of individual berries were found. In the shell microvin wines, levels of quercetin and resveratrol were found to be higher than the control. For commercial shell wines, lower levels of epicatechin, gallic acid, resveratrol and t-catechin were found compared to the control. Sensory analysis of wines from this vintage showed that compared to the un-mulched treatment, shell wines overall had lower levels of green and unripe tannins and greater smoothness and complexity.

In the final report on the two mussel shell trials at Neudorf it was concluded that the shells had affected changes in vine performance and on fruit and wine quality (Creasy *et al.* 2007). The most important effects listed included changes to phenological growth stages and to the sensory perception of the wine. Mulches were generally found to have positively influenced the phenolic and aroma profiles of the wines produced (Creasy *et al.* 2007).

Sandler *et al.* (2009), whose trial testing Extenday® and aluminised sheeting has already been mentioned, also tested the effects of Quahog shells on wine grapes. Benefits included the use of a by-product from the local fishing industry, and that the shells were also more permanent than the other mulches tested in their trial. The shells were found to increase calcium in the soil, which is in accordance with trials carried out by Crawford (2007) and Leal (2007). The increase in calcium led to an increase in berry pH and increased calcium to magnesium ratios. In addition to these findings, Sandler *et al.* (2009) also found increases in canopy density, yield, cluster number, cluster weight and °Brix. However results were not consistent and were only recorded for some of the cultivars in some of the years tested.

Reflective mulches appear to have the potential to give the benefits of other mulches such as weed suppression and water retention, whilst additionally improving the vines' access to radiant energy. Effects vary according to the material used, vine cultivar and the area in which the trial took place. The ability of these mulches to increase radiant energy in the vineyard, especially in cool climate vineyards, is important however. Increasing levels of radiant energy in the vineyard in many cases appears to have had corresponding positive effects on production as well as fruit and wine quality parameters.

2.5 Effects on wine and juice

Wine aroma is an essential component in the perception of wine quality; it can either enhance or reduce the hedonistic value of a wine. The quality of a wine's aroma is directly related to the quality of the grapes received at harvest, and this in turn is connected to ripeness (Conde *et al.* 2007). Environmental conditions and viticultural practices are fundamental in gaining high quality fruit (Jackson and Lombard 1993). Although the impacts of the mesoclimate are beyond the manipulation of the viticulturist, various strategies are available to improve the vineyard environment. Many of the methods are applicable at the microclimate level, such as exposing leaves or fruit to radiant energy through canopy modification (Smart 1985b). Wine flavour attributes also add or take away from the perception of a wine's quality. Like aroma, the quality of a wines flavour is equally related to the quality of the grapes brought in at harvest. Johnstone (1996) links flavour quality to a range of variables that exist in the vineyard, including the genetic make up of the vines and the vineyard environment.

Pinot noir, the cultivar used in this trial, is a cool climate grape variety that is most well-known for the wines produced in Burgundy, France. It is a cultivar that has a highly complex aroma profile, which is formed by a matrix of odours some of which have very low concentrations (Fang and Qian 2006). Due to this complexity little is known of the most important aroma compounds that give this wine its typicity (Fang and Qian 2005). Pinot noir is an important grape variety in New Zealand. According to the New Zealand Winegrowers annual report (2009) Pinot noir is the second most planted variety in the country and the second highest variety to be exported.

Juice and wine aroma were chosen in the Sandihurst trial as factors to identify how the reflective properties of the mulch treatments had affected the fruit. Aroma can be measured in a number of different ways including gas chromatography-mass spectrometry (GC-MS) and gas chromatography (GC). It is not always possible to detect aromas with conventional technology however (Plutowska and Wardencki 2008). For the detection of these elusive compounds it is necessary to use the more sensitive human nose. GC-O (gas chromatography-olfactory) combines analysis by gas chromatography with human detection. The one drawback of the use of the human nose is the introduction of a subjective analysis. Conventional methods that use machines objectively quantify aroma compounds, but by contrast humans introduce variation as no two noses are alike (Thorngate 1997). This variation must be taken into account in the interpretation of results.

Use of the GC-O for aroma detection has been described by Plutowska and Wardencki (2008). The odour or analyte leaves the chromatographic column and is split at the column

flow splitter thus allowing the analyte to reach two detectors at the same time. The analyte is then compared and evaluated between human detection and instrumental analysis. The concentration at which the compound becomes apparent is subsequently established. GC-O has various uses including the determination of human odour thresholds (Marin *et al.* 1988), and also in the investigation of the quality and intensity of various aromas. GC-O has been used to analyse fruit juices (Jordan *et al.* 2002, Qiao *et al.* 2008) as well as odour active compounds present in various beverages such as wine (Miranda-Lopez *et al.* 1992, Marti *et al.* 2003).

Wine acidity, a parameter linked to wine balance and one that is important for the ageing potential of the wine, is also determined in part by climatic factors (Jackson and Lombard 1993). A number of different acids are present in grapes (Fowles 1992). Their levels change during the growing season and their concentration is largely temperature dependent. The main wine acids, tartaric and malic, make up approximately 90% of a wine's total acidity, other important acids found in wine include citric and amino acids that are found in grapes (Fowles 1992). Lactic, succinic and acetic acid are generated during fermentation. During ripening, malic acid concentration reduces as sugar levels increase. In general, warm climates produce berries that have higher sugar levels and less acidity (in particular less malic acid) (Jackson 2000). Rathburn and Morris (1980) noted that the drop in acid levels is more pronounced in regions that experience warm nights. In cool climate regions, leaner, more acidic wines are made. White varieties are more frequently grown and alcohol levels may be lower (Jackson and Lombard 1993). The longer ripening period experienced in cooler climates is thought to allow for the development of more flavour and aroma compounds (Shaw 1999). Various techniques are available for the measurement of organic acids in wine, and they include volumetric, distillation and enzymatic methods (Frayne 1986). High performance liquid chromatography (HPLC) is also used and has been found to be a highly reproducible, reliable and stable method (Frayne 1986). The instrument is used for the quantification of must and wine compounds including major organic acids, sugars and alcohols (Frayne 1986). The HPLC method involves an isocratic separation using a strong cation exchange resin that divides the organic acids from the sugars and alcohols contained in the juice or wine (Frayne 1986).

Phenolics are some of the most important factors affecting a wine's mouthfeel, colour and the ability for a wine to age (Jackson and Lombard 1993). They derive from cluster stems, grape skins and seeds and because of wine making techniques are more prominent in red than white wines. Phenolics can be placed into two major groups: flavonoids and non-flavonoids (Waterhouse 2002). The flavonoids have multiple aromatic rings with hydroxyl groups and include compounds such as catechin, epicatechin, and quercetin (Waterhouse

2002). Quercetin is an example of a flavonol and is found in the berry skin. Concentrations of quercetin are increased with increases in sunlight exposure and it is thought that this compound screens the sunlight in order to protect the fruit (Price *et al.* (1995). The non-flavonoids are hydroxycinnamic acids, benzoic acids, hydroyzable tannins (from oak) and stilbenes such as resveratrol (Waterhouse 2002). Resveratrol is the principal stilbene found in grapes (Langcake and Pryce 1976 and 1977) and is thought to have health benefits such as reducing heart disease in humans (Trela and Waterhouse 1996). It is produced in response to fungal attack, injury or with exposure to UV light (Frankel *et al.* 1993). In order to achieve the highest quality fruit for winemaking it is important that phenolic ripening coincides with the ripening of other berry components such as sugars and acids. Response of berry phenolic compounds such as quercetin (Price *et al.* 1995) and resveratrol (Langcake and Pryce 1976 and 1977) to light is an important factor in the ripening of the berries and in the quality of the finished wine.

Anthocyanins are responsible for red wine colour (Jackson 2000). Many reports have detailed the positive influence of berry exposure to sunlight on the development of anthocyanins and phenols in the fruit (Price *et al.* 1995, Dookoozlian and Kliewer 1996, Keller and Hrazdina 1998, Haselgrove *et al.* 2000, Bergqvist *et al.* 2001, Spayd *et al.* 2002, Pereira *et al.* 2006). While anthocyanins are important in the colouration of wine, phenolics are responsible for colour stability (Haselgrove *et al.* 2000). They contribute to flavour and mouthfeel attributes of wine and some are additionally thought to provide cardio-protective health benefits (Conde *et al.* 2007).

Like acids, phenolics can be measured using HPLC. It is also possible to estimate wine colour and phenolics using the red wine colour and phenolic assay developed by Iland *et al.* (2000). The assay, which was used in this thesis work, is based on the fact that only a portion of pigments are in red coloured forms at any stage during development. This means that when absorbance is measured by spectroscopy at 520 nm it is possible to gain an estimated value for the concentration for the wine anthocyanins that exist in the red coloured range (Iland *et al.* 2000). Acetaldehyde is added to wine to gain an estimated value for red coloured pigments at 520 nm. Addition of sulphur dioxide to the wine provides an estimate of those pigments that are resistant to bisulphur bleaching at 520 nm. The wine is also analysed at 420 nm for an estimate of yellow brown pigmentation. Finally wine is diluted with hydrochloric acid and the sample is analysed at 520 nm to estimate total red pigment colour. The same sample is analysed again at 280 nm to give an estimate of total phenolics. Though the Iland *et al.* (2000) assay is useful to gain an idea of the difference between wines a more accurate measurement is gained using HPLC.

The possibility to introduce more light into the vineyard environment means the concentration of certain aroma compounds could be enhanced, and the development of acids and phenolics could be altered. Change to any or all of these factors will have an influence in the wine that is produced.

2.6 Recycled glass

The use of glass as a mulch in vineyards is an innovative solution to a problem involving the build up of this material in recycling stations around New Zealand. New Zealand is currently affected by a glass recycling crisis due to the fact that the amount of glass collected exceeds the capacity to process it (Anon 2009a). The crisis has particularly affected the South Island, as the only smelter in the country that can process all the glass collected is situated in Auckland. Glass must therefore be transported large distances and this has proven to be uneconomical in many cases. Glass is commonly used to package a range of products and its use has been steadily increasing (Anon 2009a). Owens-Illinois based in Penrose, Auckland processes all of New Zealand's glass waste and has just expanded to meet increasing demand. However, it has been reported that even with an increase in capacity there will still be an excess of glass waste waiting to be processed (Anon 2009a). While some of the surplus glass is shipped to Australia, what cannot be processed or exported elsewhere continues to build up in the yards of small recycling operators. Linda Norris CEO of SIFT (Sustainable Initiatives Fund Trust) estimated that only 20% of glass waste was accounted for in curbside collection with the rest either sent to landfills or shipped overseas (personal communication, December 2008). There are a number of potential benefits for using glass mulch in the vineyard. Its application completes a cycle between the wineries' use of the material to package its product and the possibility to reuse the material to take advantage of the sun's energy in the vineyard. Glass is made up mostly of silica, a material similar to sand, that is largely inert and therefore unlikely to react with any of the soil components. Glass breaks down very slowly, is heavy and is unlikely to move from the area in which it was being used. Applied as mulch, glass has the potential to last for an extended period of time, particularly in comparison to organic mulches that break down quickly. Deterioration of glass and shells is also not as much of an issue as that seen for products made from plastic or aluminium. These materials can be adversely affected by the wind tearing them easily and collecting dirt, which reduced potential reflectance (Coventry *et al.* 2005, Sandler *et al.* 2009). Mussel shells, which have been included in the trial at Sandihurst, have already been tested elsewhere (Crawford 2007, Leal 2007) and are to be used as a double control alongside a true control of bare earth. As results have already been

collected from the shells they form a point of reference in the trial and present another reflective material to which the glass can be compared.

Mulches have been promoted as a method of sustainable viticulture (Agnew and Mundy 2002). Benefits such as reducing moisture loss from the soil, reducing weeds and increasing nutrients can reduce reliance on irrigation, herbicide and fertiliser (Agnew and Mundy 2002). Reflective mulches offer greater access to radiant energy in the vineyard and therefore the potential to increase production and quality of fruit and wine. Produced from waste products, the mulches are also sustainable in a broader sense, potentially helping to reduce waste streams in New Zealand. This trial will test recycled crushed glass as a reflective material in the vineyard for the first time. In line with the current recycling crisis in New Zealand, and because wineries use glass as packaging for the wine they produce, this trial is a unique attempt at finding an alternative use for this waste product in the vineyard.

Chapter 3 - Reflective mulch effects on the grapevine environment and Pinot noir vine performance

3.1 Abstract

The effect of different reflective mulches on the grapevine environment and Pinot noir vine performance was investigated in a cool climate vineyard in Canterbury, New Zealand. Mulches were applied in 2005 as four randomised treatments divided over three replicated blocks. The treatments included a non-mulched control (CON) and mulches made from waste products including mussel shells (MS) and green (GG) and clear (CG) recycled crushed glass. MS and CG were light coloured while CON and GG were dark. Mulch effects on soil temperature, soil moisture, microbial biomass carbon, microbial activity and soil nutrients were also examined. Vine canopies were tested for nutrients, photosynthesis and leaf greenness. Fruit pH, titratable acidity, °Brix and yield parameters were tested. Pruning weights were measured, as was canopy temperature and reflected radiation from the four treatments. Mulches were found to have a direct effect on soil parameters. MS buffered soil temperature and all of the mulches were found to increase soil water retention. This was most apparent for MS, which had higher values throughout most of the growing season. CON showed the most fluctuation in soil water content. MS was found to have higher microbial biomass carbon than CG and GG. No significant differences were found for microbial activity. Soil pH levels were increased by MS while sodium levels were highest for GG. Higher levels of vine canopy boron, copper, potassium, molybdenum, phosphorous and sulphur were found for mulch treatments. No differences were noted for photosynthetic rate although differences were found for related gas exchange parameters across mulches suggesting that the mulches had affected water use by the vine. No differences were noted for leaf greenness or for any of the fruit quality parameters tested. However, cluster number and fruit weight were higher for light, compared to dark treatments. Pruning weights were also highest for lighter coloured treatments, possibly due to greater availability of radiation and water to these vines. All of the mulches were found to reflect more solar radiation into the canopy than CON. No treatment differences were found for canopy temperature at any stage during the growing season. Light parameters did however reveal highly significant differences. Photosynthetically active radiation (380 – 760 nm) was found to be higher for light compared to dark treatments. A similar pattern was noted for red to far red ratios. Finally MS, CG and CON had higher readings in the infra-red spectrum (>750 nm), while GG absorbed, rather than reflected, the most energy at these wavelengths. The findings in this trial suggest that reflective mulches increase vine access to solar radiation in the vineyard.

This has been found to improve certain aspects of the vine environment and microclimate. Findings suggest that the application of reflective mulches made from MS and CG could improve vine health and production. The mulches additionally offer an alternative use for waste products in New Zealand.

Keywords: Reflective mulch, Pinot noir, mussel shells, recycled glass, cool climate viticulture, vine performance, solar radiation.

3.2 Introduction

Mulches are most often applied in the vineyard to improve aspects of the vine environment and subsequently vine performance. Mulch has a direct impact on the soil as it is applied to the soil surface, which influences soil temperature, moisture, microbial populations and nutrition. Mulches have been found to buffer the soil, preventing large fluctuations in temperature (Agnew and Mundy 2002). They can also increase soil moisture retention (Watson 2006, Cox *et al.* 2004, Penfold 2004), and there is the potential to delay the use of irrigation (Mundy and Agnew 2001). Mulches have also been reported to positively influence the size and activity of microbial populations (Cox *et al.* 2004). The role of microbes is particularly important as they carry out decomposition of organic matter in the soil (Coleman *et al* 2004). Virtually all of the soil nutrients pass through microbial communities and these communities perform the various functions and processes that are necessary to support life in the ecosystem (Bardgett 2005). Microbes transform soil organic matter and aid soil respiration. They also carry out carbon sequestration, pollutant mitigation (Bardgett 2005) and help to develop soil structure (Abbott and Murphy 2003). They carry out biogeochemical cycling of carbon, nitrogen, phosphorous, sulphur and other nutrients (Bardgett 2005). It is through the presence and activity of microbes that nutrients become available to the vines (Pinamonti 1998). As well as microbes, soil moisture and pH also affect nutrient availability to vines (Lanyon *et al.* 2004). Mulches affect both of these factors and the degree by which the soil changes, is dependent on the material the mulch is made from. For example, less moisture can pass through plastic mulch compared to mulch made from bark, which allows water infiltration (Hostetler *et al.* 2007a). Different materials also influence changes in pH. While organic materials are less likely to affect this aspect of the soil (Agnew and Mundy 2002), mulching with alkaline products such as limestone or shells will generally cause pH levels to increase (Yokotsuka *et al.* 1999, Crawford 2007, Leal 2007).

In addition to the benefits of mulch application, reflective mulches influence vine access to radiant energy and have the potential to reflect additional radiation as light and heat into the grapevine canopy. An increase of these wavelengths, which are vital for vine performance,

can influence photosynthesis (Smart 1973), dry matter accumulation (Cartechini and Palliotti 1995) and also berry development and ripening (Smart 1987, Bergqvist *et al.* 2001, Spayd *et al.* 2002). Especially in cool climate viticulture, the use of reflective mulches provides a means to exploit these essential elements. Radiation has a major impact on the growth and development of the vine, in particular impacting on the vine's ability to photosynthesise, where energy from the sun is converted into chemical energy (Smart 1974). Light can also impact on leaf greenness and senescence. In cool climates, an extended period of photosynthesis towards the end of the season, following harvest, can be important for carbohydrate storage (Howell 2001). These stored carbohydrates will be used for initial growth in the following season. Howell (2001) reported that where vines were over-cropped or if the canopy was very sparse, the leaves had higher photosynthetic rates and aged later than leaves from more balanced vines.

This ability of the vine to make up for a lack of balance has limits however. Mansfield and Howell (1981) demonstrated that vines that had been defoliated too early in the season suffered stress because of the lack of balance between sources (the leaves) and sinks (the fruit and at the end of the season, the woody parts of the vine). Bennett *et al.* (2005) also noted that defoliation could have a detrimental impact. It was discovered that defoliation in one season would negatively affect yield in the subsequent season because of the impact of leaf removal on winter carbohydrate storage. Edson *et al.* (1993) also demonstrated that leaf area was inversely related to crop load. These theories of balancing the vine system are summarised by Smart and Robinson (1991). The balanced cycle is described where the canopy to crop ratio is correct and the vine has enough access to useful radiation. This stimulates bud-break, bunch initiation, fruit-set and berry growth. The opposite cycle is the vegetative cycle, which is imposed by shade. This depresses bud-break, bunch initiation, fruit-set and berry growth, whilst promoting vegetative growth, leading to compromised yield and quality parameters. Access to radiant energy from the sun is an essential component of vine balance, affecting vine health and having a direct influence on the crop.

Many trials have reported that sun exposure can increase berry sugar content (Kliewer *et al.* 1967, Crippen and Morrison 1986, Mabrouk and Sinoquet 1998, Reynolds *et al.* 1986). Lower titratable acidity is also related to sun exposure (Kliewer and Lider 1968, Kliewer *et al.* 1967, Reynolds *et al.*1986). Anthocyanins and simple phenolics appear to be affected by an interaction between temperature and light (Mabrouk and Sinoquet 1998). Keller and Hrazdina (1998) found that berry ripening was reduced at low light levels. In their trial, reduced light had a negative impact on accumulation of total phenolics, flavonols, anthocyanins and sugars. Smart (1988) investigated the effect of red (660 nm) to far red (730 nm) ratios on berry development. These wavelengths are absorbed by plant

photoreceptors called phytochrome and indicate the quality of light reaching the different parts of the vine canopy (Franklin and Whitelam 2007). Smart (1988) found that shade which caused smaller red to far red ratios reduced photosynthesis, which in turn reduced berry ripening, sugar concentration, phenols and anthocyanins. Ammonium levels and titratable acidity also increased in shaded fruit though it was thought that these changes were due to quantity as opposed to quality of light. However, supplementation of red light (660 nm), which increased red to far red ratios, advanced fruit colouration and increased glucose and fructose levels in berries.

In this study, it was hypothesised that application of reflective mulches to the vineyard system would improve levels of radiant energy in the grapevine canopy. It was thought that this could positively affect vine balance by increasing the vines' access to radiant energy therefore positively influencing vine balance. It was also hypothesised that the reflective mulches would increase photosynthesis and therefore the accumulation of dry matter as well as improving aspects of ripening. The mulches were expected to buffer soil temperatures, prevent evaporation and increase soil moisture retention. Soil micro-organisms would be affected by mulch effects on soil temperature, moisture and organic matter content. It was also thought that pH would increase under the mussel shell mulch because of the amount of calcium carbonate contained within this product as was reported by Crawford (2007) and Leal (2007). Calcium carbonate is alkaline which could increase the soil pH thus affecting vine nutrient uptake (Lanyon *et al.* 2004). It was hypothesised that all of the mulches would affect soil nutrition and possibly also vine nutrition because of the materials they were made from and by their influence on temperature, moisture and micro-organisms.

Vine function is influenced by factors both within and exterior to the vine. Through its positive impact on the soil and radiant environment, reflective mulches have already been shown to influence the grapevine canopy, the fruit and wine that is produced (Razungles *et al.* 1997, Robin *et al.* 1997, Sauvage *et al.* 1998, Coventry *et al.* 2005, Crawford 2007, Hostetler *et al*. 2007a, Hostetler *et al*. 2007b, Leal 2007, Reynolds *et al.* 2007, Todic *et al.* 2007, Sandler *et al.* 2009). Vines with greater access to solar radiation and a healthier soil environment are likely to be healthier and more productive with higher quality fruit.

3.3 Materials and methods

3.3.1 Trial site and management

The trial was carried out at a vineyard positioned behind the Sandihurst Winery at West Melton, Canterbury. For statistical analysis 4 treatments were applied to 3 plots arranged in a complete block design, each plot comprised of 12 vines. The block was 22 rows wide and 18

bays long with north to south row orientation. Four vines were planted per bay at 1.5 metre spacing. There were 73 vines planted per row and rows had 3 metre spacing. The trial block covered 0.68 hectares (Appendix 1). The four treatments applied in the vineyard were: an un-mulched control of bare earth (CON), clear glass mulch (CG), green glass mulch (GG) and mussel shell mulch (MS) (Figure 3.1).

Figure 3.1 – Clockwise from top left treatments include: control, clear glass, mussel shells, green glass. It should be noted that the soil in the vineyard had clover growing in it by the end of the trial.

Mulch was originally laid down in December 2005. The first glass particles used were approximately 1 - 3 mm diameter. Larger particles, measuring 3 - 5 mm, were laid down in February 2008. It was thought that using the larger sized particles would improve the reflective qualities of the glass, as well as requiring less processing than the finer particles. Each treatment replicate was laid down over three bays. The mulch continued at bay ends for two vines and was laid down on rows running parallel to the trial vines on the side facing the trial vines (Figure 3.2). This additional mulching meant the vines in the trial were surrounded by the mulch as if the treatment had been applied to the entire vineyard. The

extra mulched vines (or buffer vines), were not examined in the trial. Treatments were applied randomly within each replicate.

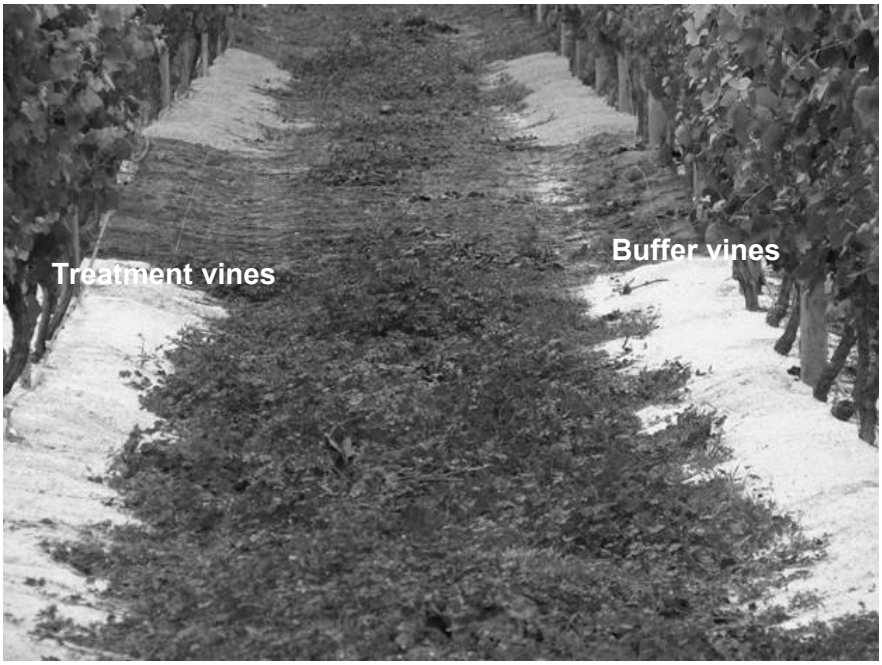

Figure 3.2 – Treatment (centre row of three) versus buffer (outside row, with mulch applied to only the inward side of the row) vines.

There were four treatments with three replicates each (Table 3.1). The trial included 141 vines in total, as three vines were missing from the trial area. Soil types in the area were Eyre shallow fine sandy loam and Templeton fine sandy loam with a moderately deep phase (Tindall 1978). The vineyard was irrigated with a drip irrigation system. During 2009 this system was only used during February at a rate of 4 L per hour for two hours on even numbered days. Growing degree days in the area are typically 900 to 1100°C (base 10°C) per year (Jackson and Schuster 1981). Average annual rainfall in the area is 600 – 650 mm per year (Kirk Bray, personal communication, December 2008).

Table 3.1 – Treatment layout of the reflective mulch trial at Sandihurst.

	Row 7	Row 11	Row 15	Row 19
Replicate 3	GG	MS	CG	CON
Replicate 2	CON	CG	GG	MS
Replicate 1	MS	GG	CON	CG

Vines were netted in March 2008 in the lead up to harvest, however some bird damage still occurred through the nets. Bird damage was recorded when yield measurements were taken and was factored in to give an idea of potential yield.

A frost which occurred in November 2008 caused severe damage to primary shoot growth. Following the frost it was decided that all primary growth of damaged and undamaged shoots should be removed in order to gain a uniform canopy of secondary growth (Creasy *et al.* 2002). Creasy *et al.* (2002) demonstrated that where a frost had occurred, removal of all primary growth provided a more balanced canopy that was easier to manage. Following the frost, a new focus was to concentrate on vine environmental and performance parameters as opposed to fruit and wine parameters. There was no harvest for the 2009 season.

3.3.2 Soil Temperature

Soil temperature was measured using Tiny Tag (Gemini Data Loggers (UK) Ltd., West Sussex, UK) data loggers connected to stainless steel probes of 10 cm length. Temperatures were recorded at 30 minute intervals. Data loggers were set up through the second replicate, so that only one set of soil temperature data was available. Without replication no statistics could be carried out on the results. Data loggers recorded information between 14 September 2007 and 24 January 2008; following this, loggers ceased collecting reliable data, so they were removed and no further information was collected.

3.3.3 Soil moisture

Soil moisture was measured using Time Domain Reflectrometry (TDR). A portable 6050X1, Trase System I was used with a 6002F1 Waveguide Connector. Twelve 6008CL45 coated waveguides were inserted into the soil in each plot throughout the vineyard to a depth of 40 cm. Readings were carried out on dry days during the season in January, February, March and April 2009.

