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**Effect of crushed glass, used as a reflective mulch, on Pinot noir
performance**

A thesis
submitted in partial fulfilment
of the requirements for the Degree of
Master of Horticultural Science

at
Lincoln University
by
Patricio Mejias-Barrera

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Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Master of Horticultural Science.

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by

Patricio Mejias-Barrera

Research conducted at Lincoln University, New Zealand, evaluated an alternative use for recycled glass in its crushed form as a mulch under grapevines. The trial comprised four treatments: clear glass, brown glass, mixed glass (which is primarily green and brown) and undisturbed soil as a control, with four replicates in a randomised block design. The vine material was twelve year-old Pinot noir 777 on 3309 rootstock. Radiation reflected back up into the fruiting zone was quantified using a Bentham spectroradiometer under clear and sunny field conditions. Reflection from the mulches was also measured under controlled conditions. The results showed that clear glass reflected the highest amount of radiation in all the spectral ranges evaluated. UV-B radiation reflected by clear glass was double that reflected by the control. UV-A reflected by clear glass was about seven times more than that of the undisturbed soil treatment. Readings for PAR were almost five times larger than the control and, for infra-red (IR), this difference was twice as much as for the control. PAR was also divided in different “colours”, with clear glass being the most reflective treatment in all PAR ranges. Similar data were obtained from the evaluation of the mulches under controlled conditions. However, the extra amounts of radiation reflected by the mulches did not have any effect on harvest parameters: number of clusters, cluster weight, potential crop, °Brix, pH or TA.

Aromatic profiles of the juice obtained from the grapes of this trial were evaluated using GC-O analysis. The panellist identified seven aroma descriptors from the samples: cut grass, mushroom, fresh peas, violet, cooked potato, rose and blackberry. Despite more aromas being detected, these were the ones most frequently sniffed by the panellist. The descriptors corresponded only to an association between panellist’s perception and a known aroma. The use of reflective mulches showed an influence on the aromatic profile of the grape. Clear

glass decreased the intensity of the cooked potato-like aroma. This treatment also enhanced the aromas described as roses and blackberry when sniffed by the panellist. GC-MS and a mix of standards were used to determine the compounds related to each of the aromas described by the panellist. Hexanal was reported as the compound related to the aroma described as cut grass, and 1-hexanol was related to the aroma described as fresh peas. The rest of the descriptors were only tentatively identified. To do that, the retention time of each descriptor was