3.3.4 Soil micro organisms, biomass and activity

The size and activity of soil microbial communities was determined by two separate experiments. Microbial biomass carbon was used to estimate the size of the microbial population and dehydrogenase enzyme activity was used to determine how active the microbes were in the soil.

3.3.4.1 Soil sample collection

Soil samples were collected from the vineyard at the beginning of March after a month of irrigation and above-average wet weather during February. The National Climate Centre

reported 165% of normal rainfall for Canterbury during February 2009 (Tait and Renwick 2009), which was reflected in the soil moisture measurements taken in the vineyard at this time. By the end of February, soil moisture levels were reported to have returned to near normal levels after a dry period in January (Tait and Renwick 2009). Soil samples were taken at this time in accordance with Forster (1995) who noted that seasonal variations including temperature and moisture have a large impact on activity and biomass of soil microbes. Forster (1995) mentioned that soil samples should not be collected directly after a period of drying or freezing as this can alter the availability of various nutrients including carbon.

All samples were taken on the same day, with three lots of approximately 100 g collected from each bay in the trial. These samples were then bulked and a sub-sample was taken, so that 36 samples were collected in total. Forster (1995) suggested that in order to achieve representative samples it is important to take as many individual samples as possible. These should then be analysed separately to cancel out heterogeneity and macro-scale variability. Samples were taken at a depth of 10 cm which was in the range (0-25 cm) suggested by Forster (1995) for agricultural soils. Samples were taken to Lincoln University and kept in sealed plastic containers at 3°C. The following day, 10 g of each sample was weighed out to three decimal places. These samples were used to determine soil moisture content by evaporation. They were put into an oven at 105°C for 24 hours and the dry samples weighed at the end of this time period. The difference between the wet and dry samples was used to calculate soil moisture levels, which would be used for the determination of microbial biomass carbon.

3.3.4.2 Microbial biomass carbon

Five days after the samples were collected, the measurement of microbial biomass carbon was carried out using the method by Cresswell and Hassall (2008, pg 16), which is based on Vance *et al.* (1987). Soil samples were weighted to 5 g, 20 mL of 0.5M potassium sulphate was added and they were then shaken for 30 minutes. One set of samples were filtered with Watman 42'' filter papers and stored at 4°C whilst another sample set were filtered with the same type of filter papers and fumigated with chloroform. All samples were analysed using a Shimadzu Total Organic Carbon Analyser TOC-5000(A) (Kyoto, Japan). The difference between the fumigated and non fumigated results was used to calculate the microbial biomass carbon.

3.3.4.3 Dehydrogenase enzyme activity

Fourteen days following collection of the samples, the measurement of dehydrogenase enzyme activity was carried out using the method by Cresswell and Hassall (2008, pg 48). This method is taken from Alef (1995) and is based on that of Thalmann (1968). In this

assay, the rate of reduction of triphenyltetrazolium chloride (TTC) to triphenyl formazan (TPF) is measured. Soil samples were weighted to 5 g suspended in 5 mL of TTC buffer was added. Test tubes were sealed and put into an incubator at 30°C for twenty-four hours. Following the incubation period, 40 mL of acetone was added. Samples were shaken and returned to the incubator set to 25°C for a further two hours and were shaken every half an hour. Samples were filtered through Watman 42'' filter papers and the filtrate was measured with a UV spectrophotometer at 546 nm. Results were compared to a standard curve. The standard curve was prepared using 50 mg of TPF dissolved in 100 mL acetone. This was distributed into six 50 mL volumetric flasks at volumes of 0, 10, 20, 30, 40 and 50 µg. To each of these amounts 8.3 mL of Tris buffer was added and the flasks were made up to the mark with acetone.

3.3.5 Soil nutrient levels

Soil samples from a depth of 10 cm (avoiding suface contamination) were taken from the vineyard in early May for nutrient analysis. Samples were collected from each bay and then bulked for each treatment in each replicate giving twelve samples in total. These were taken back to Lincoln University where samples were weighed to 500 g and frozen until they could be sent away for testing. Nutrient analysis took place in June and was carried out by Hill Laboratories (Hamilton). Samples were tested using the Mehlich 3 soil test (Mehlich 1984). It involves multi-element extraction and can be used to investigate levels of phosphorus, potassium, calcium, magnesium, sodium, iron, manganese, zinc, copper, aluminium, cobalt and boron (Calvert *et al.* 2009).

In this trial analysis was carried out for pH, aluminium, calcium, cobalt, copper, iron, potassium, magnesium, manganese, sodium, phosphorus and zinc. Boron was also tested but values in all soil samples were less than 0.5 mg/L. Hill Laboratories stated that levels below 1.5 mg/L can not be reliably measured using the Mehlich 3 soil test and therefore no results were given for boron.

3.3.6 Vine nutrient levels

An ICP-OES (Inductively Coupled Plasma-Optical Emission Plasma Spectrometer) was used to investigate vine nutrition. The instrument used was a Varian (Varian Inc. Palo Alto, California USA) 720-ES axially-viewed plasma instrument. It can identify elements by the wavelengths they emit when introduced into the plasma flame. The radiation emitted by the element is converted to an electrical signal that is measured quantitatively (Bradford and Cook 1997). The macro- and micro-elements chosen to be tested in this experiment included those important for vine development and fruit production: calcium, potassium, magnesium,

phosphorous, sulphur, boron, copper, iron, manganese, molybdenum, sodium and zinc (Jackson 2000).

Leaf samples were collected as described by Porro *et al*. (1995). Leaves were sampled at veraison from medium vigour (and where possible) fruiting shoots. It was demonstrated by da Silva *et al.* (2008) that in most cases the stage of maximum absorbance of nutrients in the vine occurs in the lead up to veraison. Nutrient levels in this trial were therefore measured at this stage.

The fourth leaf above the distal cluster was collected and two leaves were taken from every vine in the trial. Jones and Case (1990) mentioned that leaves fully exposed to sunlight should be sampled and that leaves that are covered with soil or dust, damaged or contain dead tissue should not be sampled. They recommended that the leaves be washed before analysis to remove any contaminants such as fungicides that could affect the results. Leaves were washed, as recommended by Wallace *et al*. (1980), in 0.1 – 0.3% detergent solution before rinsing with pure water. The tissue was dried straight afterwards to minimise chemical and biological change and to stop enzymatic reactions from occurring. Leaves were dried as shown by Steyn (1959) who found that drying leaves at 65°C for 24 hours resulted in the removal of moisture without thermal decomposition. The samples were then ground into fine particles with a Cyclotec leaf grinder (FOSS, Höganäs, Sweden) fitted with a 0.5 mm sieve.

Prior to analysis using ICP, ground leaf samples were weighed at approximately 5 g to three decimal places. The method for digestion followed the procedure described by Gray *et al.* (1999). ASC-grade 10 mL of nitric acid 69% was added to the sample, which was then heated on a digestion block. The temperature was increased to 140°C during a period of seven hours. Following this, the sample was made up to 20 mL using de-ionised water. Duplicate samples were made in addition to a reagent blank and a standard sample from a tomato leaf. Following the digestion stage, the stable samples were stored in the fridge until they could be analysed with the ICP.

3.3.7 Photosynthesis

A portable infrared gas analysis system, Li-Cor Model 6400 (LI-COR Biosciences, Inc, Lincoln, Nebraska, USA) was used to simultaneously conduct measurements for: net photosynthesis, stomatal conductance, intercellular carbon dioxide concentration, transpiration rate and vapour pressure deficit in leaves. Measurements were taken at three stages during the season on the 23 January 2009 (after fruit set), 4 March 2009 (after veraison) and 30 March 2009 (ripening). The equipment was fitted with a clamp-on leaf curvette that could measure 6 cm² of leaf area. Data were collected as spot measurements on clear sunny days in order to obtain consistent readings and to gain readings at as close to

maximum photosynthetic rate as possible. Conditions inside the leaf curvette were matched to external environmental conditions. Photosynthetic Photon Flux (PPF) measured on 23 January was 1200 W/m² at the beginning of the run and increased to 1800 W/m², temperature increased from 24°C to 27°C. On 4 March PAR started at 1060 W/m² and increased to 1500 µmol/m²/s, temperature increased from 19°C to 19.5°C. On 30 March PAR increased from 1000 W/m² to 1200 W/m² and temperature from 18.5°C to 19°C. For each set of measurements CO_2 was maintained at 500 µmol/sec using a LI-6400-01 CO_2 injector with a high pressure liquid CO_2 cartridge source. Data collection always started at 10.30am and continued up until before solar noon at 1pm, which meant that data was always collected from fully exposed leaves on the east side of the vine. Three different leaves were chosen per plot to take readings from. These included the 3rd, 7th and 11th vine in each plot counting from its south end. Mature, undamaged basal leaves were tested and where possible readings were taken from the leaf one up from the very basal leaf on fruitful shoots of the same age. Unfortunately a frost that occurred at the beginning of the season caused some variation in canopy age. Every effort was made to measure leaves of the same age however and these leaves were tagged so that future readings would be comparable. Readings were taken by replicate in order to gather data from each treatment within as close to the same time period as possible.

3.3.8 Leaf greenness

A Konica Minolta Chlorophyll Meter, SPAD-502 (Soil Plant Analysis Device) (Tokyo, Japan) was used to test for leaf greenness, a value correlating to the amount of chlorophyll and nitrogen the leaf contains. Fanizza *et al.* (1991) showed that SPAD readings are closely related to total leaf chlorophyll content of vines and provide a non-destructive method for measuring this parameter. The method was chosen for use in this trial to make comparisons with results for photosynthesis. It was also used to observe the onset of senescence towards the end of the season. Readings were taken from mature, undamaged basal leaves and where possible leaves selected were one up from the very basal leaf on fruitful shoots of the same age. One leaf per vine was tagged so future readings would target the same leaves and results would be comparable. Five readings were averaged per leaf to gain a final result. The same leaves tagged for leaf greenness included those tagged for photosynthesis measurements, so that comparisons would also be possible between each experiment. The SPAD was used to test vines at four stages during the season, at the end of January (after fruit set), February (after veraison), March (ripening) and April (towards harvest).

3.3.9 Fruit

As a result of damage to the vines from the frost that occurred at the beginning of the 2009 season, crop load was low and berries did not reach full ripeness by the end of the season, therefore there was no harvest for the 2009 season.

Wine was however made in 2006 and 2008. Unfortunately in each of these years, replication within each treatment was not possible due to low crop load and therefore results could not be statistically compared. Replication was however achieved for juice samples in 2008. Parameters collected from the 2008 harvest included those relating to fruit quality: pH, °Brix and titratable acidity. These measurements were taken from a sub-sample of fruit collected at harvest. Yield parameters were measured per bay. Clusters were counted and weighed in the field during the harvest. Bird-damaged clusters were also counted. These included rachis that remained on the vine with no fruit. From the number of bird damaged clusters and using the average bunch weight from each treatment, it was possible to calculate the potential yield without bird damage.

3.3.10 Pruning weights

Vines were pruned at the beginning of September 2008. They were pruned in dry weather by replicate and prunings were weighed per vine. Only the past season's growth was weighed: no old wood was included.

3.3.11 Canopy temperature

Tiny Tag (Gemini Data Loggers (UK) Ltd., West Sussex, UK) temperature loggers were used to determine whether the mulches changed the ambient temperature in the fruiting zone. The loggers were in Stevenson-type screens attached to the first foliage wire in the central bay in replicates one and three. The data loggers were set to measure the temperature at half-hour intervals throughout the season from budburst to harvest. Information was downloaded monthly from each of the devices. At the end of the season, loggers were calibrated to check variation between data logging and data was corrected for any variations.

3.3.12 Reflected radiation

Two types of spectroradiometer were used to measure mulch and control reflectance. The first was a portable StellarNet spectroradiometer, which could be taken into the vineyard and had a range spanning 200 – 1100 nm. The meter therefore offered the possibility to measure the UV, visble and near infrared spectrums. The Bentham spectroradiometer, which was not portable but was used in a model system, could measure 200 – 900 nm. Each of the spectroradiometers that were used in this trial, were fitted with a slitted screen, diffraction grating and a photodetector (Anon 2010a, Anon 2010b).

Red to far-red (R:FR) ratios were also investigated. Photoreceptors in plants called phytochrome respond to red to far red ratios (Fanklin and Whitelam 2007).

The ratio was determined by the following equation from Franklin and Whitelam (2007):

$$R:FR = \frac{\text{photon irradiance between 660 and 670 nm}}{\text{photon irradiance between 725 and 735 nm}}$$

In the vineyard, a portable StellarNet Miniature Fibre Optic Spectrometer, EPP2000C-SR-50 LT-12, with a fixed concave holographic grating and cosine corrected polymer diffuser (StellarNet, Inc, Tampa, Florida, USA) was used. The device was set up on a tripod with the sensor pointed down towards the mulch in order to measure reflectance at fruiting wire height. The height of the sensor from the ground meant that it would also have picked up some scattered light from the vineyard, especially from the centre row that consisted of mowed grass. Readings were taken on a clear sunny day in April and were recorded between 10.30 am until before solar noon (1 pm). During solar noon the shadow of the vines passes beneath the canopy and obstructs mulch reflectance. The east side of the mulch was measured and measurements were taken by replicate to ensure each replicate was measured within the closest time frame.

A bench-mounted Bentham DM150BC Double Monochromator with motorised 1800 gratings, end window photomultiplier tube detector and cosine corrected Teflon diffuser (Bentham Instruments Limited, Reading, UK) was used at the beginning of May in a model system which was set up on the roof of the Hilgendorf building at Lincoln University. Mulches were collected from the vineyard. The glass mulch was clean and could be used directly. However, shells, which often had soil stuck to them, had to be washed. Soil was also collected to be used as a control. The soil was dry at the time it was measured. Mulches were put into black trays at a depth of approximately 5 cm. The trays were then placed inside a tall black box which allowed light in from one side. The box was black to prevent other reflected light from entering the system. The spectroradiometer was set up on the other side of the entrance of the box in order to read the radiation reflecting from the mulch (Figure 3.3). Readings were taken near solar noon to find maximum reflectance of the mulches. Three replicated scans were recorded over solar noon (1pm). The reason behind using a model system was to investigate mulch reflectance without the variables from the field that could influence their reflectance. Readings were taken in the visible spectrum (380 nm-760 nm) and into the beginning of the near infra-red spectrum (760 – 950 nm). An attempt was made to record ultra-violet radiation however at the time the scan was taken the levels of ultra-violet from the sun were reduced because of the angle of the sun. The NIWA website

reported UV in the low range with a maximum reading of just 2 on the UV index scale (Anon 2009b). A low UV index made it difficult to compare reflectance below the visible spectrum and readings below 300 nm were unreliable. However, comparisons were made at the top of the UV range between 300 and 400 nm.

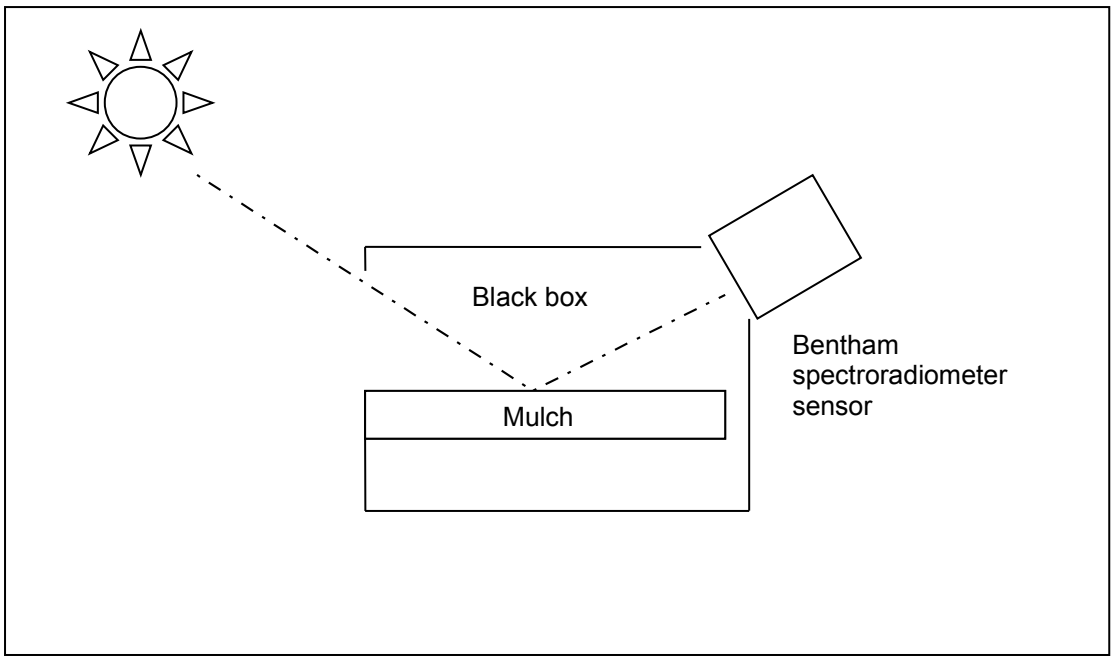

Figure 3.3 – Model system with Bentham spectroradiometer.

Surface temperature of the mulches was also recorded using an infrared thermometer, model 110C, from Everest InterScience Inc. (California, USA). The thermometer read temperatures while mulches were in their trays and when they were fully exposed to sunlight between 1 and 2pm on the 7 May 2009. Three replicates were used.

3.3.13 Statistical analysis

Results for treatment parameters from each experiment were analysed using analysis of variance (ANOVA) with GenStat for windows, version 12, VSN International Limited, Hemel Hempstead, UK (http://www.vsni.co.uk/software/genstat/). Significant differences were calculated at the 95% confidence interval where $p \leq 0.05$ using Fisher's protected LSD test.

3.4 Results and discussion

3.4.1 Soil temperature

Soil temperatures were measured between 14 September 2007 and 24 January 2008. Measurements were not replicated therefore no statistical assessment could be calculated. During this period the mussel shell treatment ranged 11.3 – 24°C. The range for clear glass was 10.4 - 29.5°C. For green glass this was 11.1 – 30.7°C and for control the fluctuation was 9.4 – 32°C. Interestingly the glass treatments, despite their thickness (5 cm deep compared to 1 cm for the mussel shells) had a more similar affect on soil temperature to control than mussel shells (Figure 3.4).

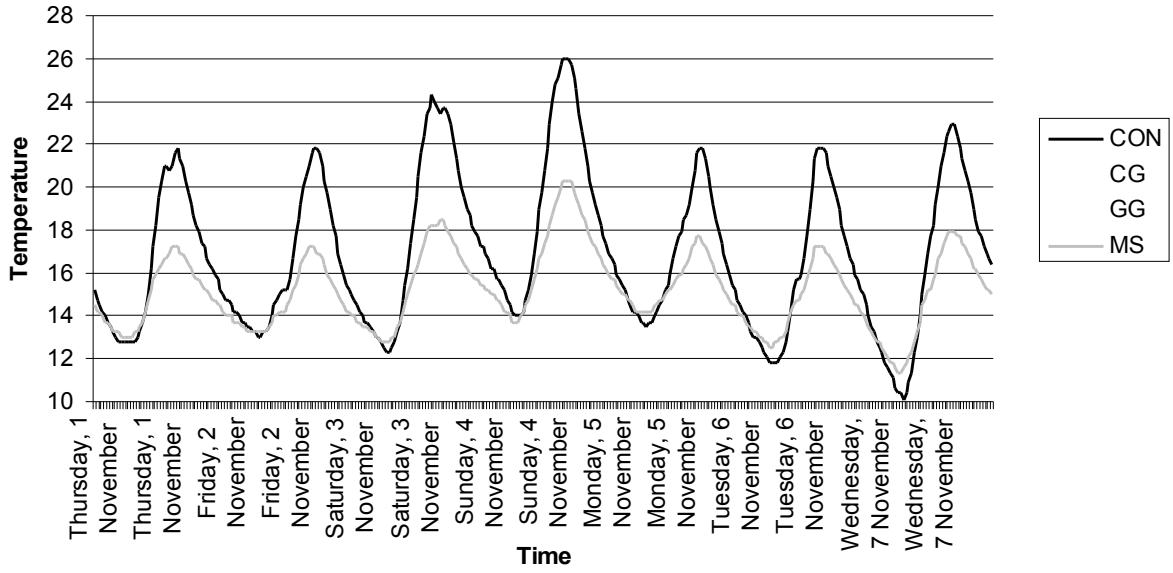

Figure 3.4 – Soil temperature 1 – 7 November 2007.

The results observed for the mussel shell mulch correspond with findings by Creasy and Crawford (2005) who showed that mussel shells kept the soil significantly cooler than the un-mulched control. They also noted that soil temperatures were buffered by the mulch and that the fluctuation in temperature was significantly less in the shells than in the control. Leal (2007), who also investigated the effect of mussel shell mulch, confirmed these findings when significantly cooler soil temperatures were found beneath this type of mulch compared to an un-mulched control. The buffering effect of the mussel shell mulch also appeared to have affected the soil in this trial. It is thought that the light colour of the mulch may have kept the soil cooler as was found in a trial by Creasy *et al.* (2003b) where white plastic over polystyrene mulch reduced soil temperature in relation to an un-mulched control.

3.4.2 Soil moisture

Soil moisture levels were tested for each treatment in each replicate at the end of the month in January, February, March and April. The mean result for soil moisture under the mulches was found to be significantly higher than the un-mulched control in January and March, the two driest months (Appendix 2). In February the mussel shells had significantly higher soil moisture levels than the glass treatments and the control and in April results were not significantly different (Table 3.2).

Table 3.2 – Soil moisture levels (%) measured using TDR with rainfall amounts from NIWA.

Treatment	January	February	March	April	Overall
CON	12.7a	19.5a	14.5a	23.3	17.5a
GG	20.2b	21.0a	19.6b	27.6	22.1b
CG	20.2b	22.1a	20.3b	25.5	22.0b
MS	25.0c	26.7b	23.6b	31.3	26.6c
p value	<.001***	0.007**	0.019*	0.077	0.001***

CON vs Mulch	January	February	March	April	Overall
CON	12.7	19.5	14.5	23.3	17.5
Mulch	21.8	23.3	21.1	28.1	23.6
p value	<.001***	0.012*	0.006**	0.053	<.001***

Glass vs MS	January	February	March	April	Overall
Glass	20.2	21.6	19.9	26.6	22.1
Shells	25.0	26.7	23.6	31.3	26.6
p value	<.001***	0.004**	0.073	0.068	0.003**

GG vs CG	January	February	March	April	Overall
GG	20.2	21.0	19.6	27.6	22.1
CG	20.2	22.1	20.3	25.5	22.0
p value	0.970	0.461	0.731	0.441	0.954

Rainfall (mm)	26	68	15	43	
% of norm	60	165	27	85	

Results are significant at $p \leq 0.05$*, $p \leq 0.01$** $p \leq 0.001$***

These results, showing significantly higher moisture levels beneath mulched treatments, are expected. Mulch provides a cover for the soil surface aiding moisture retention: it allows water infiltration whilst strongly reducing evaporation (Agnew and Mundy 2002). In line with these results, the un-mulched control showed the highest fluctuation in soil moisture (Figure 3.5).

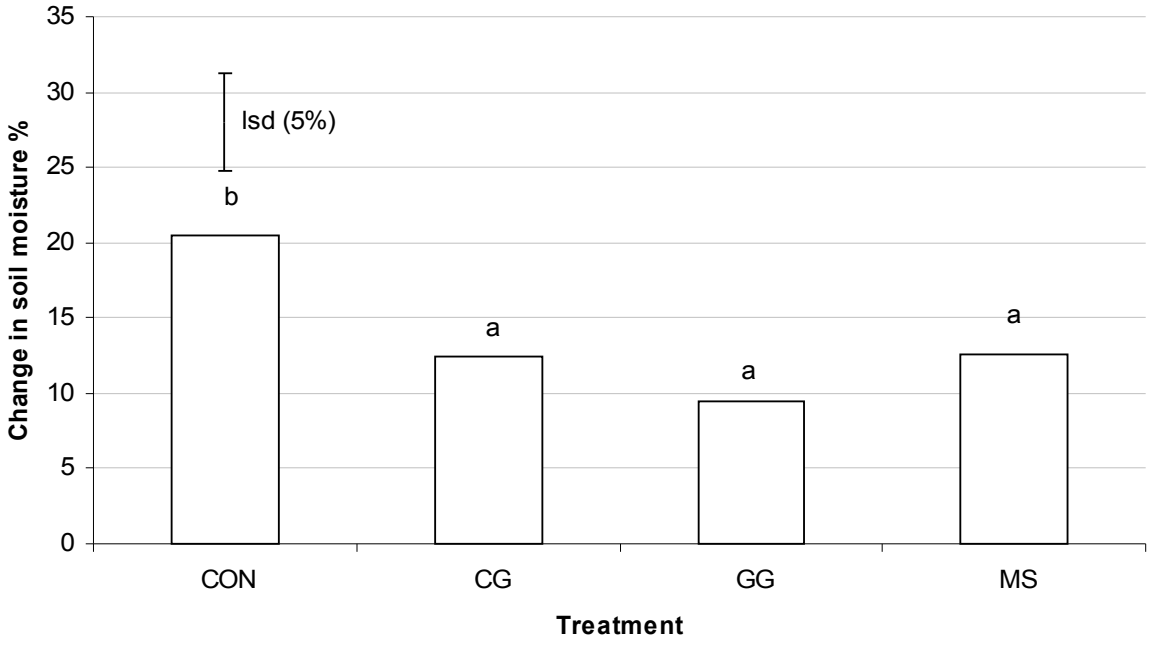

Figure 3.5 – Change in soil moisture from January to April 2009. Results are significant at $p \leq 0.05$.

An interesting difference was found in January and February where the mussel shells showed significantly higher soil moisture percentage than all other treatments (Table 3.2). In February soil moisture under the glass mulches was not found to be significantly different from the un-mulched control, while soil moisture under the mussel shells remained significantly higher. This finding suggests that the mussel shells were more efficient than the glass at water retention despite the glass being much thicker on the ground than the mussel shells. It is possible that the mussel shells have changed the composition or structure of the soil because of the material they are made from, which includes calcium. Changes to the soils nutrient status are discussed at a later stage, however it would be useful to carry out further research in this area to investigate the mussel shell effect on soil structure. Soil structure beneath the glass mulch should also be monitored. Addition of crushed glass to an agricultural soil was carried out by de Louvigny *et al.* (2002). In that trial, high glass to soil content (80% glass) was not deemed beneficial for emergence of sugar beet, and seedlings often remained trapped under the rough surface of the glass. As vines are deeply rooted, it is unlikely that glass would affect growth of mature vines, but it could impact on weed growth, possibly even preventing their emergence in glass mulched areas.

3.4.3 Soil micro organisms, biomass and activity

3.4.3.1 Microbial biomass carbon

Levels of microbial biomass carbon were found to be significantly higher in the mussel shell mulch compared to the average of the glass treatments (Table 3.3). Significant differences were also found when individual treatments were compared.

Table 3.3 – Statistical comparisons for microbial biomass carbon.

Comparison	Treatment mean CON	GG	CG	MS	*p* value
Treatment	203.3bc	170.0a	178.7ab	226.5c	0.006**
CON vs Mulch	203.3	191.7			0.224
Glass vs MS		174.4		226.5	0.001***
GG vs CG		170.0	178.7		0.443

Results are significant at $p \leq 0.05$*, $p \leq 0.01$** , $p \leq 0.001$***

The fact that microbial biomass carbon levels were significantly higher for the mussel shell treatment than the glass further illustrates that the two mulches have a very different influence on the soil. This is likely due to the different materials each is made from. Glass is an inorganic material and its largest ingredient is silica which is derived from white sand or pulverised sandstone (Anon 2009c). Small amounts of alkali such as sodium bicarbonate or potash are added to the silica glass to reduce its boiling point (Anon 2009c). A small amount of lime, from limestone, is also added to stabilise the mixture and give it strength and water resistance (Anon 2009c). Coloured glass has other compounds added to it, for example iron-chromite is added to create green glass (Anon 2009c).

Molluscan shells such as mussel shells are made up of 95-99% calcium carbonate by weight. The remaining 1-5% is an organic component which includes specialised proteins that direct the growth pattern of the shell (Currey 1999). It is possible that the significant differences between the glass and shell treatments were due to the organic component of the shells. Mussel shells would break down faster in the soil than the glass, which is largely inert. The mussel shells also had a layer of organic coating on them when they were first laid down (Figure 3.1). This coating gradually comes off and enters the soil as the mussel shells are weathered in the vineyard. By contrast, the glass which has been processed is mostly clean at the time it is laid down (Figure 3.1). It is possible that the organic matter brought in with the shells might provide food for the microbes, encouraging them to be there. Future studies on the effect of the mulches on the soil could investigate the nature of the organic

components of the mussel shells to confirm whether this had played a role in the increase in microbial biomass carbon.

Other possible reasons for increased microbial biomass carbon include effects of the mulches on soil temperature and moisture. Microbes are particularly sensitive to changes in both of these factors (Paul 2007). As has already been mentioned, soil moisture was generally higher underneath the mussel shells compared to the glass treatments and control (Table 3.2). At the end of February, just before soil samples were taken, mussel shells were found to have significantly higher soil moisture compared to the other treatments. Barros *et al.* (1995) showed that increased soil dryness inhibited microbial activity as well as growth rate.

Soil temperature appeared to be cooler beneath the mussel shell treatment when compared to the other treatments (Figure 3.4). Mussel shells also appeared to have a buffering effect which reduced temperature fluctuation (Figure 3.4). It does not appear however, that cooler temperatures encourage microbes. Kandeler *et al.* (1998) showed that increasing the soil temperature could increase microbial carbon in the upper 10 cm of the soil. Paul (2007) noted that temperature increases encouraged the activity of microbes in the soil with the highest activity occurring in the range 10 - 25°C. Mineralization, where nutrients become available to plants through microbial metabolism, was found to increase with increasing temperatures (Paul 2007). Paul (2007) also noted that, microbial community structure was affected by temperature and that as the temperature increased, so too did the amount of organic matter undergoing mineralization. Temperature monitors had been removed by the time soil samples were taken in March, however previous data from temperature monitoring would suggest that soil temperatures for all treatments would have been in an optimal range (10 - 25°C). This would have meant that the differences in soil temperature would not have been extreme enough to affect the microbial population per treatment.

Finally, mulches were found to have altered the nutrient status of the soils over which they were laid (Table 3.4). Microbes have been found to be affected by nutrients available in the soil (Martens 1995). Nutrient availability is partly regulated by soil pH (Maschmedt 2005). Wardle (1998) reported that increases in soil pH had a stabilising effect on microbial communities. In the Sandihurst trial pH was found to be significantly higher beneath the mussel shell treatment, which may have affected microbial biomass carbon.

Further research in this area could establish mechanisms responsible for increases in microbial biomass carbon in the control and mussel shell treatments compared to the glass. An explanation is needed to determine whether this was due to temperature, moisture, nutrient availability or combinations of the above.

3.4.3.2 Dehydrogenase Enzyme Activity

No statistically significant differences were found between treatments for dehydrogenase enzyme activity (Figure 3.6). High variation in this parameter is often an issue when testing soil (Forster 1995). Future testing of this parameter could use higher replication and a greater number of samples in order to achieve statistically comparable results. The pattern seen in this assay, where the glass treatments gained lower results compared to the other treatments did however mirror the observations seen in the microbial biomass carbon assay (Table 3.3), so it could be hypothesised that the glass was suppressing the microbial activity that was encouraged under the control and mussel shell treatments.

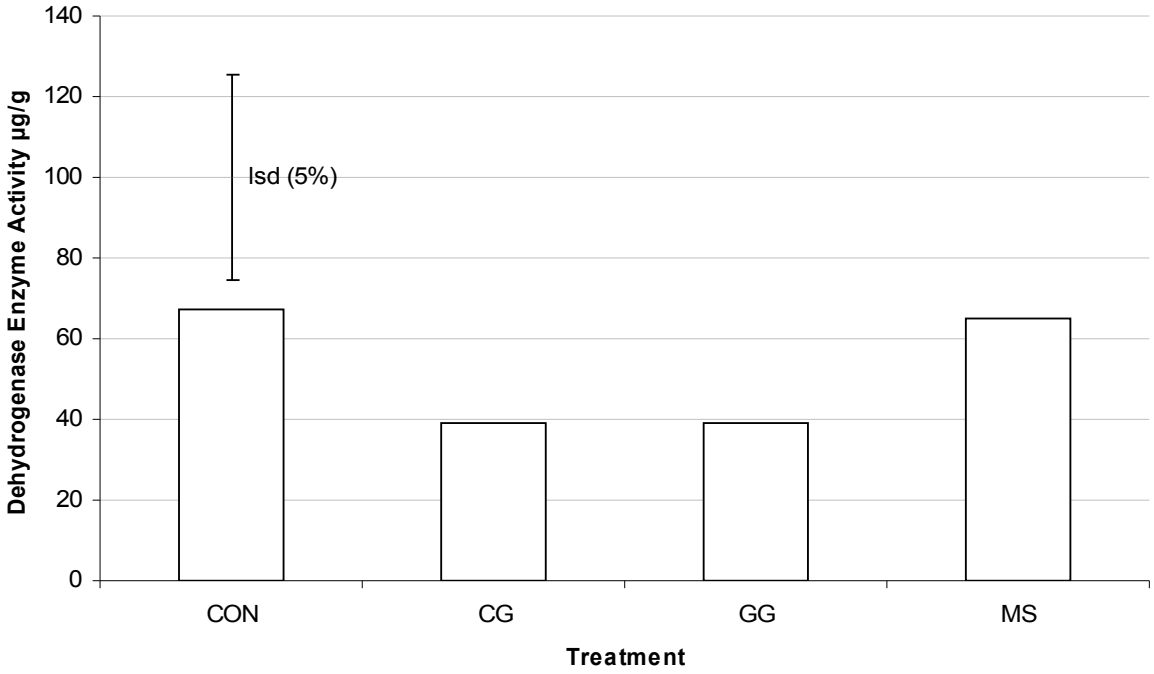

Figure 3.6 – Dehydrogenase enzyme activity comparison between treatments. Results are significant at $p \leq 0.05$.

3.4.4 Soil nutrients levels

Soil collected from beneath the mussel shells had significantly higher pH compared to the control and clear glass treatments (Table 3.4). These results were expected as mussel shells are made up of mostly calcium carbonate. Adding shells to the soil increases the soils calcium content and therefore its pH (Crawford 2007, Leal 2007, Sandler *et al.* 2009).

Table 3.4 – Soil nutrients analysed by Hill Laboratories using the Mehlich 3 soil test.

	pH	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu	Co	Al
CON	6.6a	52.0	75.7	1600	89.4	22.7a	104.0	9.9	0.81	1.200	0.133	750
GG	6.8ab	47.0	69.7	1797	63.7	46.3b	104.8	11.1	1.05	1.200	0.167	748
CG	6.6a	43.3	52.3	1667	78.4	29.7a	97.6	9.1	0.83	1.367	0.100	710
MS	7.2b	51.0	71.3	2093	72.3	22.0a	92.0	9.1	0.56	1.433	0.100	677
p value	0.027*	0.828	0.639	0.052	0.139	0.010**	0.330	0.449	0.226	0.326	0.189	0.372
CON vs Mulch	pH	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu	Co	Al
CON	6.6	52.0	75.7	1600	89.4	22.7	104.0	9.9	0.81	1.200	0.133	750
Mulch	6.9	47.1	64.4	1852	71.5	32.7	98.1	9.8	0.81	1.333	0.122	712
p value	0.062	0.583	0.491	0.074	0.056	0.053	0.352	0.883	0.985	0.290	0.670	0.321
Glass vs MS	pH	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu	Co	Al
Glass	6.7	45.2	61.0	1732	71.1	38.0	101.2	10.1	0.94	1.284	0.134	729
MS	7.2	51.0	71.3	2093	72.3	22.0	92.0	9.1	0.56	1.433	0.100	677
p value	0.014*	0.539	0.548	0.026*	0.888	0.011*	0.189	0.441	0.075	0.265	0.253	0.225
GG vs CG	pH	P	K	Ca	Mg	Na	Fe	Mn	Zn	Cu	Co	Al
GG	6.8	47.0	69.7	1797	63.7	46.3	104.8	11.1	1.05	1.200	0.167	748
CG	6.6	43.3	52.3	1667	78.4	29.7	97.6	9.1	0.83	1.367	0.100	710
p value	0.225	0.735	0.391	0.398	0.165	0.017*	0.353	0.177	0.322	0.281	0.071	0.420

Results are significant at $p \leq 0.05$*, $p \leq 0.01$**, $p \leq 0.001$***, all results are in mg/L

A soil's cation exchange capacity is closely linked to its clay mineral and organic components and is additionally controlled by soil pH (Maschmedt 2005). Where soil pH is altered this has an effect on nutrient availability (Fageria *et al* 2002). Creasy and Crawford (2005) noted that in their experiment on pinot noir that the acidic cations aluminium, iron, manganese and zinc all decreased in availability as pH increased. A similar pattern was seen in the soils collected from Sandihurst, with the control in all cases sharing higher levels for these nutrients than the mulched treatments.

In a trial carried out by Misra and Tyler (1999) that investigated the effect of different soil moisture levels on soil nutrient levels, it was demonstrated that pH could also increase with increases in soil moisture content. This result corresponds to the findings from the Sandihurst trial. Misra and Tyler (1999) also demonstrated that levels of calcium, magnesium, potassium and zinc decreased with increases in moisture while phosphorous and manganese increased. No significant differences were noted for any of these soil nutrients apart from calcium. Levels of calcium were expected to be higher in the mussel shell treatment because of the calcium carbonate contained within the shells (Table 3.4). Calcium levels were borderline significantly higher ($p \leq 0.052$) for the mussel shells compared to the other treatments (Table 3.5). These levels were significantly higher ($p \leq 0.026$) where the mean of the glass treatments was compared to the mussel shells, but were

not found to be significantly different from the control. The control had the lowest soil moisture content which may have caused these differences.

Sodium levels also showed significant differences. Higher levels were found in the green glass compared to all the other treatments (Table 3.4). This is in contrast to findings by Crawford (2007) and Leal (2007) who tested mussel shells mulch at Neudorf Vineyard in Nelson and found sodium levels to be higher in the mussel shell soil compared to the un-mulched control. The reason for the difference in only the green glass could be due to the processing of this type of glass. The green glass mulch is in fact a mixture of many different colours including brown glass. The majority of the glass in this mix is however green giving it its green appearance. It is apparent that reasons for the higher levels of sodium in this treatment warrants further investigation. It would be necessary to submerge the glass in water to test for any elements leached from the treatment. Despite the differences found in the Sandihurst vines, when sodium levels were tested in plant tissue in May, they were found to be within tolerable levels for all of the treatments.

3.4.5 Vine nutrient levels

Leaves were collected at veraison, when maximum concentration of leaf nutrients occurs (da Silva *et al.* 2008). No significant differences were noted in any comparison for levels of calcium, magnesium, iron, sodium or zinc. Differences were found between the mulched compared to the un-mulched treatments for boron, copper, potassium, molybdenum, phosphorous and sulphur. In all cases the mulched treatments had higher levels than the control (Table 3.5). Boron and manganese showed differences in the comparison between the mean of the glass treatments and the shells. Shells yielded higher levels in leaves for boron than glass while for manganese this was found to be higher in the glass treatments compared to shells (Table 3.5).

Table 3.5 – Nutrients analysed in vine leaves using inductively coupled plasma spectrometry.

	Macro-nutrients (%)						Micro-nutrients (mg/kg)					
	P	K	S	Ca	Mg	Na	Fe	Mn	Zn	Cu	B	Mo
CON	0.17 a	0.74	0.17	1.97	0.39	0.04	122.70	91.2	24.8	4.81	28.62 a	1.083 a
GG	0.20 b	0.91	0.19	2.13	0.36	0.03	129.10	98.3	26.1	6.39	35.82 bc	1.274 b
CG	0.19 b	0.91	0.19	2.00	0.35	0.03	114.00	89.4	24.1	6.55	32.92 ab	1.265 b
MS	0.19 b	0.91	0.18	2.07	0.39	0.03	119.30	55.5	25.5	5.78	39.30 c	1.272 b
p value	0.003**	0.160	0.101	0.614	0.218	0.537	0.645	0.062	0.981	0.098	0.006**	0.028*
	P	K	S	Ca	Mg	Na	Fe	Mn	Zn	Cu	B	Mo
CON	0.17	0.74	0.17	1.97	0.39	0.04	122.70	91.2	24.8	4.81	32.92	1.083
Mulch	0.19	0.91	0.19	2.07	0.36	0.03	120.80	81.1	25.2	6.24	34.58	1.270
p value	<.001***	0.035*	0.041*	0.406	0.199	0.179	0.843	0.379	0.920	0.029*	0.003**	0.005**
	P	K	S	Ca	Mg	Na	Fe	Mn	Zn	Cu	B	Mo
Glass	0.19	0.91	0.19	2.07	0.35	0.03	121.55	93.9	25.1	6.47	32.22	1.270
MS	0.19	0.91	0.18	2.07	0.39	0.03	119.30	55.5	25.5	5.78	39.30	1.272
p value	0.565	0.992	0.135	0.946	0.114	0.828	0.833	0.015*	0.929	0.238	0.022*	0.962
	P	K	S	Ca	Mg	Na	Fe	Mn	Zn	Cu	B	Mo
GG	0.19	0.91	0.19	2.13	0.35	0.03	114.00	89.4	24.1	6.55	35.82	1.265
CG	0.20	0.91	0.19	2.00	0.36	0.03	129.10	98.3	26.1	6.39	28.62	1.274
p value	0.274	0.953	0.726	0.329	0.523	0.851	0.244	0.524	0.713	0.809	0.168	0.874
Optimum range	0.20 - 0.24	1.20 - 1.40	0.30 - 0.50	2.50 - 3.50	0.23 - 0.27	0.00 - 0.10	100 - 250	30 - 200	5 - 20	5 - 20	25 - 40	0.15 - 0.50

Results are significant at $p \leq 0.05$*, $p \leq 0.01$** $p \leq 0.001$***

It is possible that the higher levels available to the vine for the mulched treatments were caused by the mulch suppressing weeds in the under vine area (Leal 2007). Hostetler *et al.* (2007b) also showed that mulches could suppress weeds and concluded that this would make more nutrients available to the vines. The mulches might also have affected microbial life in the soil. Microbes control the availability of nutrients to plants growing in the soil (Paul 2007). Investigations into levels of microbial biomass carbon showed that the control and the mussel shells had the highest levels (Table 3.3). Although higher microbial biomass carbon for mussel shells coincides with higher nutrient levels for this treatment (Table 3.3), it does not do this with data for the control. The control was also found to have high levels of microbial biomass carbon however within this comparison the control had lower levels of the nutrients tested.

Higher levels of leaf nutrients might also have been caused by the presence of mycorrhizal fungi. Phosphorous in particular may have been affected by an increase in the amount of mycorrhizal fungi beneath the mulches. Significantly higher levels of fungi were found in a mulch trial carried out by Agnew and Mundy (2002). Mycorrhizal fungi increase plant root surface area (Agnew and Mundy 2002) and are known to increase phosphorous uptake (Cavagnaro *et al.* 2006). Mycorrhizal fungi have also been found to increase uptake of

nutrients such as potassium, copper and zinc (Marschner and Dell 1994). In contrast to findings by Agnew and Mundy (2002) Crawford (2007) found that ectomycorrhizal colonisation and vesicular arbusclar mycorrhizal colonisation was less beneath mussel shell mulch compared to the control. However in the trial at Sandihurst, although no significant differences were noted for zinc, levels of potassium and copper were both significantly higher in the mulched treatments compared to the control. Soil moisture also plays a role in nutrient uptake by vines. Misra and Tyler (1999) reported increases in the uptake of some nutrients with increased soil moisture content while the concentrations of other nutrients decreased. The results were different for different plants however, and vines were not tested in this experiment.

Evidence that mulches could increase nutrient levels has implications for vine and fruit development. Boron is required in nucleic acid synthesis, it is important for cell membrane maintenance, and is involved in calcium metabolism in the vine (Jackson, 2000). Boron is also important at flowering in relation to pollination and fertilisation and has a role in carbohydrate metabolism (Mullins *et al.* 2002). Copper is involved in oxidative reactions within the vine it is also involved in respiration and synthesis of proteins, carbohydrates and chlorophyll (Jackson 2000). Potassium is required for cellular osmotic and ionic balance, neutralisation of organic acids, stomatal function regulation, cell division, enzyme activation, protein synthesis and synthesis and translocation of sugars (Jackson 2000). Potassium also makes up a considerable part of the dry weight of the grapevine (up to 3%) and is an important component of grape juice. It is involved in the internal vacuole of the plant cell where it is the most important cation. It provides electrical balance for organic and inorganic anions, indirectly maintaining the structure of the non-woody parts of the vine through its effect on cell turgor (Robinson 2006). Phosphorous is required in sugar metabolism and is found in seeds, fruit and meristematic regions of the grapevine (Jackson, 2000). It makes up an important component of cell membrane lipids, nucleic acids and energy carriers such as ATP. It is also an important component of some proteins (Jackson, 2000). Molybdenum is important in the process of nitrate reduction and where proteins and chlorophyll are synthesised by the vine (Jackson, 2000). Sulphur is a component of proteins and enzyme co-factors (Robinson 2006).

Levels of the nutrients phosphorous, potassium, sulphur and copper were higher in mulched compared to un-mulched vine leaves (Table 3.5). All of the vines were found to be deficient in potassium where they had < 1% (Fregoni 1985). Similarly for phosphorous all of the treatments were found to be in the below optimum range (0.15 – 0.2 %) (Fregoni 1985), however, while the control had 0.17 %, the mulch treatments had 0.19 %. For sulphur, all treatments were deficient although the mulched treatments were significantly higher than the

control (Table 3.5). Copper also showed higher levels in the mulched compared to the non-mulched treatments. Mulched treatments had 6.24 mg/kg placing them in the optimum range (5 - 30 mg/kg) (Fregoni 1985) while the control was found to be in the below optimum range with 4.81 mg/kg. Boron also shared higher results for the mulched compared to the non-mulched vines and all levels were in the optimum range. Finally for Molybdenum the mulched vines had higher results than the non-mulched vines and molybdenum was found to be above optimum levels for all treatments (Table 3.5). Further study should be carried out on the effect of the mulches on leaf nutrient values when these nutrients are not limiting.

3.4.6 Photosynthesis

Photosynthesis was measured using a LiCor 6400 photosynthesis gas exchange system on 23 January, 4 March and 30 March 2009. Large variation in the data set meant that no significant differences were found for net photosynthesis between treatments. The variation is likely to have been caused by the frost that occurred at the beginning of the season, on the growth of the canopy. Significant results were found however, for readings taken on 4 March for some of the other parameters measured by the LiCor 6400. Table 3.6 shows results for all parameters including: net photosynthesis (Photosyn), stomatal conductance (Cond), intercellular carbon dioxide concentration (Ci), transpiration (Trmmol), vapour pressure deficit (VpdL) and WUE (water use efficiency). Units for Photosyn are: μmol $CO_2/m^2/s$, units for Cond are: mol $H_2O/m^2/s$, units for Ci are: μmol CO_2/mol, units for Trmmol are: mmol $H_2O/m^2/s$, units for VpdL are: kPa, units for WUE are μmol CO_2/mmol H_2O.

Table 3.6 – LiCor results taken on 4 March 2009.

Comparison	Photosyn	Cond	Ci	Trmmol	VpdL	WUE
CON	15.24	0.963b	342.6	3.612	0.521	4.23
GG	14.73	0.719a	336.6	3.223	0.572	4.62
CG	14.24	0.726a	339.7	3.316	0.575	4.31
MS	14.24	0.677a	335.1	2.989	0.573	4.82
p value	0.397	0.012*	0.061	0.084	0.080	0.189
Dark	14.99	0.841	339.6	3.418	0.547	4.42
Light	14.24	0.702	337.4	3.153	0.574	4.56
p value	0.143	0.018*	0.225	0.097	0.087	0.479
CON	15.24	0.963	342.6	3.612	0.521	4.23
Mulch	14.40	0.707	337.1	3.176	0.573	4.58
p value	0.152	0.002**	0.026*	0.031*	0.016*	0.152
Glass	14.49	0.723	338.2	3.270	0.574	4.46
MS	14.24	0.677	335.1	2.989	0.573	4.82
p value	0.667	0.423	0.177	0.141	0.956	0.165
GG	14.73	0.719	336.6	3.223	0.572	4.62
CG	14.24	0.726	339.7	3.316	0.575	4.31
p value	0.464	0.907	0.219	0.647	0.853	0.281

Results are significant at $p \leq 0.05$*, $p \leq 0.01$**, $p \leq 0.001$***

Stomatal conductance was increased by 27% in the control compared to the mulched treatments (Table 3.6). Stomata are important for gas exchange and main factors affecting their function include light and water (Mullins *et al.* 2002). Stomata are closed when it is dark and open from a tenth of full sunlight (Mullins *et al.* 2002). Where vines are water stressed, conductance is normally found to be lowest in the stressed vines, as stomata close in order to prevent water loss and water use efficiency by the vine is therefore increased (Stoll *et al.* 2000, de Souza *et al.* 2005). Other parameters measured also showed significant differences between the mean of the mulches and the control (Table 3.6). Vapour pressure deficit is related to leaf moisture content. Results were lowest for the control which was less by 9% compared to mulch treatments. This may have been caused by less available soil moisture for the control compared to mulched treatments therefore the roots in control vines did not transport the same amount of water to the leaves as was possible for the other treatments. Intercellular leaf carbon dioxide concentration for mulch treatments showed a 2% decrease compared to the control and transpiration rates were reduced by 12% for the mulches compared to the control. These results for the control correspond to those found for conductance. The control, which had drier soil and the lowest leaf vapour pressure deficit, had significantly the highest transpiration rate compared to the mulched treatments.

The fact that stomatal conductance was highest in the control vines compared to the mulched treatments at Sandihurst is perhaps indicative of the fact that the vines were not water stressed despite having drier soil. The mussel shell mulch, which had a significantly higher soil moisture content compared to the control, also had significantly lower stomatal conductance possibly caused by increased water use efficiency by these vines (Table 3.6). Water use efficiency has been shown to be affected by partial rootzone drying. Stoll *et al.* (2000) showed that drying roots of vines irrigated by this method signalled for stomatal closure via abscisic acid. The mulches in the Sandihurst trial appear to have had a similar effect on vines by reducing conductance. However this can not have been caused by signalling from drying roots as soil moisture was highest in the mulched soils. Also no significant differences were found for water use efficiency in any of the treatments (Table 3.6).

As conductance did not appear to have been affected by water it may have been affected by the extra light being reflected from the mulches. It is possible that the extra light caused the leaves to function more efficiently. The lower conductance in the mulched treatments suggests that the stomata in these vine leaves were more closed than those of the control yet all of the vines carried out photosynthesis at a rate that was not significantly different. In future studies it could be interesting to further investigate leaf function over these treatments using chlorophyll fluorescence analysis. Chlorophyll fluorescence is a measure of how light is used by the vine. It detects how much light is used to drive photosynthesis and what is lost as heat and light (Maxwell and Johnson 2000).

3.4.7 Leaf greenness

Leaf greenness was tested using a soil plant analysis device (SPAD) in January, February, March and April. There were no significant differences for any of the months in which comparisons were made or between any of the treatments tested. The variability was possibly due to the fact that only one leaf per vine was measured and it may have been better to measure a greater number of leaves in order to gain significant results. The vine canopy had also been affected by the frost at the beginning of the season. This may have introduced variation that would have affected development of individual leaves. A pattern was seen however towards the end of the season when photosynthesis was in decline. SPAD readings also appeared to be in decline from this time. The largest decline for SPAD was witnessed between the end of March and the end of April with the greatest fall seen for the control (Figure 3.7).

By April, leaf fall had begun and it was observed that the control was the first to begin losing its tagged leaves (data not shown). In the first replicate, the control treatment had lost all of

its tagged leaves by the time final measurements were taken. It is likely that this was due to the fact that this treatment in this replicate had been the driest throughout the growing season (Table 3.2).

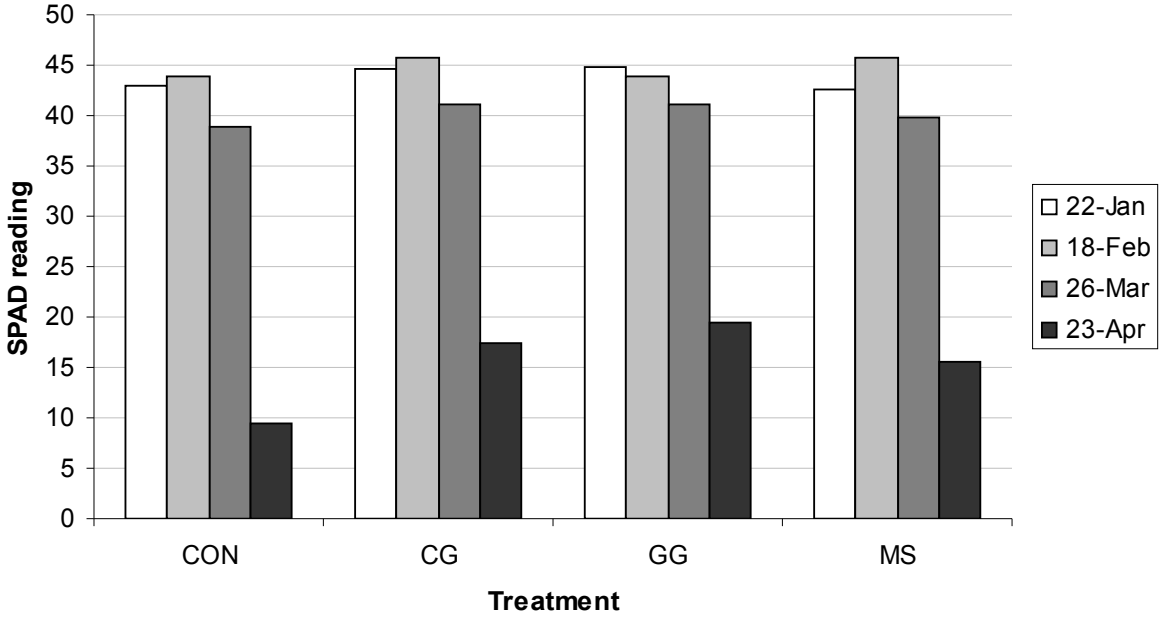

Figure 3.7 – Leaf SPAD readings from January to February 2009. Differences within each month are not significant.

3.4.8 Fruit

Fruit was harvested on 7 May 2008. Measurements of pH, titratable acidity (TA) and °Brix were taken from a sub-sample of fruit (Table 3.7). No significant differences were found for any of the parameters tested or for any of the comparisons made. It was hypothesised at the beginning of the trial that the additional light introduced by the mulch treatments would affect acid metabolism and sugar accumulation in the berries. Possibly the increase in available light was not enough to affect changes large enough to be significantly different.

Table 3.7 – Cluster sample berry parameters for 2008 harvest. No significant differences were found.

Treatment	pH	TA (mg/L)	°Brix	Cluster (g)
CON	3.29	9.77	22.47	30.58
GG	3.31	11.53	21.77	28.54
CG	3.33	10.97	21.80	29.00
MS	3.28	10.90	23.00	25.47
p value	0.415	0.403	0.405	0.234
Dark vs light	pH	TA	Brix	Cluster (g)
Dark	3.30	10.65	22.12	29.56
Light	3.30	10.94	22.40	27.24
p value	0.776	0.696	0.626	0.186
CON vs Mulch	pH	TA	Brix	Cluster (g)
CON	3.29	9.77	22.47	30.58
Mulch	3.30	11.13	22.19	27.67
p value	0.517	0.137	0.678	0.157
Glass vs MS	pH	TA	Brix	Cluster (g)
Glass	3.32	11.25	21.79	28.77
MS	3.28	10.90	23.00	25.47
p value	0.179	0.693	0.122	0.134
GG vs CG	pH	TA	Brix	Cluster (g)
GG	3.31	11.53	21.77	28.54
CG	3.33	10.97	21.80	29.00
p value	0.488	0.583	0.967	0.843

Results are significant at $p \leq 0.05$*, $p \leq 0.01$**, $p \leq 0.001$***

Yield measurements were collected per bay during the harvest in 2008. Parameters included cluster number and amount of bird damaged fruit from which the potential number of clusters could be calculated. Fruit weight data was also collected and potential yield as weight was calculated (Table 3.8). Significant differences were found for cluster number per bay and fruit weight, however once bird damage was factored in the results were no longer significant. The green glass was reduced by 37% compared to the other treatments for cluster numbers per bay when bird damage was not factored in. This treatment also had significantly lower results for fruit weight 46% compared to the other treatments. It might be expected that yields would be lower for this treatment due to the colour of the mulch. Shade which reduces the red to far red ratio available to plants also reduces the rate of photosynthesis (Smart *et al.* 1988). It is speculated that green glass, because of its colour, had absorbed rather than reflected energy that would have been used by the vines to increase production.

Table 3.8 – Yield parameters per bay for 2008 harvest.

Treatment	Harvested cluster #	Bird damaged cluster #	Total potential cluster #	Harvested fruit weight (Kg)	Potential fruit weight (Kg)
CON	36b	14	49	1.77b	2.41
GG	22a	8	29	1.00a	1.32
CG	38b	10	48	2.20b	2.78
MS	32b	16	46	1.60ab	2.3
p value	0.021*	0.734	0.236	0.023*	0.195
Dark vs Light	Cluster number	Bird damage	Potential yield	Fruit weight (Kg)	Potential yield (Kg)
Dark	29	11	39	1.39	1.87
Light	35	13	47	1.90	2.54
p value	0.069	0.763	0.327	0.036*	0.155
CON vs Mulch	Cluster number	Bird damage	Potential yield	Fruit weight (Kg)	Potential yield (Kg)
CON	36	14	49	1.77	2.41
Mulch	31	11	41	1.60	2.13
p value	0.130	0.571	0.313	0.466	0.629
Glass vs MS	Cluster number	Bird damage	Potential yield	Fruit weight (Kg)	Potential yield (Kg)
Glass	30	9	39	1.60	2.05
MS	32	16	46	1.60	2.3
p value	0.591	0.415	0.427	0.993	0.657
GG vs CG	Cluster number	Bird damage	Potential yield	Fruit weight (Kg)	Potential yield (Kg)
GG	22	8	29	1.00	1.32
CG	38	10	48	2.20	2.78
p value	0.005**	0.681	0.104	0.004**	0.050*

Results are significant at $p \leq 0.05$*, $p \leq 0.01$**, $p \leq 0.001$***

3.4.9 Pruning weights

Pruning dry weights are presented in Table 3.9. The treatments all had an effect on vine pruning weight. Vine weights over the control were 75% of those collected from mulched vines. The lighter coloured mulches produced cane weights that were 9% higher than darker coloured mulches. Two factors may have affected the increase in cane weight. Firstly an increase in light availability from the mulches has likely increased the potential for greater photosynthesis by the vines, resulting in more dry matter produced. Photosynthesis produces carbon which contributes to growth and to the storage of carbohydrates within the woody structures of the vine (Mullins *et al.* 2002). Significantly higher rates of photosynthesis were not recorded during the 2009 season, which would suggest that this was not a factor, however, photosynthesis was not measured during the 2008 harvest, so the fact that this might have been due to higher rates of photosynthesis can not be ruled out entirely. Soil moisture may also have been involved in increasing pruning weights. Cane weights in the mulch treatments, which had higher soil moisture (Table 3.2), were 25% higher than the control. Increasing availability of water to the vines can increase vegetative vigour. Flexas *et al.* (1999) found a highly significant correlation between pre-dawn leaf water potential and net carbon dioxide assimilation, therefore higher levels of moisture in the soil also are likely to encourage photosynthesis to increase dry matter accumulation.

Table 3.9 – Statistical differences for pruning weights (Kg/vine) gathered September 2008.

Comparison	Treatment mean CON	GG	CG	MS	*p* value
Treatment	0.89	1.25	1.12	1.22	0.075
Dark vs Light	1.07		1.17		0.032*
CON vs Mulch	0.89	1.19			0.019*
Glass vs MS		1.18		1.22	0.767
GG vs CG		1.25	1.12		0.300

Results are significant at $p \leq 0.05$*, $p \leq 0.01$**, $p \leq 0.001$***

3.4.10 Canopy temperature

Canopy temperatures were measured using data loggers from October 2008 until May 2009 or from just before budburst through to a theoretical harvest date based on the previous year's harvest. Temperatures were converted to growing degree days (GDD) with a base temperature of 10°C. No significant differences were found between the treatments or for any of the comparisons made (Table 3.10). It would be necessary to carry out a long-term study over a number of years and through different seasonal conditions to substantiate findings in this area however.

Table 3.10 – Total accumulated GDD per treatment from October 2008 to May 2009.

Comparison	Treatment comparison CON	GG	CG	MS	*p* value
Treatment	1040	1021	1063	1080	0.538
Dark vs Light	1031		1072		0.231
CON vs Mulch	1040	1055			0.667
Glass vs MS		1042		1080	0.341
GG vs CG		1021	1063		0.361
Diff from CON		-19	23	40	

Results are significant at $p \leq 0.05$*, $p \leq 0.01$**, $p \leq 0.001$***

Surface temperatures of each type of mulch were also measured and showed significant differences (Figure 3.8). The darker treatments: control and green glass were found to absorb more heat than the clear glass and mussel shells. The cooler surface temperatures of the lighter coloured mulch types suggests that they were reflecting heat as opposed to absorbing it. This finding corresponds to that mussel shell soil temperature which appeared to be cooler than the other treatments. The clear glass also appeared to lag behind green

glass and control treatments in relation to soil temperature (Figure 3.4). Light coloured mulches reflect radiation, which has a cooling effect on the soil (Creasy *et al.* 2003b, Crawford 2007, Leal 2007). Mulch surface temperatures in this study were only tested outside of the vineyard. It would be important to repeat this analysis in the vineyard in order to fully understand the effect of the different colours and materials on mulch temperature.

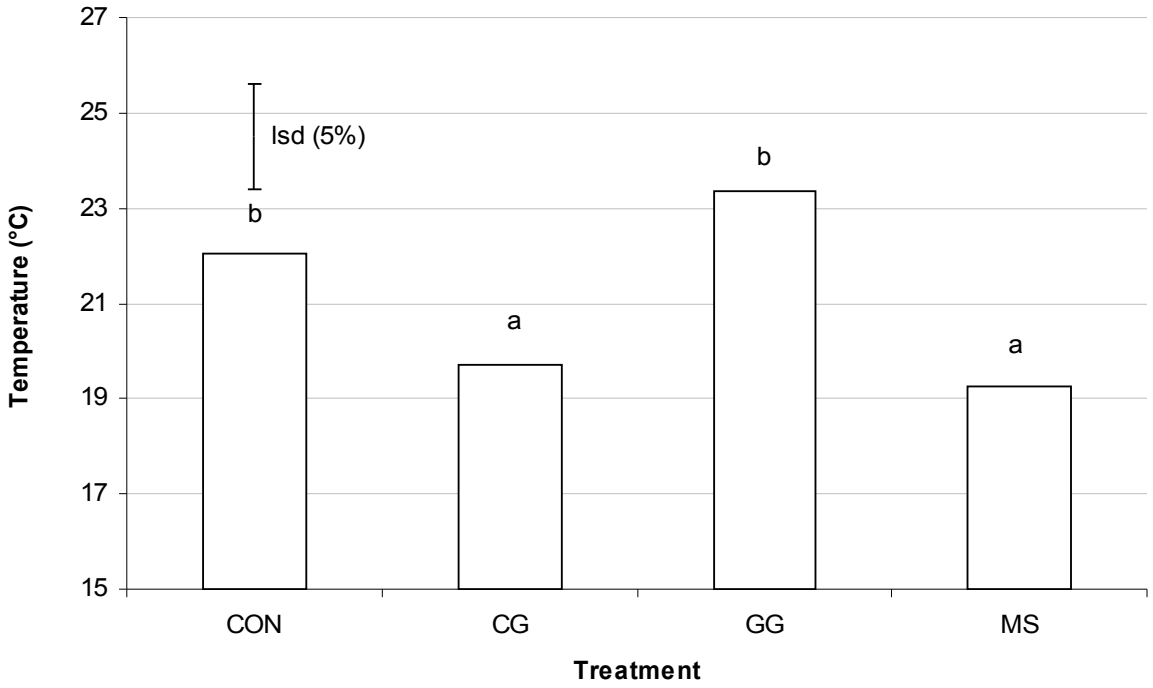

Figure 3.8 – Mulch surface temperature measured on 7 May 2009 between 1pm and 2pm Results are significant at $p \leq 0.05$.

The pattern between the affect of the various treatments on canopy temperature can also be viewed during the season (Figure 3.9). The mussel shells and clear glass treatments more often appeared to reflect heat when compared to the control. The green glass treatment more often absorbed heat when compared to the other treatments. It is interesting to note that the green glass appeared to absorb the most radiant energy (5% more than control) during flowering, an important period upon which crop levels are dependent. Harvested crop levels were significantly reduced in the green glass treatment.

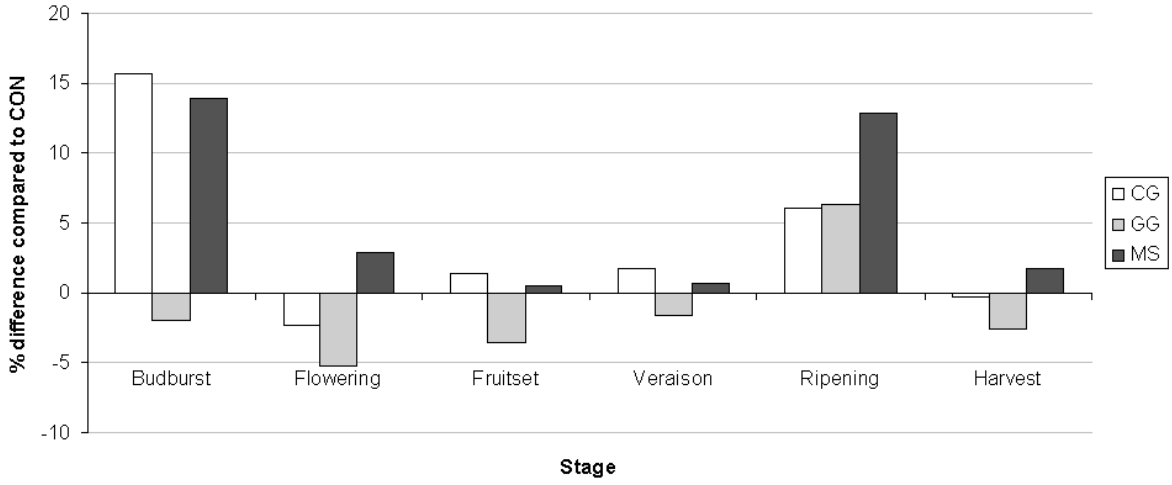

Figure 3.9 – GDD % difference from CON at each stage during the growing season, results are not significantly different.

Creasy and Crawford (2005) mentioned frost risk in relation to the mussel shell mulch as they noticed that this type of mulch generally caused cooler night-time temperatures. During the frost event that occurred for the Sandihurst trial differences between treatments were not found to be statistially significant. All treatments experienced around the same base temperature, the clear glass and mussel shells both reached -2.5°C, the control got to -2.2°C and the green glass reached -2.1°C (Figure 3.10). Although results were not significant, variation at the microclimate level can have a large impact on frost severity (Halley *et al.* 2003). As no frost is the same it would be useful to monitor the various mulch effects on temperautre during other frost events.

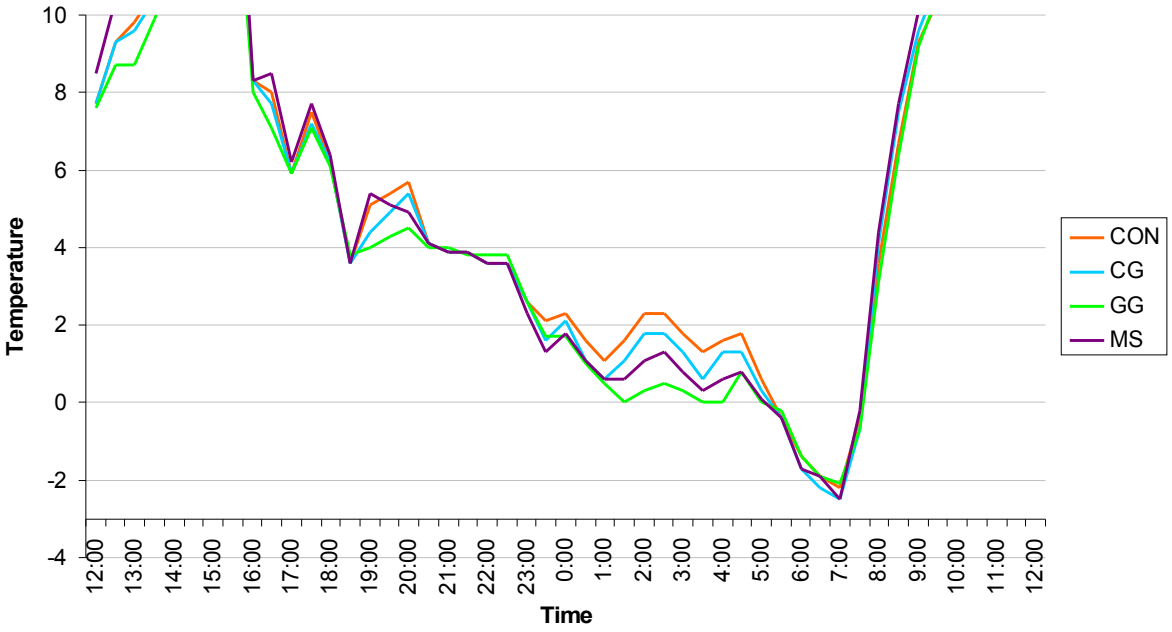

Figure 3.10 – Frost temperature data experienced at fruiting wire height on morning of 6 November 2008 in replicate 3. Results from both replicates were not significant.

3.4.11 Reflected radiation

Data were taken using a StellarNet Miniature Fibre Optic Spectrometer in the vineyard on 2 April. As results for each replicate were similar, only replicate one is presented (Figure 3.11).

The graph shows that the clear glass gave the most reflectance in the visible range of the spectrum (380 nm – 760 nm) followed by the mussel shells and finally the green glass and control. On a clear day the sun ranges from 1.5 – 2.0 W/m². In comparison to light levels received at fruiting wire height over each of the treatments, the control received 16% of solar radiation in the visible range, green glass received 20%, mussel shells received 40% and clear glass 42%. In the near infrared spectrum (760 – 950 nm) control, clear glass and mussel shells had the highest readings. Green glass received 26% less infrared compared to the other treatments together. High readings for the control in this part of the spectrum are likely to have been caused by infra-red reflectance from the soil and also from weeds that were starting to grow under these vines at the time the scan was taken. The green glass absorbed the greatest amount of energy in the red and infra-red spectrum compared to the other treatments. This is shown clearly in the graph with the lowest readings in this part of the spectrum having been experienced by the green glass treatment.

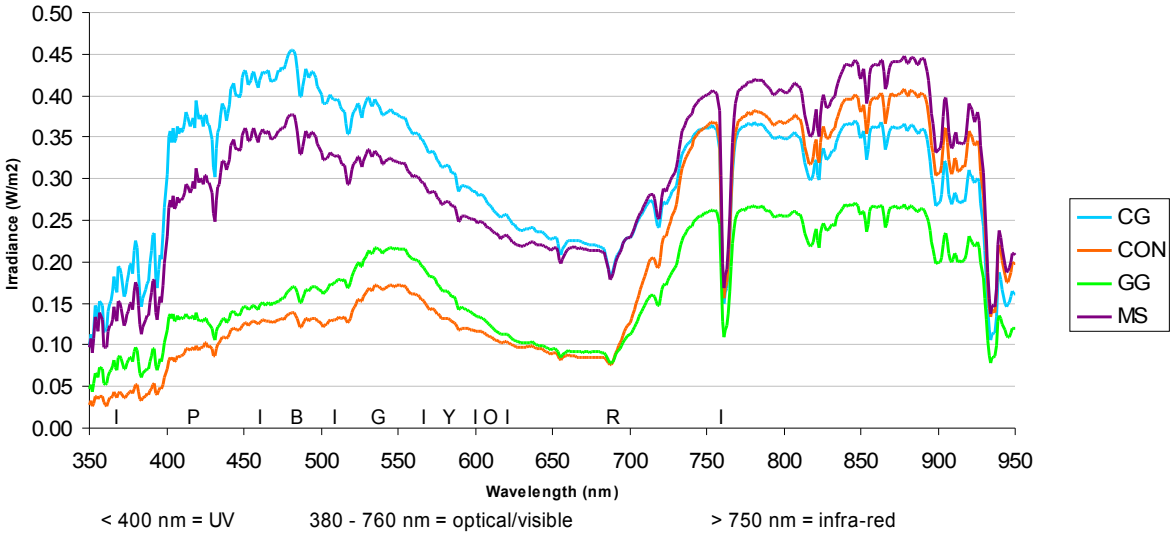

Figure 3.11 – Replicate 1 measurements from the StellarNet spectroradiometer scan of reflectance from each treatment in the vineyard.

From the top of the UV spectrum (300 – 400 nm) readings were highest for the clear and mussel shell treatments, which reflected respectively 83% and 82% more UV than the control, the green glass also reflected higher UV in this range than the than the control at 63% (Figure 3.12). Greater access of UV can influence levels of phenolics such as quercetin (Price *et al.* 1995) and resveratrol (Langcake and Pryce 1976 and 1977). Anthocyanin concentration also increases with exposure to UV (Kataoka *et al.* 2003).

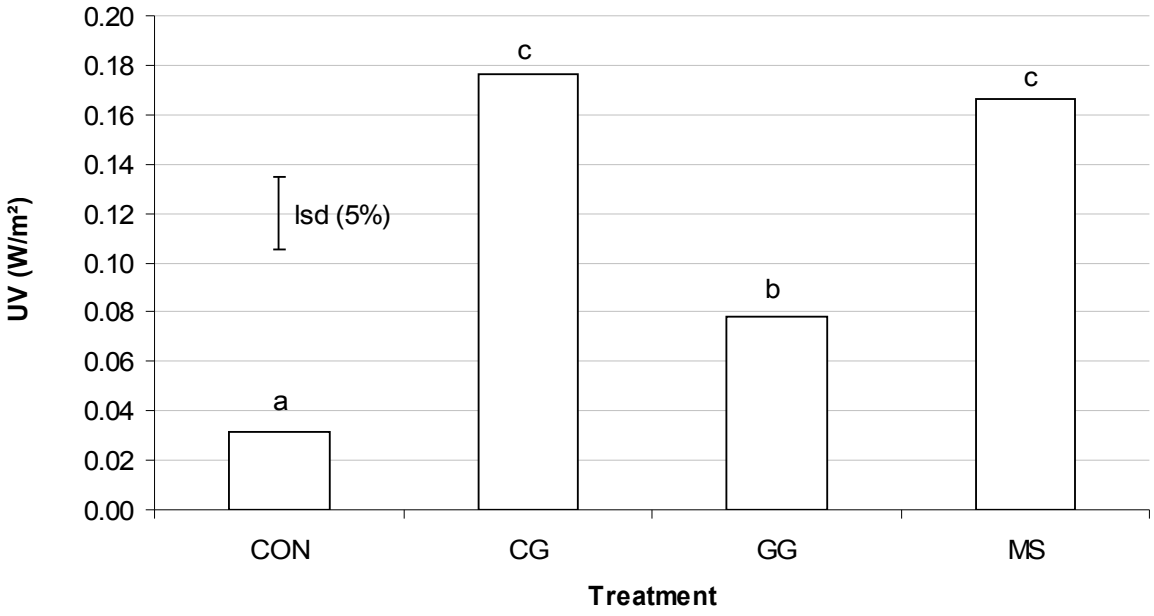

Figure 3.12 – UV readings per treatment in the range at the top of the UV spectrum (300 – 400 nm). Measurements were taken in the vineyard using the StellarNet spectroradiometer. Significant at $p \leq 0.05$.

Another set of scans were taken using a Bentham spectroradiometer on 7 May 2009 (Figure 3.13 and 3.14). Three replicated scans were taken with similar results, and therefore only replicate one has been shown. Comparisons made between treatments in the UV range between 300 and 400 nm showed significant differences for all treatments (data not shown). Control reflected the lowest amount of UV in this range followed by green glass, mussel shells and clear glass results were significant at $p \leq 0.001$.

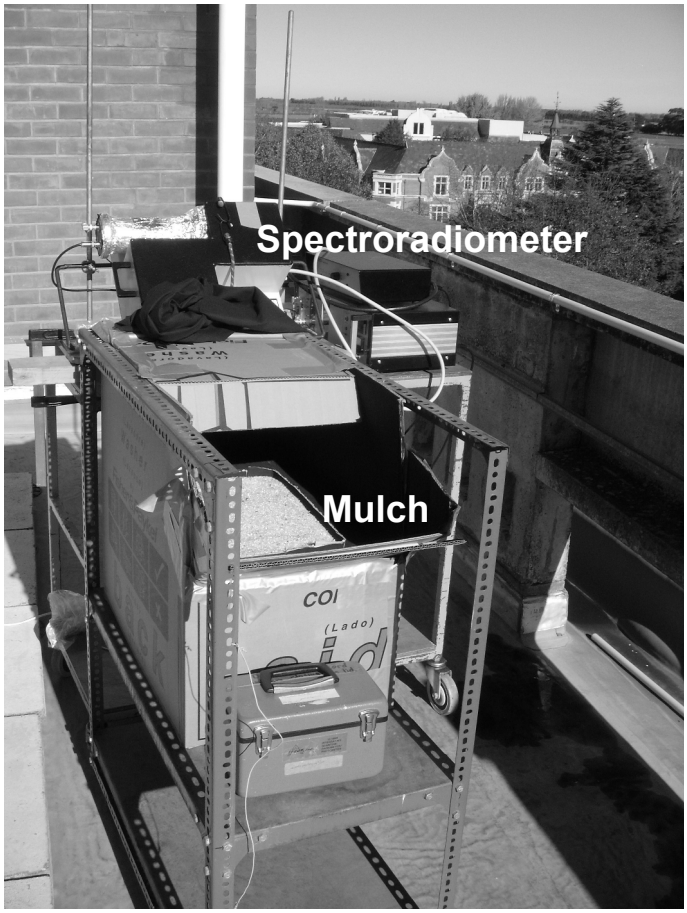

Figure 3.13 – Photograph of the Bentham spectroradiometer set up on the roof of the Hilgendorf building at Lincoln University.

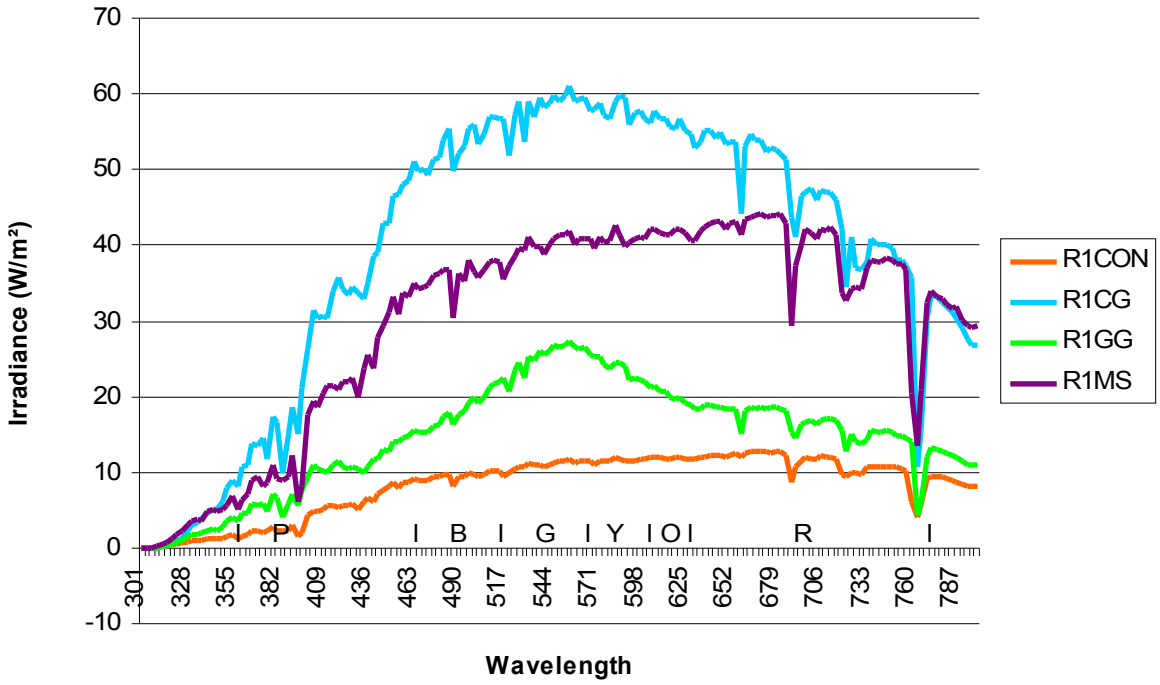

Figure 3.14 – Replicate 1 Bentham spectroradiometer scan of reflectance for each treatment in the model system.

It should be noted that vineyard and model scans were taken using two different spectroradiometers and thus comparisons between these two different situations should be interpreted with caution. The readings taken in the vineyard were also taken during the morning up to solar noon, to avoid canopy shade which obstructed reflectance as the sun moved over the top of the vines. By contrast, the model was set up to measure maximum reflectance and readings were taken over solar noon. Despite differences between the readings taken, it is possible to make some generalised comparisons between each data set based on what is known about each situation.

In the model system, without the presence of other plants and scattered light it is possible to see an increase in irradiance as the green glass passes the green part of the spectrum (500 – 570 nm) (Figure 3.14). An increase was seen over this part of the spectrum in the vineyard for all treatments (Figure 3.11). The model system also showed that the control had the lowest reading overall and did not increase as the scan moved into the infra-red spectrum. This may be due to the fact that weeds were present beneath this treatment in the vineyard, which were not present in the model system. Another difference between the vineyard and the model scan was the intensity of reflectance. However this was likely due to the time of day each scan was taken. Discrepancies may have additionally been caused by the different

types of equipment used. A final major difference was seen in the shape of the curve generated with each scan. The Bentham spectroradiometer appeared to detect a greater amount of red light in the model system compared to the StellarNet instrument in the vineyard. The reduced amount of red light detected by the StellarNet may have been due to the absence of scattered light in the model system which was present in the vineyard. It was also likely to have been due to use of different equipment and different times of day each scan was taken. Finally, for the control treatment in the vineyard the soil was untouched versus for the model the soil that had been collected was loose and dried having been taken out of the vineyard. Therefore reflectance was likely to have differed between the controls in each situation.

Figure 3.14 shows that the clear glass gave the most reflectance in the model system followed by the mussel shells. A similar pattern was seen in the field with the clear glass reflecting more light. The mussel shell mulch was not as different between the vineyard and model scans as the clear glass. The clear glass reflected 30% less PAR in the model system (compared to the vineyard) while the mussel shells reflected 12% less PAR in the model compared to the vineyard (Figure 3.15). It was expected that higher reflectance would occur in the model system as the light intensity is greater towards solar noon and there was no interference from scattered light. The fact that the difference was not as great for the mussel shells is thought to have been caused by soil that was collected with the shells when they were taken from the vineyard, the soil persisted with washing and would have interfered with the reflective properties of the shells. Normally, the shells surface is bright in the vineyard, with the undersides being darker due to the soil. Darker coloured treatments were found to have similar results in the vineyard and within the model system (Figures 3.15). The control was found to reflect less light in the model system than in the vineyard this may have been due to the presence of scattered light in the vineyard.

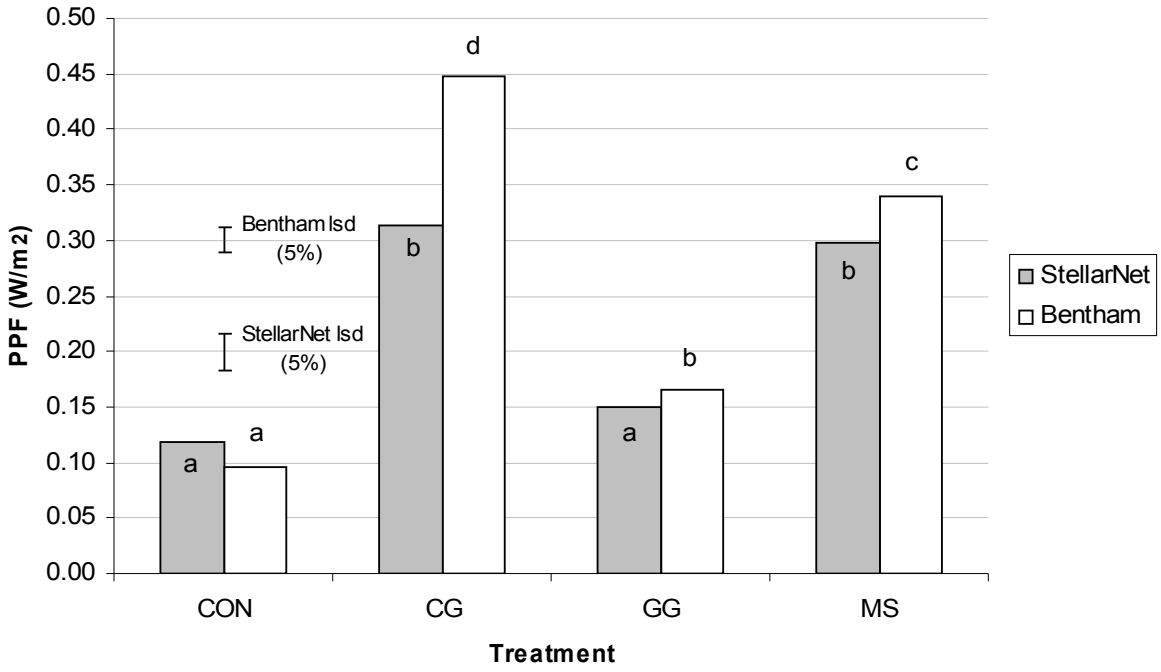

Figure 3.15 – Photosynthetically active radiation (PAR) average per treatment comparison between vineyard scan using StellarNet spectrometer and model scan using Bentham spectroradiometer. Results are significant at $p \leq 0.05$.

One final parameter investigated in relation to reflected radiation was the red to far red ratio (R:FR) for each treatment. In the vineyard higher levels of R:FR light were found for the lighter coloured treatments. High ratios are desirable as red light is more easily absorbed by the canopy compared to far red light (Franklin and Whitelam 2007). Red light alters the balance of active phytochromes, which can lead to a physiological response by the vine. Reduced R:FR ratios are synonymous with shading which causes vegetative growth as the vine shoots try to escape the shaded environment (Franklin and Whitelam 2007). Direct sunlight R:FR ratios are typically 1.15 while the R:FR ratio for a canopy shaded from close spacing can be as low as 0.22 (Smart 1987). Readings taken using the StellarNet spectrometer in the vineyard (Figure 3.16) showed R:FR ratios experienced by clear glass and mussel shells were highest at respectively 63% and 62% of the sun's ratio. Green glass was 35% of the sun's ratio and the control received the lowest figure of 0.22, the same as for a shaded canopy, which was 19% of the sun's ratio (Figure 3.16). Red to far red ratios calculated from the model system (data not shown) varied from the pattern seen in the vineyard whereby the order for treatment ratio was clear glass > green glass > control > mussel shells. The ratios overall were also 60% higher when recorded in the model system

compared to the vineyard. With no scattered light and no plants present in the model system the treatments reflected almost all of the red light from the sun towards the sensor accounting for this result.

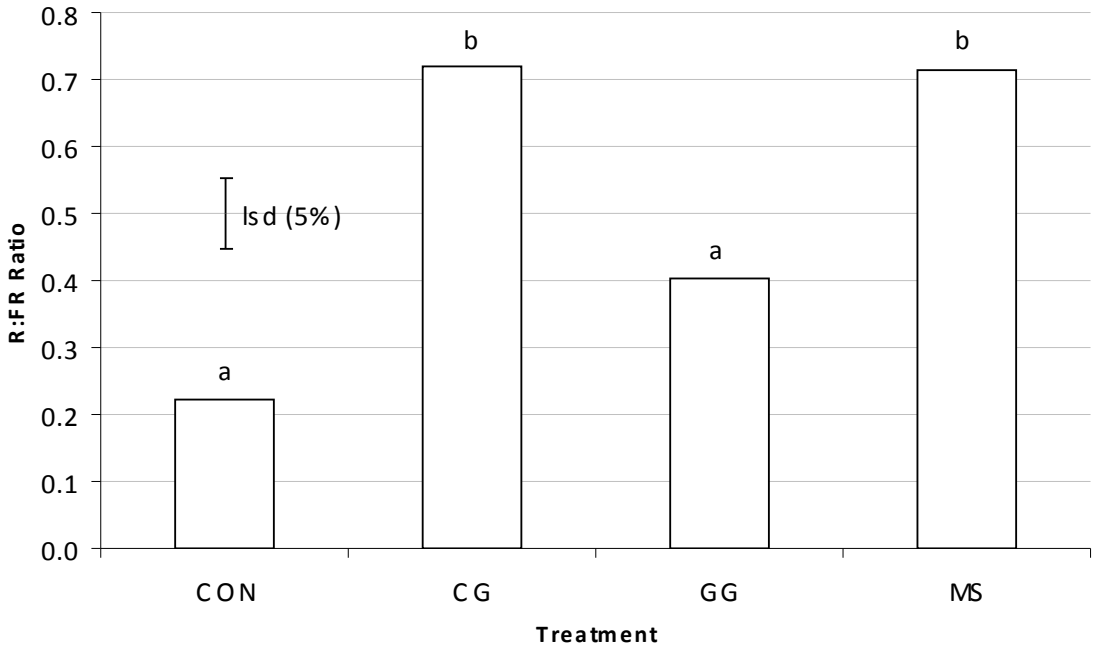

Figure 3.16 – Red to far red ratio per treatment vineyard data from StellarNet spectrometer. Results are significant at $p \leq 0.05$.

Lighter coloured treatments reflected the most light into the grapevine canopy, which was seen in levels of PAR and R:FR ratios. It is speculated that the additional light would help grapes to ripen by increasing sugar levels (Kliewer *et al.* 1967, Crippen and Morrison 1986, Mabrouk and Sinoquet 1998, Reynolds *et al.* 1986, Smart 1987) and reducing titratable acid (Kliewer and Lider 1968, Kliewer *et al.* 1967, Reynolds *et al.*1986). The increase in light could also aid in the development of certain phenolic compounds such as flavonols (Price *et al.* 1995). Higher levels of Flavonols such as Quercetin found in berries exposed to increased levels of light have potential health benefits for humans (Price *et al.* 1995). In subsequent seasons the additional light might be expected to increase yields. Baldwin (1964) reported that light intensity played a part in light dependent reactions within the bud to increase fruitfulness. Sommer *et al.* (2000) reported that greater carbohydrate reserves during winter resulted in increased shoot fruitfulness in the following season. Where reflective mulches had been used, Hostetler *et al.* (2007a) reported larger clusters in vines

that had been mulched with white geotextile material. Number of clusters per vine in this trial were not significantly different between the mulched and un-mulched vines however. Robin *et al.* (1997) also reported higher yields in fruit that had been grown over reflective mulch. In that trial mulched berries were heavier and there were more berries per cluster. The same effect was seen in the 2008 harvest at Sandihurst where yields were higher over lighter coloured mulches. The greater potential for photosynthesis over reflective mulch would also be expected to increase dry matter content, as was seen in the 2008 pruning data where the lighter mulches had higher pruning weights compared to darker treatments (Table 3.9). This could positively influence growth in the following season (Howell 2001) and bud fruitfulness (Sommer *et al.* 2000).

3.5 Conclusions

Mulch effects on the soil were mostly connected to vine health and more positive influence on soil parameters were seen from the mussel shell mulch compared to the glass treatments. The addition of calcium carbonate from the shells is likely to have caused higher pH levels that would have increased the availability of certain nutrients to the vine. The mussel shells also showed higher levels of microbial biomass carbon, suggesting there were more microbes under this mulch compared to the glass treatments. All mulches retained soil moisture content compared to the control and nutrients in the canopy were seen to be higher for the mulched compared to the un-mulched control. In the canopy, though photosynthesis and leaf greenness showed no significant differences, conductance, internal leaf carbon dioxide concentration and transpiration rates were higher in control compared to mulch treated vines while vapour pressure deficit was higher in the mulched compared to the control vines. This may have been caused by additional light in the fruiting zone which could have increased leaf efficiency and should be tested in future research using chlorophyll fluorescence. No significant differences were found for fruit quality parameters, however yield parameters were higher for light coloured treatments compared to dark treatments. Green glass resulted in lower overall yield when compared to the other treatments. The green glass may have absorbed radiation from the red part of the spectrum that might normally encourage production. Mulches and reflected light also appeared to increase pruning weights. Where reflected radiation was tested in the vineyard and using a model system, photosynthetically active radiation was higher for lighter coloured mulches. Red to far red ratios measured in the vineyard were also highest over light, compared to dark coloured treatments.

The mulches have had clearly influenced the soil environment. The introduction of any product into the vineyard system warrants consideration of long term effects to the soil and

ecosystem. One of the benefits of using mulches made from mussel shells, and especially from the inorganic product glass, is that they do not break down as quickly as organic mulches and therefore do not need to be replaced as frequently. The mulches will however eventually work their way into the soil and it would be worth monitoring the effects of this over an extended period of time. It is hypothesised that the glass might eventually change the structure of the soil especially as it is added in such high quantities though this could also be beneficial especially if the rough surface of the glass was found to prevent the emergence of weeds in glass mulched areas. As mulches are often used to suppress weeds, future research could also investigate the effect of the mulches on weed growth under the vines. Further research could also be carried out on the effect of the mussel shells on soil structure, which appeared to increase water retention in this experiment. The effect of the organic component of the mussel shells on higher microbial biomass carbon also warrants further investigation as does the effect of the green glass treatment on higher sodium levels in the soil. Canopy temperature measurements which were not found to be significantly different between treatments could be repeated with a greater number of replicates to re-assess the differences. It would also be useful to monitor to the effect of the mulches during several different years and frost events.

In this trial the mulches have been found to have various beneficial effects on the vine environment. They offer options to improve soil health and function while at the same time increasing the vines' access to sunlight. This investigation has also demonstrated important differences in the use of different coloured products. This was especially noticeable for the green glass mulch which did not gain positive results for yield. By comparison, lighter coloured mulches were found to have reflective properties that could offer viticulturists the opportunity to optimise vine access to sunlight for improving vine health, productivity and potentially having a positive influence on wine quality.

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Chapter 4 - Reflective mulch effects on juice and wine characteristics

4.1 Abstract

The effect of different reflective mulches on Pinot noir juice and wine was investigated in a cool climate vineyard in Canterbury, New Zealand. Treatments included a non-mulched control (CON) and mulches made from waste products including mussel shells (MS) and green (GG) and clear (CG) recycled crushed glass. Increasing access by vines to light is known to aid in the ripening of fruit and to increase quality parameters such as development of desirable aromas. This can be achieved by the application of reflective mulches. The effects of the mulches in the vineyard have caused changes seen in the grape juice and subsequently in the wine. Colour, phenolics and acids were tested for wines made in 2006 and 2008. A tasting was also held for each of these wines. Wine samples were not replicated and therefore results for these tests are preliminary to further research on replicated samples. Gas chromatography – olfactory (GC-O) was used to investigate the aroma attributes of juice from each treatment. Two panellists acted as aroma detectors and sniffed juice samples that had been collected during the 2008 harvest. Differences in juice aromas were reported slightly differently by each panellist who detected them, but significant results were found within each data set. The most statistically significant differences were found for the vegetative peak at 18.26, the raspberry/marzipan peak at 26.06, the geranium/leafy peak at 27.45 and the berry/floral peak at 45.59. The intensity of the vegetative peak was found to be highest for CON followed by CG, MS and finally GG. CON had greater length than the other treatments for the raspberry/marzipan peak and had a higher intensity compared to other mulches for the geranium/leafy peak. Finally for the berry/floral peak MS, when compared to the other treatments had greater length. Wines were also analysed using GC-O though samples were not replicated. Analysis of the different wine attributes: colour, phenolics, acids and aroma suggest there had been a treatment effect on the wine. A blind tasting of these wines also alluded to this conclusion, that application of reflective mulches in the vineyard can alter the flavour, aroma and mouthfeel of the wine. GC-O analysis of juice from each treatment demonstrated the effects of the mulches on fruit aroma. Aroma profiling also showed the effectiveness of the GC-O method as a means of understanding the human experience of wine aroma. It will be necessary in future trials to include a greater number of panellists in order to improve result resolution and increase the number of possible comparisons. It will also be important to test replicated wine samples. Results thus far

however, form an important basis for further research and show that primary fruit aromas in berry juice are affected through the use of these mulches.

Keywords

Pinot noir, reflective mulch, mussel shells, recycled glass, GC-O, aroma profiling, phenolics, anthocyanins.

4.2 Introduction

Gaining quality and ripeness of fruit in the vineyard at harvest can be challenging, especially in a cool climate region. Basic quality measurements for ripeness used in the lead up to harvest include °Brix, titratable acidity (TA) and pH (Jackson 1986). Other important factors include phenolics, flavours and aromas (Conde *et al.* 2007). Phenolic compounds are extracted from the grapes during winemaking, and they derive from the seeds, skins and rachis of the clusters (Jackson and Lombard 1993). Anthocyanins develop during ripening in the skins of red grape varieties and are a major component of red wine colour (Jackson and Lombard 1993). The two major groups of phenolics include the flavonoids and the non-flavonoids (Waterhouse 2002). Other phenolic compounds are important in the development of wine flavour and aroma (Jackson and Lombard 1993). Phenolics are affected by vine access to light in the vineyard. The flavonoid quercetin (Price *et al.* 1995) and the stilbene resveratrol (Stein 1984 cited in Creasy and Coffee 1988) are produced in the fruit when the vine is exposed to ultra-violet.

Dokoozlian and Kliewer (1996) discovered that early exposure to light, between fruit set and the beginning of berry softening, could increase development of anthocyanins and other phenolics from berry softening to maturity. Haselgrove *et al.* (2000) also demonstrated that a minimum level of light was needed for anthocyanin development during the first stages of ripening. During later stages, it was discovered that high temperatures (>35°C) would inhibit or even degrade anthocyanins. Bergqvist *et al.* (2001) reaffirmed that grape berry access to sunlight is an important factor of fruit and wine quality, however in hot regions the high temperatures experienced by fully exposed fruit were detrimental for berry colouration. In cool climates, high temperatures are not likely to be as much of an issue, but the initial period of light is highly important. Spayd *et al.* (2002) also demonstrated that anthocyanin development was dependent on light when they found higher concentrations in exposed berries that had been cooled to the same temperature as shade berries. The control berries

that had not been cooled had lower anthocyanin concentration as did shade berries that had been heated. Cohen *et al.* (2008) showed that phenolic metabolism, including anthocyanin synthesis, was affected by temperature. Where temperatures were damped by cooling during the day and heating at night, berries were larger and more coloured than other berries at veraison. Damping also encouraged berry ripening with higher sugar content, berry weight and anthocyanin concentration at harvest (Cohen *et al.* 2008). Proanthocyanidins increased with higher night temperatures, but cooler daytime temperatures resulted in the lowest proanthocyanidin accumulation. It was concluded that higher overall temperatures are beneficial in terms of phenolic accumulation and berry colour. Fruit colour is important as it is related to other ripeness parameters. Francis *et al.* (1999) demonstrated this with a positive correlation between grape extract colour and wine flavour intensity score.

Light has also been shown to be important for the development of phenolic compounds. Haselgrove *et al.* (2000) investigated development of quercetin-3-glucoside, a flavonol likely to be involved in co-pigmentation, increasing colour stability in wine. It was found that this compound was enhanced by greater light exposure. By contrast, the stilbene resveratrol was found to be less affected by light levels (Haselgrove *et al.* 2000). Price *et al.* (1995), who investigated the development of different phenolics under different light exposures, found that phenolic development was highly related to sun exposure. Flavonols tested showed higher accumulation in exposed berries. These berries had a higher degree of light stress caused by increased amounts of UV, which flavonols absorb. Spayd *et al.* (2002) found lower levels of flavonol concentration in berries that had been grown beneath screens that block UV, confirming that this appears to be a limiting factor for the development of flavonols. Anthocyanin concentration is also affected by increasing levels of UV radiation (Kataoka *et al.* 2003). Bergqvist *et al.* (2001) reported a positive correlation between increasing photosynthetically active radiation (PAR) and total phenolics. Keller *et al.* (1998) tracked the development of total phenols, total flavonols and total anthocyanins in berries that were exposed to 100%, 20% and 2% sunlight for three weeks at veraison. It was found that levels of each type of phenolic compound were lower per berry, when light levels had been reduced during veraison. Although the vine can in some cases recover from stress situations such as a lack of access to light during ripening, this did not occur in this particular situation and berries that had been exposed to the lower light levels during veraison finished with lower phenolic levels at harvest.

Light exposure to berries or degree of shade also has an effect on wine flavour and aroma. Morrison and Noble (1990) reported significant differences between wines that had been made from shade fruit compared to an unshaded control. In this trial tasters were able to discriminate between control wines from those made with shade treatments. Bureau *et al.*

(2000) reported lower levels of monoterpenols and C13 norisoprenoids in the aromatic white variety Muscat of Frontignan where berries were artificially shaded. This fruit had less free and bound terpenols. In this trial whole vine shading had less effect on aroma than artificial shading of individual clusters. The paper concluded that for this variety, cluster light and temperature environment had a greater influence on aroma development than did vine environment. Ristic *et al.* (2007) tested the effects of shading on Shiraz grapes and found that shade bunches had fewer levels of flavonols. Wine made from shade fruit contained fewer levels of total anthocyanins, total phenolics and tannins. Wine colour density was also reduced. Wines from shade environments additionally had lower ratings for desirable mouthfeel characteristics and fruit flavour. The effects of shading fruit was found to have reduced norisoprenoid levels in the wine and it was therefore concluded that shading negatively impacted on wine composition.

Other trials that reported on the influence of light on wine flavour and aroma profiles did so in relation to the application of reflective mulches in the vineyard. Sandler *et al.* (2009) reported that wines that had been mulched with quahog shells had reduced floral and pomegranate aromas but increased earthy aromas. For taste, cherry and earthy flavours were reduced in the shell treatment compared to control. Reynolds *et al.* (2007), who investigated the effect of aluminised mulch on wine aroma and flavour, reported reduced vegetative aromas for Cabernet franc, Cabernet Sauvignon and Pinot meunier wines made from mulched treatments. Cabernet Sauvignon wines were also found to have reduced colour intensity in response to mulching. Pinot noir mulched vines had more intense plum aroma, currant flavour, colour and reduced herbaceous aromas (Reynolds *et al.* 2007).

Crawford (2007) and Leal (2007) both investigated the effects of mussel shell mulch on Pinot noir. Crawford (2007) reported that 29 out of 39 winemakers who tasted micro-vinification wines made from trial fruit preferred those made from the mulched treatments. Results from the blind tasting, which focused on mouthfeel and colour characteristics, showed that mulched wines had higher scores for surface-smoothness, texture, heat and complexity while unripe tannins, drying tannins and acidity were rated lower in the mulched compared to control wines. When commercial wines made from the mulch treatment fruit were tasted however, 66% of tasters preferred the control wines. Shell commercial wines were found to be less ripe, complex and dynamic with higher acidity. Leal (2007) also reported on wines made in another season: micro-vinifications, and those made commercially, both made from the trial plot. For the micro-vinifications total phenolics and phenolic ripeness were reported higher in mussel shell treatment wines, hue and colour density also appeared to be higher, whereas ripe fruit, palate texture and overall quality were slightly higher in control wines. Commercial wines from the mulched area were rated higher for total phenolics, phenolic

ripeness, colour density, ripe fruit, bitterness, palate texture and overall quality; only hue was found to be the same as the control (Leal 2007).

As well as tasting it is important to quantify the compounds present in the juice or wine. One method often used for the quantification of aroma compounds is gas chromatography – mass spectrometry (GC-MS) (Brander *et al.* 1980). However there still exist some odours that can not be quantified using this technology. For these aromas it is possible to combine instrumental analysis with a more highly selective tool: the human nose (Qiao *et al.* 2008). The method is useful in the determination of odour type, the time for which the odour is active and its intensity. GC-O is highly useful in gaining an understanding of the activity of individual wine components. It allows observation of the relationship between the odour and the chemical composition of its volatile fraction (Plutoska and Wardencki 2008). GC-O also gives an idea of human thresholds for the aroma compounds present and is therefore revealing of their sensory importance (Marin *et al.* 1988). The GC-O technique was selected for this trial to quantitatively measure wine aromas in grapes growing above the various mulch treatments. In light of previous research it was thought that the reflective properties of the mulches would impact on the development of phenolic compounds in the fruit and that this would subsequently impact on juice aroma to affect wine quality. This trial is one of the few that has investigated grape juice aroma using the GC-O method.

4.3 Materials and methods

4.3.1 Trial site and management

The trial was carried out at a vineyard positioned behind the Sandihurst Winery at West Melton, Canterbury. Four treatments were applied randomly within three replicates in the vineyard (Table 3.1 and Appendix 1). Treatments included: an un-mulched control of bare earth (CON), clear glass mulch (CG), green glass mulch (GG) and mussel shell mulch (MS) (Figure 3.1).

Mulches were laid down in December 2005 covering slightly more than three bays length. The mulch continued at bay ends for two vines and was laid down on the near side of rows running parallel to treatment vines on the side facing them. The additional mulching meant the vines in the trial were surrounded by the mulch as if the treatment had been applied to the entire vineyard. The extra mulched vines, or buffer vines, were not examined in the trial.

Microvin wines were made in 2006 and 2008 at the Lincoln University Winery. In 2006, the berry harvest occurred on 19 April. In 2008 berries were harvested on 7 May. In each year treatments were handled separately, but all ferments were treated the same. For winemaking notes see Appendix 3.

4.3.2 Wine phenolics

4.3.2.1 Colour and phenolics

Wine colour and phenolics were measured using the method described by Iland *et al.* (2000 pg 99). Wine was measured into three test tubes at 2 mL per tube. The first test tube had nothing extra added to it. The second test tube had 20 µL 10% w/v acetaldehyde added; this was left for 45 minutes before being measured. The third test tube had 30 µL 25% w/v sodium metabisulphite added to it; this was left for 3 hours before being measured. To the fourth test tube, 10 mL of hydrochloric acid was added before 100 µL of wine was added.

Absorbance was measured at four different wavelengths using a Helium Alpha UV-Visble Spectrometer made by Unicam UV-Visible Spectrometry, UK. The wine, wine + acetaldehyde, wine + sodium metabisulphite and wine + hydrochloric acid were measured at 520 nm. The wine, wine + acetaldehyde and wine + sodium metabisulphite were measured at 420 nm. Finally the wine + hydrochloric acid was measured at 280 nm.

4.3.2.2 High performance liquid chromatography

Phenolic profiling of wines was carried out using High Performance Liquid Chromatography – Diode Array Detector (HPLC-DAD) as described by Keller *et al.* (2000). The model used was a Waters 600-MS system controller, Waters 717 plus autosampler and Waters 996 photodiode array detector. Millennium software was used for chromatographic analysis. The column was a Phenomenex (Auckland, New Zealand) Luna 5µ C18 (2) 100A 250 mm length by 4.6 mm ID. Standards were used to quantify specific phenolic compounds in the treatment samples. Standards used for identification included the flavonoids catechin, epicatechin, quercetin and rutin, and the nonflavonoids protoctechuic acid, *p*-coumaric acid and *t*-resveratrol.

4.3.3 Wine acid composition

Wine acidity was analysed using HPLC. A Shimadzu LC-10AD with a SPD-M10A diode-array detector was used with Simadzu LC-10 software for the analysis. A Phenomenex Synergi 4micrometer Hydro-RP column (250 x 4.6mm) was used to separate organic acids. Wine from the 2006 and 2008 vintages were both analysed. Acids investigated included: tartaric, malic, lactic, acetic, citric, succinic. The method used was that as described by Kordiš-Krapež *et al.* (2001).

4.3.4 Wine tasting

An informal blind tasting was carried out on wines from vintages 2006 and 2008 in December 2008 at Sandihurst Winery. A tasting panel of six wine experts were used. Each taster was

asked to complete a tasting scorecard which had been adapted from Leal (2007) and is included in Appendix 4. The scorecard covered aspects such as appearance in colour and hue; aroma depending on ripe fruit character and mouth-feel relating to palate length and bitterness. Tasters were also asked for descriptors relating to flavour and mouthfeel and to give a score for the overall balance of the wine and their personal preference. A copy of the Wine Aroma Wheel for red wine (Noble *et al.* 1987) was included in the tasting sheet, as was a copy of the red wine Mouth-feel Wheel (Gawel 2000). Data were analysed using a ranking system. The order in which wines were ranked by tasters were put into a spreadsheet to give a percentage for tasters who rated each wine in each category. Aroma profiles were generated by taking descriptors used by tasters and categorising them under headings taken from the inner circle of the Wine Aroma Wheel (Noble *et al.* 1987). These categories included: fruity, spicy, floral, microbiological, oxidised, pungent, chemical, earthy, woody, caramelised, nutty and vegetative. As an example if a taster chose the descriptor cherry this would be categorised as fruit, capsicum would fall into the vegetative category and clove in the spicy category. The percentage use of descriptors in each category, were then turned into percentages and the profiles illustrated as pie charts.

As wines were not replicated it was not possible to run statistical analysis on the data. Although wines were not replicated a tasting was still considered a valuable exercise especially as work following the tasting would focus on wine aromas with the use of gas chromatography-olfactory. The tasting also allowed first steps towards identifying true differences between the treatments and formed an important basis for future research.

4.3.5 GC-O grape juice analysis

Gas chromatography – olfactory (GC-O) analysis of juice aroma was completed in October. The GC-O makes it possible to single out aroma compounds from the juice. These aromas can then be investigated separately using both instrumental analysis and the human nose.

4.3.5.1 Sample preparation

Juice samples were collected during the 2008 harvest and were frozen as bulk samples. These were subsequently thawed and distributed into coded GC-O vials at 9 mL per vial. The codes related to the treatment and samples were sniffed by replicate. Once samples had been distributed into the GC-O vials they were refrozen and stored until they were needed. Samples were taken out of the freezer one and a half hours before the GC-O run was due to begin. This was done in order to allow samples time to thaw at room temperature. Deionised water (9 mL) was added to the sample while it was still frozen in order to dilute the sample to a total volume of 18 mL. This was necessary to create a headspace ratio of 1:1 in the 40 mL SPME (solid phase micro extraction) vial after 4.5g

sodium chloride was added. The salt was added to force the volatile aroma compounds into the headspace of the vial. A small hole was then pierced through the septa in the lid of the vial. Through this, a tube enclosing a SPME fibre (Stableflex 2 cm 50/30 µm DVB/CAR/PDMS, p/n 57348-U from Supelco, Bellefonte, PA, USA) was introduced, the fibre extended to its full 2 cm length and held inside the vial in the headspace above the juice. The sample was then placed into a water bath held at 50°C. At the end of 40 minutes the fibre was retracted and removed from the vial before being inserted into the injection port of the GC-O where it was desorbed for 10 minutes at 250°C in splitless mode.

4.3.5.2 Panellists

Replicated juice samples from each of the mulched treatments were analysed. Two panellists were chosen to sniff each sample from each replicate and each treatment three times making a total of 36 samples per panellist. Samples were randomised within each replicate and each replicate was sniffed separately, panellists did not know which treatment they were sniffing (Table 4.1). Because resources were limited, more thorough replication was chosen over having a greater number of panellists. Panellists were wine experts who already had training in the language used to describe different aromas relating to grapes and wine. One of the panellists was the winemaker at Lincoln University and the other was the author of this thesis.

Table 4.1 – Order in which treatment samples were sniffed by each panellist.

Panellist 1		
Rep1		
CG	CON	MS
CON	GG	CON
GG	CG	MS
CG	GG	MS
Rep2		
CON	GG	CON
MS	MS	CG
CON	GG	MS
CG	CG	GG
Rep3		
MS	GG	MS
CG	GG	CG
CON	CON	GG
CON	CG	MS

Panellist 2		
Rep1		
CON	CG	GG
MS	GG	CON
CG	GG	MS
CON	MS	CG
Rep2		
MS	CON	GG
MS	CON	GG
CON	CG	CG
MS	GG	CG
Rep3		
MS	CON	CG
GG	GG	MS
GG	CG	CON
CG	MS	CON

4.3.5.3 GC-O analysis

Juice aroma compounds were analysed using a Shimadzu GC-2010 (Shimadzu Corporation Kyoto Japan) gas chromatograph equipped with an Rtx-Wax 30 m x 0.32 mm i.d. x 1µm film thickness (polar phase, polyethylene glycol) capillary GC column (Restek, Bellefonte, PA, USA) connected to an olfactory port (OP-275 from ATAS GL Sciences, Eindhoven, The Netherlands). The GC effluent was split 1:1 between FID detector and the olfactory port with the GC column flow set at 24.6 cm/sec in linear velocity mode. The olfactory port and FID detector were set up at a split of 1:1 based on the length of deactivated tubing used. This ratio changed slightly within the run due to the temperature difference between the GC oven and the interface heater of the olfactory port. At an oven temperature equal to the interface temperature the split was 1:1. However where the GC oven temperature was lower than this, the split operated in favour of the FID detector. At temperatures greater than the interface temperature the split favoured the olfactory port (Table 4.2).

Table 4.2 – Ratio at which the effluent was split depending on GC oven temperature. Olfactory port heated to 200°C.

GC oven temperature °C	Flow ratio – Olfactory:FID detector
40	1:1.63
100	1:1.27
150	1:1.08
200	1:0.96
240	1:0.89

The injector and FID detector temperatures were set at 250°C. The GC column oven temperature was initially held at 40°C for 3 minutes. Following this, the temperature was ramped to 118°C at 5°C/min, then to 148°C at 2°C/min, and further ramped to 240°C at 5°C/min. It was held at 240°C for 15 minutes making a total runtime of 74.3 minutes. The transfer line to the olfactory port was held at 200°C, with humidified air added to the nose cone of the olfactory port at a flow of 8.2 mL/min. Total lag time between the olfactory port and the FID port ranged from 0.53 seconds at 40°C to 0.29 seconds at 240°C (126°C gave a zero second lag time).

Aroma intensity indicated by panellists was recorded using a Velleman K8055 USB Interface Board with a potentiometer attached to a laptop computer. A dial was attached to the potentiometer, which the panellist could turn to show how intense an aroma was in real time. The extent to which the dial was turned was visible to the panellist on the laptop screen

using K8055twusblicht1.0.0 and K8055TWUsb2.4 software (http://www.wenzlaff.de/twusb.html). A USB Logitech microphone was used in conjunction with the open source computer programme Audacity (http://audacity.sourceforge.net/) to record spoken aroma descriptors. Intensity and descriptor information were matched in a spreadsheet program at a later stage. Similar aromas repeated at least twice by panellists were used for further analysis.

4.3.5.4 Analysis of data

As in Jordan *et al.* (2002) there were three samples of each treatment for each panellist to sniff. Jordan *et al.* (2002) collected descriptors that had been used three times by at least one panellist in their trail. In the Sandihurst trial as there were only two panellists and descriptors that were used twice were also considered. The first statistical comparison looked at individual treatments, the second at lighter coloured treatments (clear glass and mussel shells), compared to darker coloured treatments (green glass and control), and the third investigated the difference between the average of all of the mulches and the control. A fourth comparison looked at the average of the glass treatments compared to the shells and finally clear glass was compared with green glass. Differences were significant at $p \leq 0.05$, those $p \leq 0.1$ were also considered because of the low number of panellists.

Aroma compounds that had been repeated by panellists were tentatively identified after juice samples were analysed using gas chromatography-mass spectometry (GC-MS). This data was then compared to the data collected from the GC-O. Where peaks were closely related by time of occurrence and aroma description (from www.flavournet.org) they were identified as a possible match. It should be noted that the compounds identified can not be confirmed as correct and that further analysis, by running compound standards through GC-MS, is required to validate the data.

4.3.6 GC-O wine analysis

Gas Chromatography-Olfactory was also used to analyse wine aromas. Wine samples were not replicated so no statistical analysis was carried out on this data. The method used for the wine was exactly the same as that for the juice except for a dilution factor as has been used in previous trials (Marti *et al.* 2003, Kotseridis *et al.* 2008). The presence of ethanol in the wine can affect the sorption of volatile aromas onto the SPME fibre used to extract them (Kotseridis *et al.* 2008). Ethanol can out-compete other volatiles for sites on the SPME fibre (J. Breitmeyer, personal communication, 2009. Wine also contains high concentration of some volatiles that can overload or saturate the SPME fibre resulting in a non linear response (J. Breitmeyer, personal communication, 2009). Wine samples were therefore diluted by a factor of five in order to reduce these effects. The dilution factor of 1:5 meant

that 3.6 mL of wine was diluted to a total volume of 18 mL with deionised water. The addition of NaCl and other operations were similar as for the juice samples. Panellist responses were divided into categories taken from the centre of Wine Aroma Wheel (Noble *et al.* 1987) and included: fruity, spicy, floral, microbiological, oxidised, pungent, chemical, earthy, woody, caramelised, nutty and vegetative.

4.3.7 Statistical analysis

Results from each experiment, where applicable were analysed using analysis of variance (ANOVA) with GenStat for Windows, version 12, VSN International Limited, Hemel Hempstead, UK (http://www.vsni.co.uk/software/genstat/). Significant differences were calculated at a 95% confidence interval, where $p \leq 0.05$ using Fisher's protected LSD test. Where GC-O data was analysed, results significant at $p \geq 0.1$ (90% confidence level) were also considered due to the greater variation inherent in the panellist data. Considering data at $p \leq 0.1$ may point to further refinement of data collection techniques that could reveal more robust statistically significant differences.

4.4 Results and discussion

4.4.1 Wine phenolics

4.4.1.1 Colour and phenolics

Unfortunately a lack of replication of wines meant that statistics could not be carried out on the data collected. Data interpretation in this section should therefore be treated with caution. However, different patterns were seen for wines made from each year. Lighter coloured mulches did not necessarily have the highest wine colour density or total phenolics (Figure 4.1 and 4.2) as might have been expected as these mulches reflected the most radiation into the grapevine canopy. The increase in photosynthetically active radiation should have aided in the development of both of these factors (Dookoozlian and Kliewer 1996, Keller *et al.* 1998, Haselgrove *et al.* 2000, Bergqvist *et al.* 2001, Spayd *et al.* 2002, Pereira *et al.* 2006). Interestingly in the 2006 wines the green glass achieved similar results to the mussel shells for each parameter despite this mulch being found to reflect significantly less light than the lighter coloured treatments (Figure 3.11). Possibly this was caused by exterior factors for example a lighter canopy in the green glass treatment may have increased light availability to the fruit. Unfortunately only wine was made in this year and vineyard parameters were not tested.

In the 2008 wines mussel shells once again had the highest concentrations for wine colour density and total phenolics (Figure 4.2). However, in contrast to the 2006 wines, the control

also had high concentrations for these parameters with lower concentrations seen for the glass treatment wines, especially the green glass. The disparity between the two data sets may have been caused by climatic variation between the two seasons. Average temperatures in each season were the same and rainfall was only slightly higher in the 2008 season, however, the 2008 season also had 261 more sunshine hours than the 2006 season (Appendix 2). In 2006, the control had the lowest phenolic concentration suggesting that the mulches may have encouraged phenolic synthesis by reflecting more light into the canopy. Higher levels of phenolic compounds have been found in trials that investigated the effects of increasing berry exposure to light (Keller and Hrazdina 1998, Price *et al.* 1995, Haselgrove *et al.* 2000, Kolb *et al.* 2003). In 2008 when the mulches had been on the ground for longer, it could be speculated that the extra water in the soil had encouraged more vegetative growth in mulched vines and therefore these vines, had greater canopy density. Pruning weights, which were reduced in the control vines could suggest that this treatment would have had a more open canopy than the mulched vines. The extra sunlight experienced during this season may have encouraged phenolic synthesis in control fruit if these vines had greater access to light through a more open canopy. It is unclear however, why the clear glass in each season did not gain the same concentration of total phenolics and wine colour density as the mussel shells when they both reflected greater amounts of light into the grapevine canopy.

The other factor that can affect the synthesis of phenolics in grape berries is water. Kondouras *et al.* (2006) showed that limiting the availability of water to the vines could increase concentrations of phenolics and affect aromatic compounds in the fruit. Mulch treatments kept greater amounts of water in the soil than the control during most of the growing season (Table 3.2) which may account for higher phenolic concentrations seen in the 2008 control wines. However this does not explain the higher concentration also seen in the mussel shell treatment (Figure 4.1), which usually had the highest soil moisture content during the season (Table 3.2).

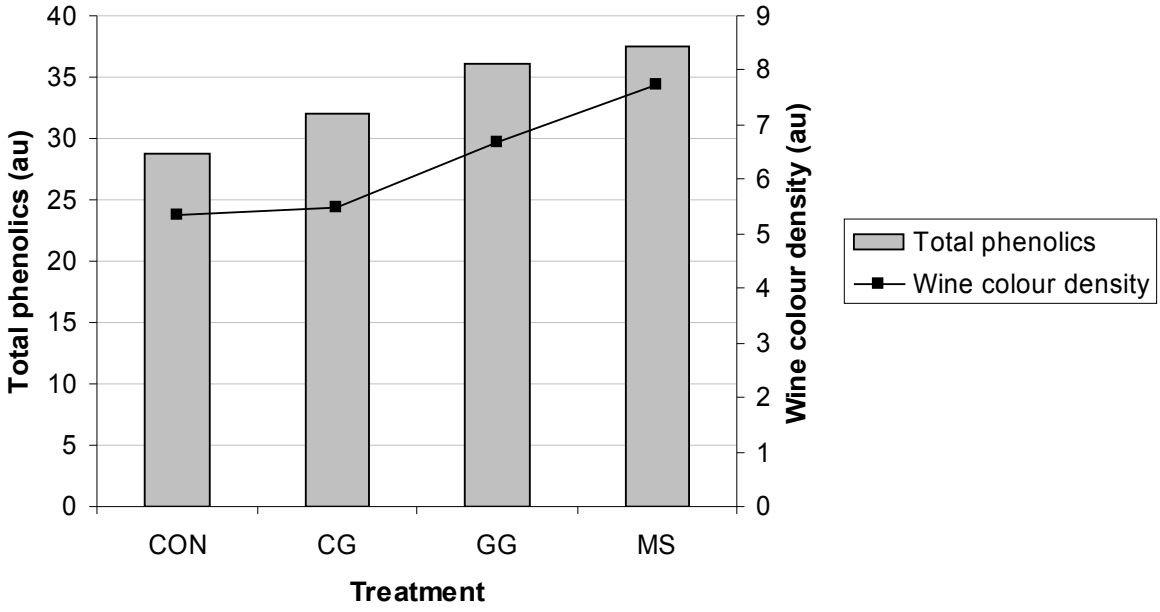


Figure 4.1 – Wine colour density and total phenolics for 2006 wines. Wine samples were not replicated.

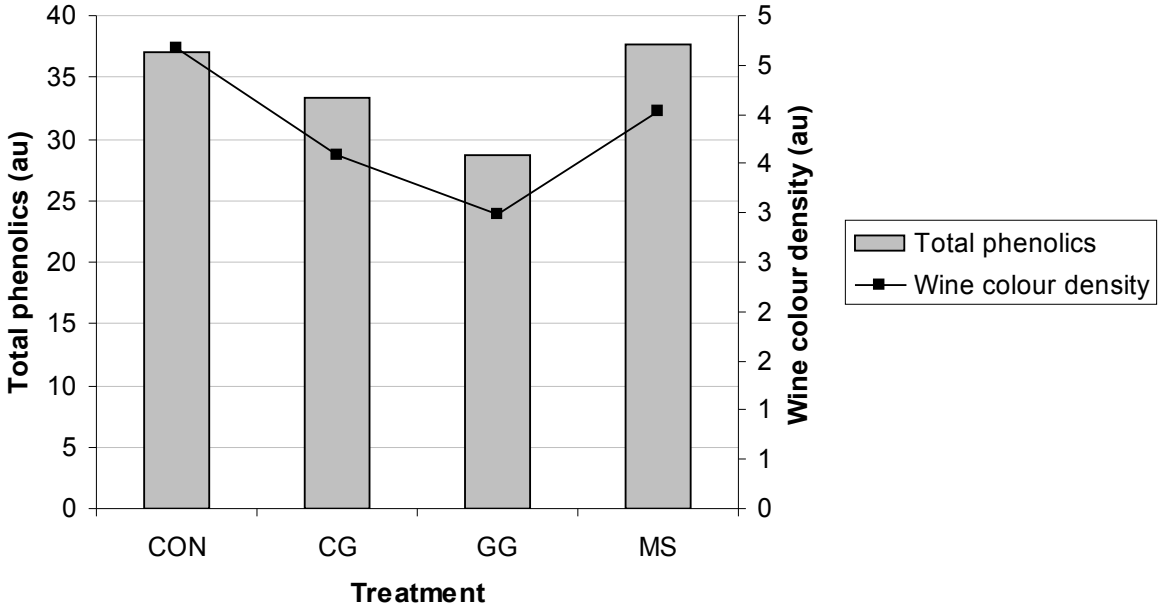


Figure 4.2 – Wine colour density and total phenolics for 2008 wines. Wine samples were not replicated.

The mulch treatments may also have affected the wines' ability to age, causing variation between the treatments from each vintage. As wines age, polymerisation and co-pigmentation reactions take place between phenolic compounds (Castillo-Sánchez *et al.* 2008). As these compounds may have been affected by the different light environments, it is possible that this influenced the wines ageing potential. As wine ages, the amount of polymeric pigments increase, at the same time the amount of anthocyanins decrease (Jackson *et al.* 1978). The reaction depends on the anthocyanin to tannin ratio at the beginning of the process (Fulcrand *et al.* 1996).

4.4.1.2 High performance liquid chromatography

In the 2006 wines (Figure 4.3) most of the mulch treatments were similar to the control. However, a possible difference appeared for the non-flavonoid protocatechuic acid in the mulch treatments. This particular compound is found in plant cells and is abundantly present in the rhizosphere (Venturi *et al.* 1998). It is also a phenolic acid, which makes it a precursor to various wine aromas (Rapp *et al.* 1977). Curiously, less quercetin appeared to be present in the mulch treatments, whereas it would have been expected that more quercetin would be there as a result of the increased radiation to the fruit (Price *et al.* 1995). The reduced amount of this compound was most apparent in the green glass and mussel shells treatments which had concentration respectively of 6.8mg/L and 6.7mg/L. The clear glass also had a lower amount of this compound with a concentration of 7.4mg/L. The mussel shell and green glass treatments also appeared to have less of the procyanidins catechin and epicatechin compared to the control. Mussel shells had a concentration of 68.1mg/L for catechin and 35.4mg/L for epicatechin, while green glass had concentrations of 85.2mg/L for catechin and 46.7mg/L for epicatechin. Catechin and epicatechin are common building blocks of grape tannins (Adams 2006). Although the mussel shells had less than the control for many of the compounds tested, for resveratrol the pattern was different and the treatment appeared to have a higher concentration than the control.

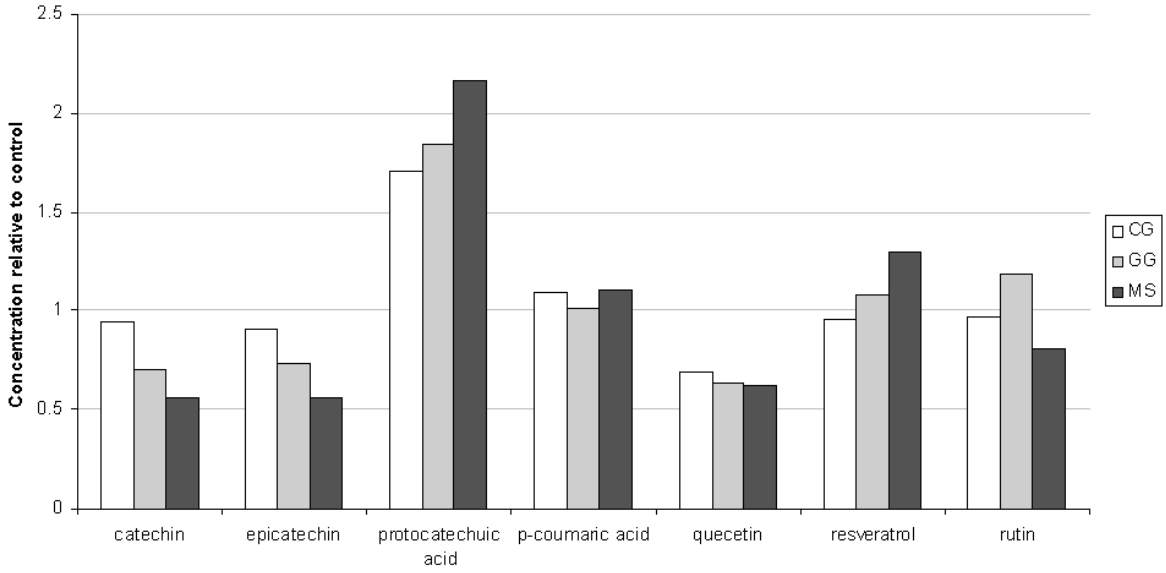


Figure 4.3 – 2006 wine phenolic concentrations relative to CON (CON=1). Wine samples were not replicated.

Figure 4.4 shows the results from the 2008 wines. In general, the concentrations of the monitored compounds were much more similar across the treatments than they were for the 2006 wines. Resveratrol, a compound linked to the 'French Paradox' and lowered risk of coronary heart disease (Trela and Waterhouse 1996). The concentration of resveratrol appeared to be less for all mulch treatments compared to control, although lighter coloured mulches mussel shells and clear glass were closer to the control's concentration than green glass. For rutin, clear glass and mussel shell treatments appeared to have higher concentrations than the control at 6.3mg/L and 6.5mg/L respectively. Green glass appeared to have less than the control with 3.6mg/L. Rutin is a derivative of quercetin and its concentraton is sensitive to light exposure of the fruit (Price *et al.* 1995). An increase in concentrations of rutin and reduction in the concentrations of quercetin indicates an enzymatic change of the quercetin into rutin. The concentration of quercetin, however, did not change in the same way that rutin did. Quercetin appeared to be highest in the clear glass compared to the control. The clear glass treatment also appeared to have the highest concentrations of catechin and epicatechin. It is possible that environmental factors played a role in the outcome for these results. Chaves and Escudero (1999) noted that stress caused by lack of water could increase synthesis of phenolics in vines. The control may have experienced water stress during January before irrigation was used and when soil moisture levels for this treatment were significantly lower than in the mulched treatments (Table 3.2). Compared to the green glass the lighter coloured treatments appeared to have slightly

increased amounts of flavonols possibly through increased light available to these vines. UV radiation was another stress factor listed by Chaves and Escudero (1999) that can induce phenolic synthesis. Kondouras *et al.* (2006) also demonstrated that limiting soil moisture content could increase anthocyanins and total phenolics.

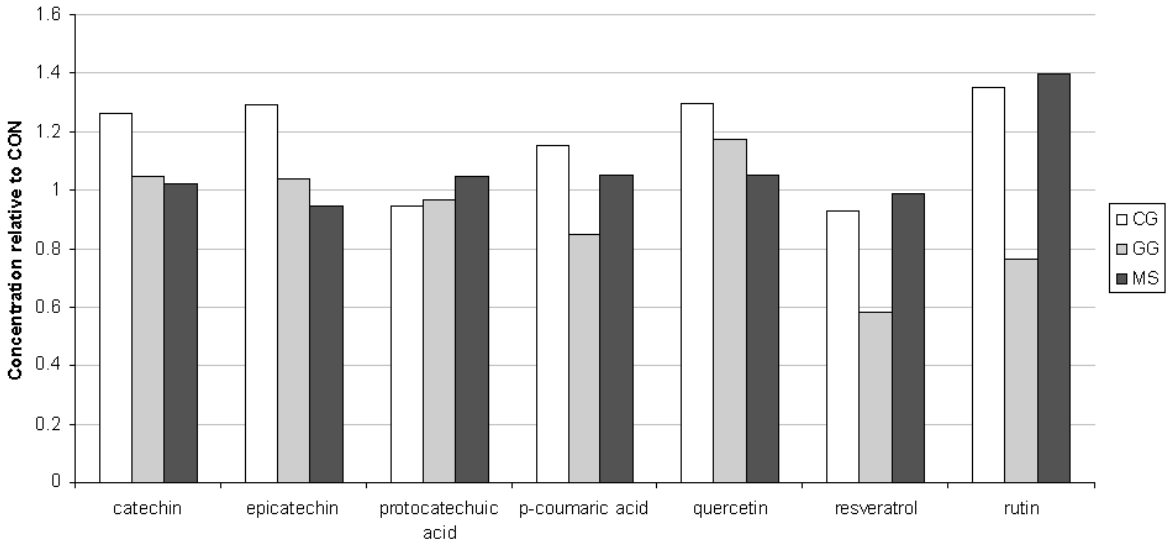


Figure 4.4 – 2008 wine phenolic concentrations relative to CON (CON=1). Wine samples were not replicated.

4.4.2 Wine acid composition

Organic acids in the 2008 wines were tested using HPLC (Table 4.3). No statistical comparisons could be made as wines were not replicated. When compared to results from juice samples for the same harvest the control appears to have lower levels of these acids. While these differences could not be examined statistically the pattern of lower acidity was in the control was also found for tartaric, malic, lactic, acetic and citric acids (Table 4.3). Reynolds *et al.* (1986), Sauvage *et al.* (1998) and Crawford (2007) reported lower levels of acidity for vines that had been mulched with reflective material. Kliewer and Lider (1968) reported malate concentrations two to three times higher in shade fruit compared to fruit exposed to sunshine. Tartrate levels were also higher in shade fruit, but did not appear to be as affected by sun exposure. Pereira *et al.* 2006 reported that acids were not affected by the availability of light as much as they were affected by temperature. Conde *et al.* (2007) also reported that acid levels were regulated by temperature with higher levels present in grapes grown in cool climates as opposed to those grown in warm climates. Mulches in the Sandihurst trial did not have significant effects on temperature, so this may not be a factor in

this instance. It is interesting however that mulched vines appeared to gain higher acidity than un-mulched vines. It should be noted however that if the control is removed the lighter treatments still gained higher results than the darker coloured green glass. Further research should be carried out using replicated wine samples, in order to understand whether this pattern is repeatable. Another item of note is that in Table 4.3, the TA was consistently greater than the sum total of the individually measured acids by a factor of 1.4 g/L. This is likely to have been due to the different methods used to measure the acids.

Table 4.3 – Organic acids for 2008 wine analysed using HPLC and 2008 juice titratable acidity (TA). Acids are in g/L. Wine samples were not replicated.

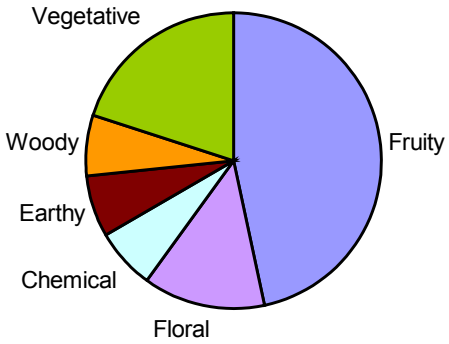
	Tartaric	Malic	Lactic	Acetic	Citric	Succinic	TA
CON	3.56	0.86	6.34	0.03	0.37	2.45	9.77
GG	3.92	1.19	7.36	0.04	0.51	2.84	11.53
CG	3.93	1.07	7.31	0.04	0.43	2.63	10.97
MS	4.33	1.14	7.21	0.04	0.47	2.32	10.90

4.4.3 Wine tasting

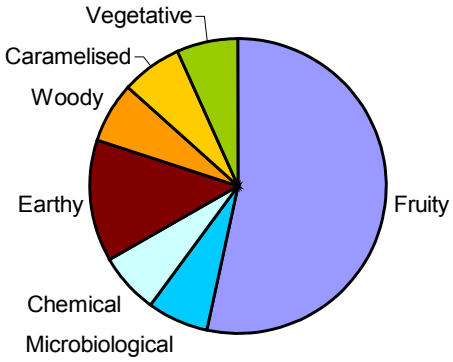
Six wine experts attended a tasting on 2 December 2008 at Sandihurst winery. Wines from each of the trial treatments: green glass, clear glass, mussel shells and control, were tasted blind in two flights. The first flight consisted of wines made from the 2006 vintage; the second of wines made from the 2008 vintage. Data were collected using a scorecard (Appendix 4) that covered the categories of: appearance, aroma, flavour, mouthfeel and overall balance. Wines were ranked under these categories and tasters were additionally asked to give three descriptors for aroma and mouthfeel. Data from these sheets were taken and used to create profiles for each wine. No statistical analysis was available as wines were not replicated. However, clear trends were noted between the wines, especially for the 2006 vintage. The fact that there were noticeable trends between the wines tasted underlines the fact that further investigation is warranted.

In the first flight (2006 vintage), wines from the mussel shell mulch were the found to be the most preferred of all the treatments. Green glass was the second most preferred wine followed by control and finally the clear glass. For appearance, the shell wine was picked as being the darkest and was found to have a more purple hue. Green and clear glass treatments were noted as being closer to red whilst the control tended more towards brown.

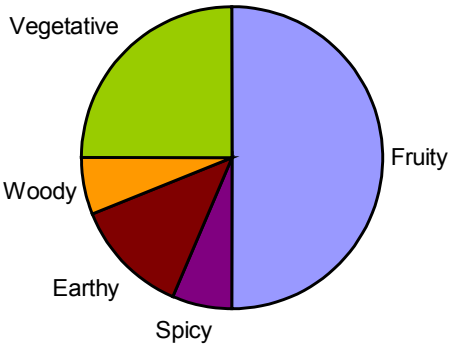
Aroma profiles from this tasting are illustrated in Figure 4.5. The shell treatment was classified as having the most ripe fruit character in its aroma and descriptors for this wine were associated with ripe, dark berry fruit. Green glass carried dusty, tobacco, black olive and pepper characters. The clear glass wine was described with a range of descriptors from berry through to mushroom. The control also had a range spanning from berry/floral through to straw/stemmy characters. The control and green glass both had the highest frequency of vegetative descriptors, whereas for the mussel shell wine no vegetative aromas were mentioned suggesting that this wine was made from the ripest fruit.



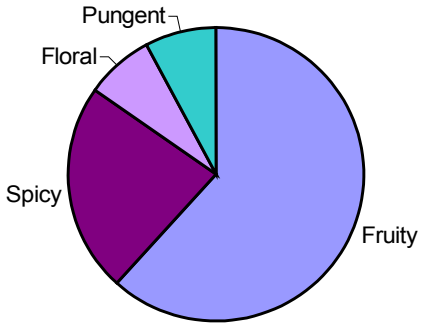
CON 2006



CG 2006



GG 2006



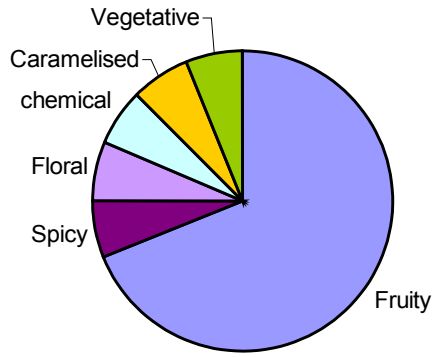
MS 2006

Figure 4.5 – Aroma profiles of 2006 vintage wines. Descriptors taken from a group of six wine experts and categorised using the Wine Aroma Wheel from Noble *et al.* (1987). Wine samples were not replicated.

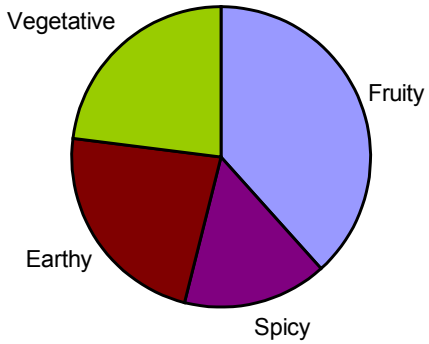
The shell treatment was classified as having the most intense flavour followed by green glass. The shell wine was also picked as the most acidic followed by clear glass. For mouthfeel, shell wine showed the most length followed by the green glass wine. Clear and green glass were picked as having the most astringency, while the shell treatment had the least. Mouthfeel descriptors for the shell treatment included velvet, syrup, full and warm. The clear glass had more grippy, fleshy, spicy and bitter notes. Green glass showed metallic, pucker, sappy and prickle. Finally control had suede, plaster, creamy and green descriptors for its mouthfeel.

For the 2008 vintage wines were more similar to each other than those from 2006 however, trends were still noticeable. This time it was the control that was preferred by the majority. Clear glass came second and was followed by mussel shells and green glass. Control wine was found to be darker in appearance with a more purple hue. The clear glass and mussel shell wines were closer in hue to red with the green glass tending more towards brown.

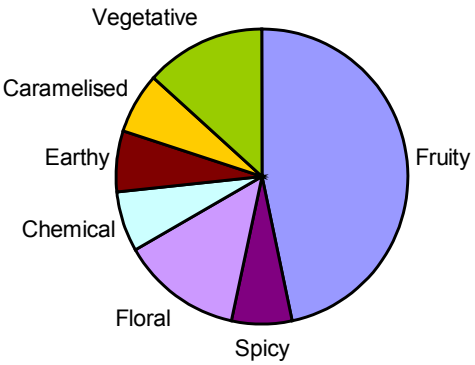
Aroma profiles from the 2008 vintage are illustrated in Figure 4.6. The control treatment showed more ripe fruit characters in its aroma and green glass had the second highest score for this parameter. Descriptors for the control included violets, cassis, berries, cloves and tobacco leaf. Clear glass had more earthy, mushroom, black olive characters with red berry fruit and lifted notes of mint or cloves. Shells showed black olive, tar and raisin characters with herbs and spices also mentioned. Finally green glass had darker characters of prune and soy sauce with mentions of spice, rubber and citrus. Control and green glass had the greatest range of descriptors used. In contrast to the 2006 wines the 2008 clear glass and mussel shell wines had a greater frequency of vegetative aroma descriptors. The 2008 clear glass wine had the smallest range of descriptors compared to the 2006 wine which had the most in that flight.



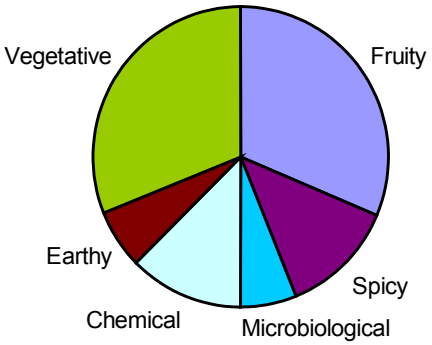
CON 2008



CG 2008



GG 2008



MS 2008

Figure 4.6 – Aroma profiles of 2008 vintage wines. Descriptors taken from a group of six wine experts and categorised using the Wine Aroma Wheel from Noble *et al.* (1987). Wine samples were not replicated.

For the flavour and mouthfeel of the wine, control and mussel shells had the highest intensity rating. Green glass showed the highest acidity. The control wine had the most length and highest astringency. Mouthfeel descriptors for the control included creamy, chamois, mouth-coating and grippy. Clear glass had descriptors including drying, suede, hot and firm. The mussel shell wine showed more sour, aggressive, hot, chewy and astringent descriptors. Finally the green glass was found to be sherberty, metallic, fleshy and full.

The two flights indicated differences between years. There also appeared to be clear trends between the treatments in each year. This suggests that there could be a treatment effect on

wines, but that this is influenced by year and perhaps also by the way in which the wines age. Lack of replication of the wines in this trial meant that these findings are only indicative. Further research using replicated wine samples would be needed to confirm the findings.

4.4.4 GC-O grape juice analysis

Aromas that were repeated by panellists are listed in Table 4.4. These have been tentatively identified after juice samples were analysed using gas chromatography-mass spectometry (GC-MS) and then compared to the data collected from the GC-O. Identified compounds have not yet been confirmed and named compounds are, as yet, hypothetical.

Table 4.4 – Repeated aromas with descriptors used by panellists and possible aroma causing compounds.

Aroma time of occurrence	Descriptor used by panellist	Possible compound (from flavournetinfo)	Compound descriptor (from flavournetinfo)
16.08	Cut grass	Hexanal	Grass tallow fat
18.26	Vegetative	2-hexanal	Freen leaf
24.29	Mushroom	(E) 2-penten-1-ol	Mushroom
26.06	Raspberry/marzipan	1-Hexanol	Resin flower green
27.45	Geranium/leafy	2-hexen-1-ol	Green leaf walnut
31.49	Potato	2,5-dimethyl-3-ethylpyrazine	Potato roast
38.23	Floral/berry		
40.85	Wood/spice/burnt	Hexanoic acid	Sweat
44.06	Lactic	Propanoic acid	Pungent rancid soy
45.59	Berry/floral	Benzyl alcohol/ß damascenone	Sweet flower apple rose honey
48.33	Citrus/fruit	(E) 2-hexenoic acid	Must fat

Analysis carried out on findings by panellists was based on three aspects. The first was peak area, the second aroma duration which related to the time the panellist was able to detect the aroma irrelevant of how strong it was. The third aspect was peak intensity this was found by dividing the peak area by the aroma duration.

For panellist 1 comparisons were found for the following peaks: vegetative at 18.3 minutes, mushroom at 24.3, geranium/leafy at 27.4, potato at 31.5 and floral/berry at 38.2 (Table 4.5).

Table 4.5 – Significantly different aroma peaks for panellist 1. Evaluation of 2008 vintage grape juices.

Panellist 1	Comparison	Treatment means CON	GG	CG	MS	sed	lsd	Significance
Vegetative - 2-Hexenal (green, leaf) 18.26								
Aroma duration	GG vs CG		0.25	0.44		0.0896	0.2192	0.084*
Mushroom - (E) 2-penten-1-ol (mushroom) 24.29								
Intensity	Glass vs MS		32167		35801	1780.5	4356.6	0.087*
Peak area	CON vs Mulch	15538	11541			1989.7	4868.6	0.091*
Peak area	GG vs CG		9397	14428		2436.9	5962.7	0.085*
Geranium/leafy: 2-Hexen-1-ol (green, leaf, walnut) 27.45								
Intensity	CON vs Mulch	27769	34340			2258.6	5526.6	0.027**
Potato - 2,5-dimethyl-3-ethylpyrazine (potato, roast) 31.49								
Peak area	CON vs Mulch	24832	19838			2283.4	5587.2	0.071*
Aroma duration	CON vs Mulch	0.64	0.56			0.0334	0.0817	0.056*
Floral/berry - ? 38.23								
Intensity	CON vs Mulch	28389	22995			2169.9	6024.3	0.068*

Result are significant at $p \leq 0.1$*, $p \leq 0.05$**, $p \leq 0.01$***

The outstanding feature here was the repeated appearance of the difference between the control and the mean of the three mulches (i.e. did mulch of any kind have an effect over the control). This occurred for four of the five peaks. For the control the earthy aromas mushroom and potato both showed greater peak area compared to the mulch treatments ($p \leq 0.1$). For the potato peak the control also had a longer aroma duration ($p \leq 0.1$). The floral/berry aroma that occurred at 38.2 minutes showed a greater intensity for the control compared to the mulches. The geranium/leafy peak showed significantly higher intensity for the mulches compared to the control ($p \leq 0.05$). Differences were also noted between the green and clear glasses so that peak area for the mushroom aroma was found to be higher in the clear glass treatment compared to the green glass ($p \leq 0.1$). The vegetative peak at 18.3 minutes showed a longer aroma duration for the clear glass compared to the green glass ($p \leq 0.1$). Finally for the intensity of the mushroom peak a difference was noted between the mean of the glass treatments and the mussel shells with the mussel shells showing higher intensity compared to the glass treatments ($p \leq 0.1$).

For panellist 2 comparisons were found for the following peaks: cut grass 16.1 minutes, vegetative 18.3, raspberry/marzipan 26.1, potato 31.5 and berry/floral 45.6 (Table 4.6).

Table 4.6 – Significantly different peaks for panellist 2. Evaluation of 2008 vintage grape juices.

Panellist 2	Comparison	Treatment means CON	GG	CG	MS	sed	lsd	Significance
Cut grass - Hexanal (grass, tallow, fat) 16.08								
Peak area	Glass vs MS		7510		9849	1199.9	2936	0.099*
Aroma duration	Glass vs MS		0.28		0.35	0.0349	0.0854	0.067*
Vegetative - 2-Hexenol (green, leaf) 18.26								
Intensity	Treatment	23459c	14934a	18982b	17611ab	1408.4	3910.1	0.016**
Intensity	CON vs Mulch	23459	17176			1149.9	3192.6	0.005**
Intensity	GG vs CG		14934	18982		1408.4	3910.1	0.045**
Raspberry/marzipan - 1-Hexanol (resin, flower, green) 26.06								
Aroma duration	Treatment	0.28b	0.22a	0.22a	0.17a	0.0222	0.0543	0.018**
Aroma duration	Dark vs Light	0.25		0.20		0.0222	0.0543	0.017**
Aroma duration	CON vs Mulch	0.28	0.20			0.0181	0.0443	0.007**
Potato - 2,5-dimethyl-3-ethylpyrazine (potato, roast) 31.49								
Peak area	Glass vs MS		18699		26565	3425.2	8381	0.056*
Berry/floral - Benzyl alcohol (sweet, flower) 45.59								
Peak area	Dark vs Light	7537		9463		1254.5	3224.8	0.082*
Peak area	CON vs Mulch	6518	9160			1024.3	2633.1	0.049**
Aroma duration	Treatment	0.29a	0.34a	0.32a	0.47b	0.0383	0.0985	0.021**
Aroma duration	Dark vs Light	0.32		0.40		0.0383	0.0985	0.036**
Aroma duration	CON vs Mulch	0.29	0.38			0.0313	0.0804	0.036**
Aroma duration	Glass vs MS		0.33		0.47	0.0332	0.0853	0.009**

Result are significant at $p \leq 0.05$*, $p \leq 0.01$**, $p \leq 0.001$***

The comparison between control and mulch featured once again. A significantly higher intensity was found for the control compared to the mulch treatments for the vegetative peak at 18.3 minutes ($p \leq 0.05$). For the raspberry/marzipan peak the control showed a significantly longer aroma duration than the mulch treatments ($p \leq 0.05$). Finally for the berry/floral peak at 45.6 minutes the mulches had a significantly longer aroma duration and peak area compared to the control ($p \leq 0.05$).

The berry/floral peak at 45.6 minutes showed a number of other interesting differences. The mean of the lighter coloured treatments was found to have a higher peak area compared to the mean of the darker coloured treatments ($p \leq 0.1$). For the same parameter aroma duration was significantly longer for the lighter coloured treatments ($p \leq 0.05$). An individual comparison showed the mussel shell mulch to have a significantly longer aroma duration compared to each of the other treatments ($p \leq 0.05$). Finally for this peak the mussel shells were found to have significantly longer aroma duration than the glass treatments ($p \leq 0.05$).

The glass versus mussel shells comparison also showed differences for the potato and cut grass peaks. For the potato aroma, peak area was higher for the mussel shells compared to

the glass treatments ($p \leq 0.05$). For cut grass, peak area and aroma duration were both higher for mussel shells compared to the glass treatments ($p \leq 0.05$).

The vegetative and raspberry/marzipan peaks both showed significant differences where individual treatments were compared. For the vegetative peak that occurred at 18.3 minutes the control had the highest intensity, clear glass was significantly less intense than the control, the green glass had the lowest intensity while the mussel shell treatment came between the clear and green glass ($p \leq 0.05$). For the raspberry/marzipan peak the control had a significantly longer aroma duration compared to the other treatments ($p \leq 0.05$).

Two final comparisons featured. The clear glass was found to have a higher intensity than green glass for the vegetative peak at 18.3 minutes ($p \leq 0.05$). For the raspberry/marzipan peak, where light and dark treatments were compared, the dark coloured treatments were found to have longer aroma duration ($p \leq 0.05$).

Results for panellist 1 suggest that differences in aroma have been caused by the mulch treatments irrelevant of what the mulch was made from. An increase in the green floral aroma geranium/leafy was recorded for mulched vines but by contrast the floral/berry aroma, which occurred at 38.2 minutes, was found to be less for the mulched vines. The earthy aromas potato and mushroom were found to be higher for control compared to the mulched treatments. It could be speculated that this was due to the higher moisture content in the soil of the mulched treatments. During the period January – April soil moisture was 20% higher in each of the glass treatments and 34% higher for the mussel shells compared to the control (Table 3.2). Possibly the increased available soil moisture caused a subsequent increase in the vegetative growth of these vines, which may have caused shading and therefore affected fruit composition. Pruning weights taken in 2008 (Table 3.9) reinforce this, as they were significantly higher for the mulched vines compared to the un-mulched control, indicating greater vegetative growth for these vines. Shading has been found to reduce grape and wine quality (Reynolds *et al.* 1986, Smart 1987, Jackson and Lombard 1993). Increasing vine access to light, through the use of reflective mulches, has been found to reduce the incidence of vegetative aromas in the wine produced from that fruit (Reynolds *et al.* 2007) and to improve sensory characteristics (Robin *et al.*1997). The shell trials also showed positive results in some cases where wines had been mulched with this reflective material (Creasy *et al.* 2007). Sandler *et al.* (2009) however found that wines made from fruit that had been treated with quahog shells, had reduced floral aromas and increased earthy aromas. It was additionally reported in that trial that the mulches had increased canopy density; perhaps this had created a more shaded environment for the fruit.

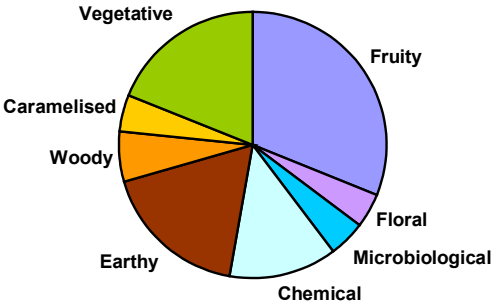
The reduced floral aroma found by panellist 1 in the Sandihurst trial correlates with these finding. However, data for panellist 1 also showed that the earthy aromas 'potato' and 'mushroom' had been reduced for mulched compared to un-mulched vines. A higher soil moisture level is a likely explanation for the differences in the aromas between the mulched and non-mulched treatments in this case. Koundouras *et al.* (2006) studied grape composition in vines that had been subjected to different levels of water deficit. In that trial it was found that the vines that had the greatest water limitation had the most positive influence on the development of anthocyanins and phenolics in grape berry skins. This influence was carried through into the wines that gained the highest scores in a tasting. In the Sandihurst trial for the 2008 wines the control appeared to have a higher colour density and total phenolic concentration compared to the other treatments in the Iland colour and phenolics assay (Figure 4.2). Phenolics tested using HPLC (Figures 4.3 and 4.4) indicated that phenolic composition of the mulched compared to the non-mulched vines were similar. Differences were not statistically comparable as samples had not been replicated. For example quercetin, which increases with higher levels of UV (Price *et al.* 1995), was found to be less concentrated than the control in the 2006 wines (Figure 4.3) and only slightly higher in the mulched treatments in the 2008 wines (Figure 4.4). From these findings it could be concluded that the light environment was possibly influenced in this trial by higher moisture levels in the soil that may have increased vigour in the vine canopy. In future research it would be important to monitor the canopy density during the season in relation to the mulch treatments. This was not possible in this trial due to early season frost damage altering the canopy structure.

For panellist 2 a slightly different pattern was found for aroma levels. The vegetative peak at 18.3 minutes was significantly higher in the control compared to the mulched vines, while the berry/floral peak at 45.6 minutes showed significantly higher results in the mulched compared to the control vines. This could suggest that the mulches were having a positive effect on the wine aroma by reducing a vegetative aroma and increasing desirable floral fruity aromas. The berry/floral peak at 45.6 minutes stands out as having the most significant differences of all of the peaks perceived by panellist 2. In each comparison, results are weighted away from the control and towards the mussel shells suggesting that mulch could increase aroma duration and peak area. Another comparison indicates that the aroma duration and peak area are increased by lighter coloured mulches and finally the mussel shell mulch has gained the highest results for these parameters (Table 4.6). By contrast however, for the raspberry/marzipan peak, the control had a significantly higher reading than the mulched treatments. The effect of the mulches appears to be more complex than simply encouraging positive aromas and suppressing negative ones and is further complicated by

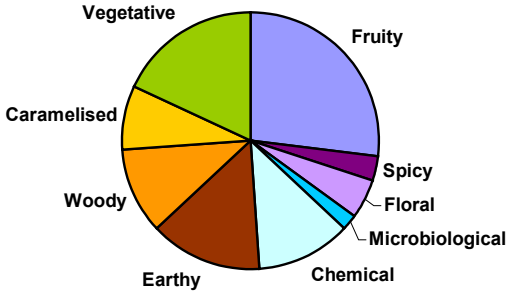
the different results found by each panellist. It should be noted that data from only a small proportion of the peaks identified by the two panellists has been analysed. There are many other peaks that have been consistently recorded by each individual panellist, but not by both; for this reason more panellists are required in future studies. The difference in results found for each panellist is likely connected to the ability of individuals to detect aromas, which is known to vary widely (Thorngate 1997). Therefore the actual impression of the juice samples, either through GC-O or even in smelling them from a glass, may be different for different people. In future research replicated wine samples should be measured and use of the GC-O should be tied in with a tasting of these wines to identify how treatment effects on the different aromas work together in the finished product. Findings by each panellist illustrate the complexity of the aroma profile of the Pinot noir variety. Pinot noir wine aroma remains less understood due to this complexity (Fang and Qian 2005). Fang and Qian (2006) have further demonstrated that Pinot noir aroma is highly dependant on grape maturity and seasonal conditions.

4.4.5 GC-O wine analysis

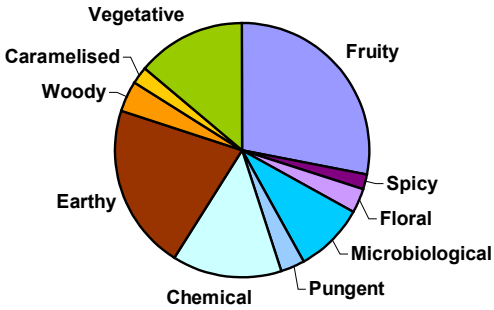
Although not replicated, the wine profiles give an idea of the type of aroma profile gained for each treatment. All treatments in the 2006 wines were found to have similar scores for fruity aromas, but control and green glass had slightly higher scores than clear glass and mussel shells. Clear glass had the lowest incidence of earthy aromas, but had high vegetative ones. Mussel shells was high in earthy aroma frequency, low in vegetative aromas and had floral aromas detected, unlike for any of the other treatments (Figure 4.7).



CON 2006



CG 2006



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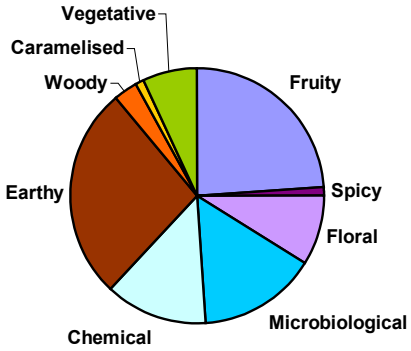
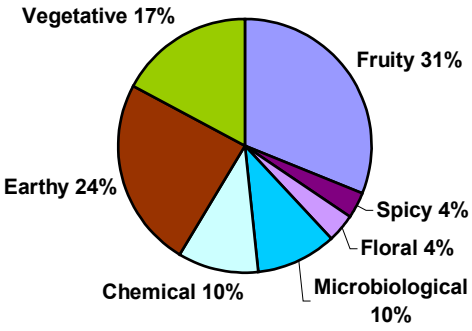


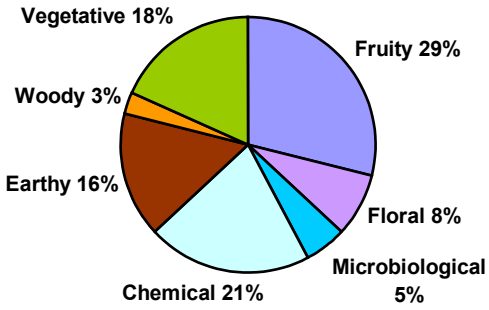
MS 2006

Figure 4.7 – GC-O analysis of 2006 wines. Descriptors were categorised using the Wine Aroma Wheel from Noble *et al.* (1987). Wine samples were not replicated.

For the 2008 wines, mussel shells had higher frequency of fruity aromas and less vegetative ones compared to all other treatments (Figure 4.8). Mussel shells also showed greater use of woody aromas and had a similar amount to the green glass treatment. Higher levels of chemical aromas were noted for both of the glass treatments, while the control showed more microbiological descriptors. Floral aromas were recorded more for the clear glass mulch compared to other treatments.



CON 2008



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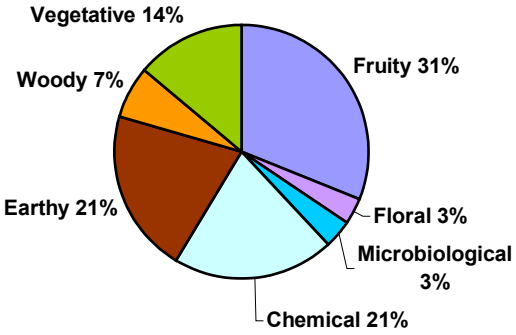

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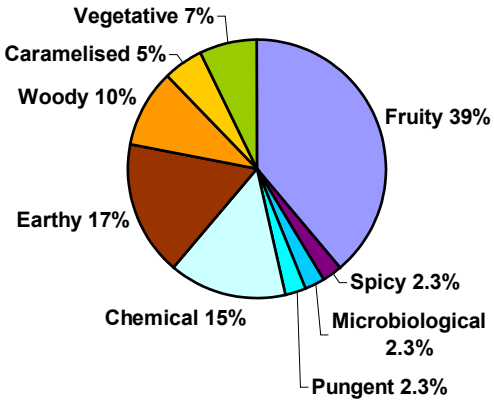

MS 2008

Figure 4.8 – GC-O analysis of 2008 wines. Descriptors were categorised using the Wine Aroma Wheel from Noble *et al.* (1987). Wine samples were not replicated.

Compared to the aroma profiles generated from the tasting, the GC-O profiles are more similar amongst wines and between years. Further research would include a greater number of panellists to make a greater number of comparisons. It will also be necessary in the future to make replicated wines from the trial so that wines as opposed to juices can be analysed. Although juices give an idea of the development of aroma compounds in the fruit, these aroma compounds change and are released during the fermentation process. It would be interesting to compare replicated juice and wine results to gain an understanding of what happens to the juice through the fermentation process.

4.5 Conclusions

The mulches used in this trial significantly increased radiation levels in the grapevine canopy, whilst no significant differences were noted for temperature (Figure 3.9). The limiting factor in the development of phenolics has been reported as berry exposure to sunlight by Haselgrove *et al.* (2000) and Spayd *et al.* (2002). The use of reflective mulches in cool climate regions is therefore a useful way of manipulating the light environment and enhancing it during the important early part of the season. Where phenolics and colour were tested in non-replicated wine samples, the mussel shell mulch gained similar results in each vintage for each parameter. Curiously clear glass, which also reflected significantly more light into the grapevine canopy, did not follow the same trend as seen in the mussel shell treatment. Green glass also showed unusual results for total phenolics and wine colour density appearing to gain relatively high levels in the 2006 wines, a trend which was reversed in the 2008 wines. This may have been due to the effect of the mulch treatments on compounds such as polymeric pigments that change during the ageing process. It would be interesting to carry out further trials on replicated samples of wine to find out if the differences were signficant.

Where phenolics were tested in wine samples using HPLC the outstanding result for the 2006 wines was the increase in the levels of protocatechuic acid. Other phenolic compounds tested were similar to the control. Quercetin, which is known to be affected by the light environment in the vineyard (Price *et al.* 1995), was unexpectedly reduced in berries over the mulched treatments as were the procyanidins, catechin and epicatechin. In the 2008 wines, results for all of the phenolic compounds tested were similar to control. Lower levels of resveratrol were found in the green glass treatment compared to the other treatments. For rutin, higher levels were found for the lighter coloured mulches, compared to control and green glass. Phenolic accumulation may have been affected by soil moisture status with greater water availability to mulched vines causing less phenolic synthesis in these vines (Chaves and Escudero 1999, Koundouras *et al.* 2006).

Findings for wine acidity were also contrary to expectations, with the mulched treatments gaining higher levels than the un-mulched control. Acidity is more affected by temperature in the vineyard (Conde *et al*. 2007). No significant differences were noted for canopy temperature between treatments (Figure 3.9) and acidity results were not replicated therefore results need to be regarded with caution. The topic does bear further investigation however, due to the importance of excess acid in fruit in cool climate growing areas.

Juice samples were analysed using GC-O and panellists found significant differences between the various treatments. Panellist 1 reported differences between control versus mulch treatments, suggesting that mulched treatments had increased a green floral aroma whilst suppressing a floral/berry peak. Higher levels were also found for potato and mushroom aromas in the control. This was possibly explained by the soil moisture levels being higher in the mulched treatments. Panellist 2 found a similar result for a raspberry/marzipan peak, which was reduced by the mulched treatments compared to the control, however, other findings by this panellist were different. One vegetative peak was reduced over the mulched treatments, while a berry/floral peak was increased and another fruity peak was reduced. It is therefore difficult to say at this stage how the mulches have affected the juice. These results show the complexity of the factors affecting the aroma profiles from each treatment. In addition to this, variation has also been introduced by panellists who detect aromas differently. The human nose is highly sensitive to aromas, some of which cannot be measured by the GC (Ferreira *et al.* 1998). This is why the GC-O, which allows both instrumental and human measurement of aromas, is such an important tool. It allows us to detect and measure these elusive odours. Disparity in the results, as seen in this trial is an expected outcome however.

Ultimately, but beyond the scope of the present research project, the idea would be to use GC-MS to analyse the samples and identify those aroma peaks that appear to be most different (and desirable) between the treatments. Research would also have to be carried out on replicated wine samples and a greater number of panellists would need to be employed. Further characterisation of individual aroma compounds would then increase the understanding of how these mulches can affect the grapevine environment and subsequently grapevine physiology, fruit composition, and ultimately wine quality. Linking these effects would allow the possibility to tailor vineyard management towards development of fruit that meets winegrower and market needs.

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Chapter 5 - Summary

5.1 Main findings

Soil moisture was highest for mulches from January to March whereas no significant differences were noted amongst treatments in April. Soil moisture levels under mussel shells were highest in January, February and overall for the four months tested.

Levels of microbial biomass carbon were highest for mussel shells followed by control, clear and then green glass. Levels of microbial biomass carbon in the soil beneath the mussel shell mulch were significantly higher than for the glass treatments. No significant differences were noted for dehydrogenase enzyme activity.

Mussel shell mulch resulted in the highest soil pH and this was significantly different from control and clear glass. Calcium tended to be higher in the soil beneath the mussel shells and was higher for the mussel shells compared to the glass treatments. Sodium was highest the soil beneath the green glass and was even significantly higher in the green compared to the clear glass showing that there are differences in the way the different glass treatments interact with the soil. No significant differences were noted for any of the other soil nutrients tested including: aluminium, colbalt, copper, iron, potassium, magnesium, molybdenum, phosphorous or zinc.

Mulches significantly increased a number of vine nutrients tested, including potassium, phosphorous, sulphur, boron, copper and molybdenum. No significant differences were noted for calcium, magnesium, iron, manganese, sodium or zinc. The fact that mulches increased levels of these nutrients in the vines was particularly important for potassium for which the vines were all considered to be deficient in. Potassium almost reached optimum levels where vines were mulched while the control remained in the below optimum range. Mulches put copper in the optimum range while in the control treatment they remained below optimum.

No significant differences were found for leaf photosynthetic rate at the end of January or at the beginning or end of March. Significant differences were found at the beginning of March however, for related factors including stomatal conductance, internal leaf carbon dioxide, transpiration and leaf vapour pressure deficit. Conductance was highest for the control compared to mulch treatments and highest for dark compared to light treatments. Internal leaf carbon dioxide and transpiration were highest in the control compared to the mulch treatments. Vapour pressure deficit of the leaves was highest for the mulch treatments compared to the control.

No significant differences were found where leaf greenness was tested, using a SPAD meter, throughout the growing season.

No significant differences were found for fruit from the 2008 harvest for parameters including pH, titratable acidity, °Brix, or sample cluster weight. For yield parameters, significant differences were found for number of clusters harvested and harvested fruit weight. Green glass had the lowest cluster number and was significantly lower than clear glass and control for fruit weight. Significantly higher fruit weights were found over light coloured treatments compared to dark treatments. In terms of colour, where green and clear glass were compared, clear glass had more clusters and greater fruit weight than green glass, potential yield as weight was also higher for clear compared to green glass. In other comparisons between individual treatments, differences were not significant when bird damaged clusters were factored in. Bird damage was not found to be significant however.

Pruning weights were significantly higher in the mulched treatments compared to the control and they were also significantly higher in the light compared to the dark treatments.

Canopy temperatures were not significantly different between treatments at any stage during the growing season.

Reflected radiation was tested in the vineyard and using a model system. In the vineayard the top of the UV spectrum (300 – 400 nm) was found to be highest for clear glass and mussel shells followed by green glass and finally control. Photosynthetically active radiation (PAR) averages were also higher for lighter coloured mulches, clear glass and mussel shells, compared to control and green glass. Red to far red ratios (R:FR) were also higher for light treatments compared to green glass and then control. Similar patterns were seen in the model system for UV and PAR however in the model system all treatments were statistically different from one another in the order: clear glass > mussel shells > green glass > control. For R:FR ratios, possibly because of soil on the shell surface which was thought to have reduced their reflective qualities. The order for model red to far red ratio was clear glass > green glass > control and mussel shells.

Replicated juice samples were measured for aroma compounds using GC-O. Results were gained for two panellists. Panellist 1 found differences between the control and mulch treatments with the control having a highest peak area for the mushroom aroma, the highest peak area and aroma length for potato also the highest intensity for the floral/berry peak. Mulches had the highest intensity for the geranium/leafy peak. Other differences recorded by panellist 1 included an increase in aroma duration for the vegetative peak for clear compared to green glass. Mushroom peak area was also highest over clear compared to green glass. A

final difference was found in the comparison between glass and mussel shells, mushroom intensity was highest for mussel shells compared to the glass treatments.

For panellist 2, differences were also found. Where control was compared to the mulch treatments, vegetative intensity was highest for control as was raspberry/marzipan aroma length. Mulches had higher peak area and length for the berry/floral peak. Where glass treatments were compared to mussel shells, the shells had higher results for cut grass peak area and aroma length, potato peak area and berry/floral aroma length. Vegetative intensity was found to be highest for clear glass when clear glass was compared to green glass. In comparisons made between light and dark treatments, dark treatments had the greatest aroma length in the dark treatment while for the berry/floral peak, peak area and aroma length were greatest in the light treatments.

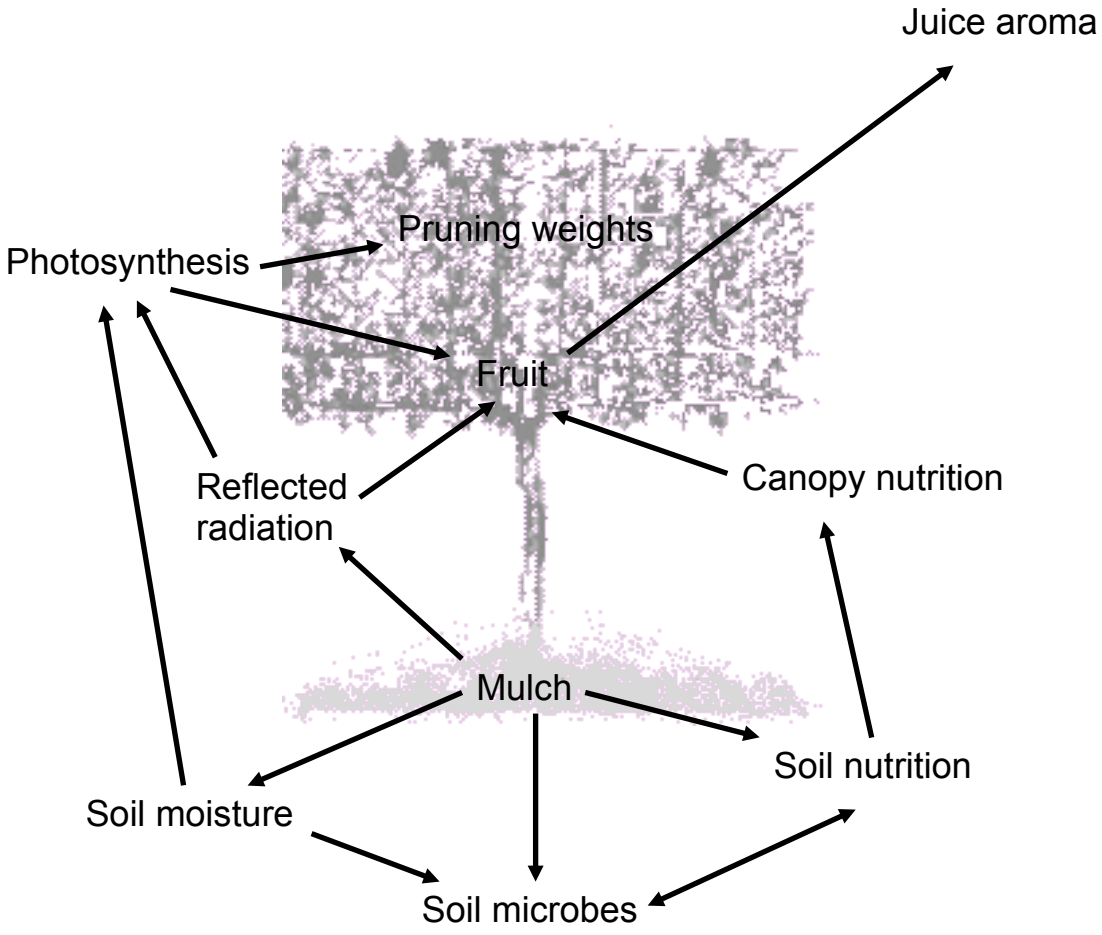

Figure 5.1 - Diagram summarising direct relationships between each of the factors tested in this thesis work. Only those factors that showed significant differences have been included.

5.2 Future perspectives

There are many areas for further research relating to this project. The following is a summary of some of the questions that arose during the research and suggested areas that could warrant continuing investigation.

Reflective mulch effects on the grapevine environment and Pinot noir vine performance

Soil moisture was highest for the mussel shells and was greater than the glass mulches despite the fact they were thicker. It is hypothesised that the shells affected the soil structure to keep more moisture in. Therefore further investigation could be carried out on the effect of the shells on the soil structure. It would also be necessary to monitor the effects of the mulches on the soil over an extended period of time in order to understand how sustainable they were.

It would be interesting to investigate the effect of the glass on weed growth. Weeds were not examined in this trial. However a trial that looked at the effects of growing plants in soil that had glass added to it found that at high concentration, the glass prevented weeds from emerging (de Louvigny *et al.* 2002). It has also been reported that weeds were reduced by mussel shell mulch (Leal 2007).

Where microbial biomass carbon was tested, increases were found underneath the mussel shells and control treatments but not for the glass. A similar trend was found for dehydrogenase enzyme activity, however these results were not statistically significant. Due to heterogeneity and macro-scale variation in the soil it can be difficult to gain statistical differences when measuring micro-organisms. It might therefore be useful to repeat the experiment with a greater number of samples.

One outstanding feature when soil nutrients were tested was the consistently higher sodium levels for the green glass treatment. It would be interesting for find out whether the sodium had come from the glass and how it had entered the soil.

Vine leaf macro-nutrient levels that were found to be significantly different all occurred in vines that were deficient in these nutrients. It could be beneficial to repeat the analysis where these nutrients were not limiting.

No significant differences were noted for photosynthesis, however differences were found in early March for related parameters: stomatal conductance, internal leaf carbon dioxide content, transpiration and leaf vapour pressure deficit. Further testing would be necessary to determine whether this was related to additional light being reflected on the vines from the mulch treatments. Chlorophyll fluorescence could be used to test leaf photosynthetic

efficiency. It might also be useful to test the vines earlier in the season and to investigate vines that had not suffered frost damage. In addition it could be beneficial to measure a greater number of leaves.

SPAD results were not significantly different because of high variation. Variation in the canopy is likely to have been caused by frost that occurred early in the season. It would be worth repeating this section of the trial on vines that had not been subjected to frost. It could also be useful to measure a greater number of leaves per vine.

Although the mulches did not significantly change the temperature in the grapevine canopy they did have small effects on temperature. It would be interesting to monitor the effect on canopy temperature over a number of years and through different seasonal conditions to gain a full understanding of the mulch effect on the ambient environment. Also as these small changes can have large effects during a frost event, it might be necessary to monitor the effect of the mulches during several frost events to understand their impact in this type of situation. Mulch surface temperature should also be further investigated in the vineyard as it was only measured outside of the vineyard in this study.

Results from the spectroradiometers showed that the mulches did reflect greater levels of radiation as UV, photosynthetically active radiation and red to far red radios. It would be necessary to repeat these readings in January when there are higher levels of UV in order to measure a greater range from this spectrum. It is particularly important to measure UV in New Zealand as higher levels are received in this country and as this spectrum has been shown to influence vine growth and production this has implications for New Zealand's wine growing industry.

Reflective mulch effects on wine and juice characteristics

Although it was not possible in this trial due to frost, it would be necessary to make replicated samples of wine in order to confirm differences for wine colour, phenolics and acids. Replication would also be necessary for another tasting of the wines to investigate true treatments effects.

Gas chromatography-mass spectrometry would need to be carried out on samples to confirm the identity of aromas detected by panellists.

Gas chromatography-olfactory would need to be repeated with a higher number of panellists to allow for a greater degree of comparison. It would also be interesting in the future to work with wine as opposed to juice as there are a higher number of volatile compounds in the wine.

The aim of this thesis work was to investigate the effects of different reflective mulches on the vine environment, Pinot noir vine performance and wine and juice characteristics. The mulches were found to significantly affect a number of environmental and performance parameters. They directly influenced soil and light parameters, which affected changes in the vine. In addition to this mulches influenced changes in the fruit, which subsequently impacted on grape juice aroma. Reflective mulches work based on the concept that light can be managed in the vineyard so that the sky view is not the only factor involved in optimising access to radiant energy. This research is the first to consider glass waste as a reflective product in the vineyard. As glass is used by the wine industry to package its products it is an appropriate material to investigate for other uses within this industry especially as there is a glass recycling crisis in New Zealand. The project is one of the few investigating juice aromas with GC-O and is an important first step along the way to understanding how juice aromas are related to the aromas that develop in the wine. Knowledge in this area could allow for a greater understanding of aroma development through fermentation also which aromas are desirable in ripening fruit and will be the most beneficial for wine production. In particular for Pinot noir production as this cultivar has a very complex matrix of aroma compounds and is an important New Zealand red variety. Understanding the development of aroma compounds in wine grapes and how they could be managed in the vineyard are especially relevant for winegrowers in cool climates. These winegrowers deal with greater seasonal variation which can affect production and wine quality.

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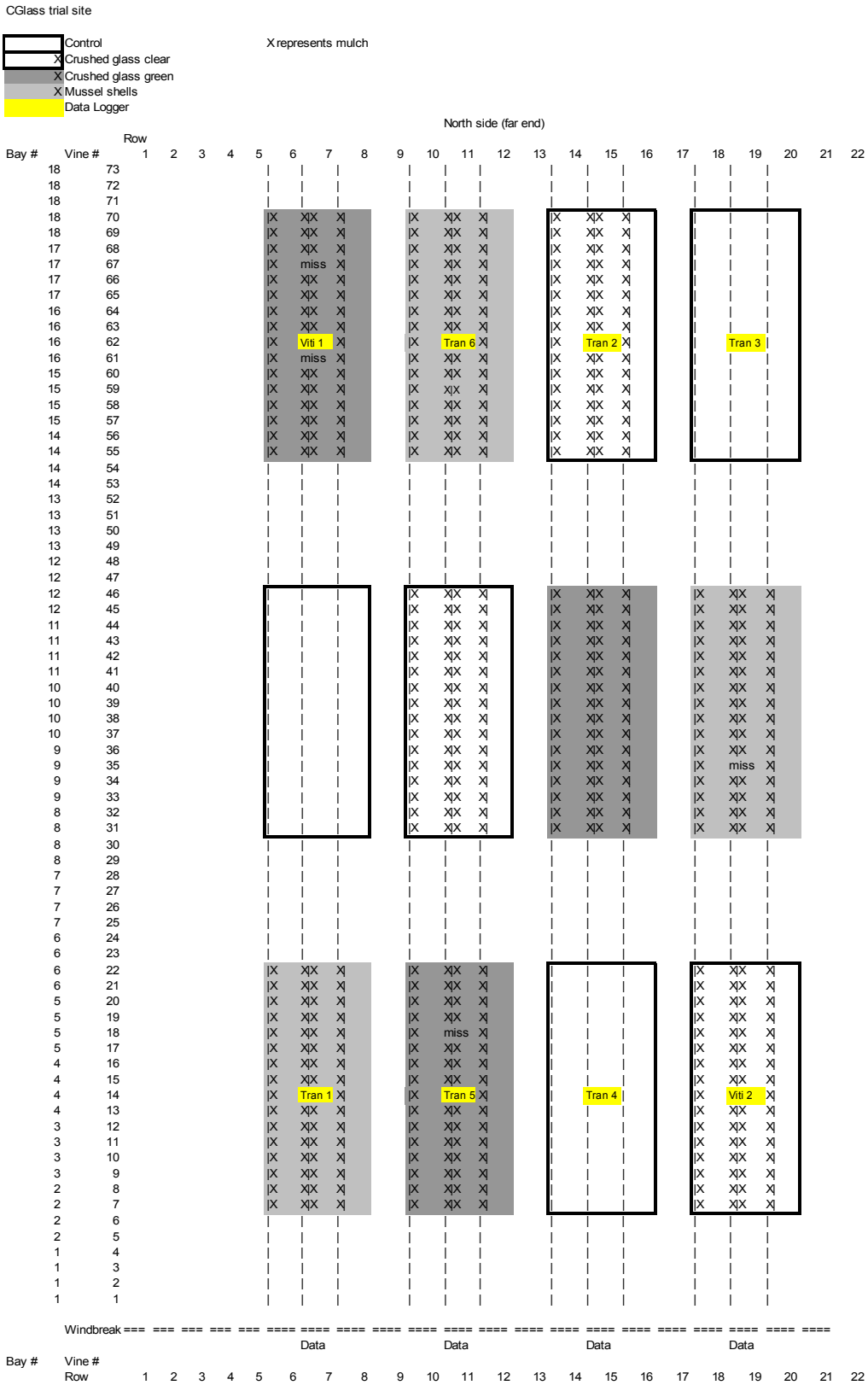
Appendices

A.1 – Layout of trial area



A. 2 – Season summary information from NIWA.

Weather station located at Christchurch International Airport – Sallinger *et al.* (2005, 2006a, 2006b, 2007, 2008a 2008b).

Season Summary	Spring (Sep - Nov) 2005	Summer (Dec - Feb) 2005/2006	Autumn (Mar - May) 2006
Temperature	11°C	17°C	11.6°C
	-0.5°C	+0.4°C	-0.4°C
	Below average	Above average	Below average
Rainfall	101 mm	114 mm	221 mm
	72%	87%	138%
	Below average	Near average	Above average
Sunshine	541 hours	629 hours	425 hours
	114%	97%	88%
	Near average	Near average	Below average

Season Summary	Spring (Sep - Nov) 2007	Summer (Dec - Feb) 2007/2008	Autumn (Mar - May) 2008
Temperature	11.1°C	16.9°C	11.5°C
	-0.4°	+0.3°	-0.5°C
	Below average	Above average	Below average
Rainfall	135 mm	188 mm	118 mm
	96%	144%	74%
	Near average	Well above average	Below average
Sunshine	662 hours	694 hours	500 hours
	115%	107%	103%
	Well above average	Near average	Near average

A.3 – Wine making plan for 2006 and 2008 vintage wines

Lincoln University
Te Whare Wānaka o Aoraki
CHRISTCHURCH•NEW ZEALAND
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Processing Steps

Research Red Wine Production

Note: in order to maintain the vineyard differences between treatments, no manipulation of the must occurred. Pre and post ferment maceration were not used or any standard additions of nutrient.

- Fruit was hand harvested.
- Due to the small quantities, reps were mixed together.
- Fruit was crushed and destemmed, with the crusher being washed down in between treatments.
- Due to the poor condition of the fruit (disease), an addition of 30ppm SO2 was made.
- Fruit was neither cooled nor warmed as it was at room temperature which was suitable for inoculation.
- Must was inoculated with 200ppm Elegance yeast (Institute of Burgundy).
- Musts were placed in the 28 degree room to complete fermentation.
- Gentle hand plunging was done 3 times a day.
- Temperature and Brix levels were done once a day. If the ferments showed signs of stress nutrients were added at the next punch down.

- As soon as the wines reached dryness (Clinitest), they were pressed off (maximum 2 bar; 30 minutes press time)
- The wines were put in the 18 degree room and inoculated with malolactic culture (Viniflora, CHR Hansen) and monitored twice weekly using TLC.
- Once MLF was completed, sulphur was added (50ppm) and maintained at between 15-20ppm free SO2. The wines were taken out of the 18 degree room and sat in the winery cellar.
- Wines were racked off gross lees once before a final rack at bottling.
- Bottling was completed using a one-head filler (Enolmatic) and closed with screw caps before being stored in the winery cellar.

A.4 – Tasting score sheet

NAME…….

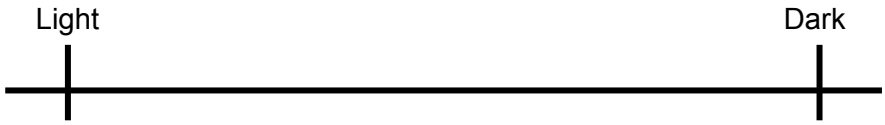
Glass Mulch Pinot Noir Wine Scorecard

You will be presented with two flights of four wines, each with a number assigned to it.

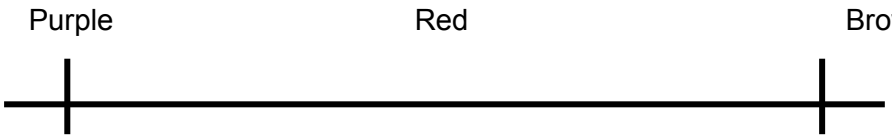
Please fill out the following page for each flight. You can answer by drawing a line where you perceive each to sit on the relative scale. Please label each mark with the wine's code.

Appearance

- **Depth of colour**



- **Hue**



Aroma

- Ripe Fruit Character

Please record the code for each of the wines you are tasting in the spaces below and select three descriptors from the aroma wheel which you feel best describe the wine's aroma.

Wine……

Wine...……...

Wine……

Wine……

Please record any notes regarding the duration, intensity, development or varietal character of the wines aroma:

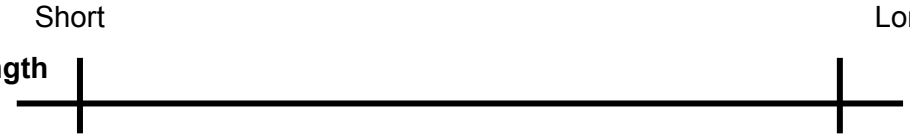
Flavour

- Overall **Intensity**

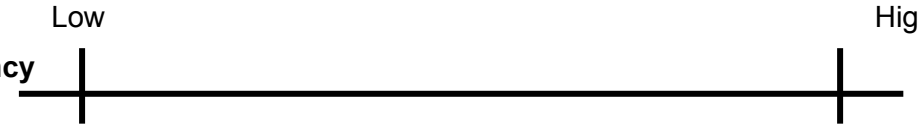
- **Acidity**

Mouthfeel

- **Palate length**



- **Astringency**



Please record the code for each of the wines you are tasting in the spaces below and select three descriptors from the mouthfeel wheel which you feel best describe the wine's mouthfeel.

Wine……

Wine……...

Wine……

Wine……

Overall Balance

Preference

Please write down the code of the wine that you preferred

……

Additional Comments:

The Aroma Wheel

Removed for copyright reasons.

Please refer to Figure 1 from Noble, A.C., Arnold, R. A., Buechsenstein, J., Leach, E. J., Schmidt, J. O. and Stern, P. M. (1987). Modification of a Standardized System of Wine Aroma Terminology. Am. J. Enol. Vitic. 38, 143-146.

The Mouthfeel Wheel

Removed for copyright reasons.

Please refer Figure 2 from to 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.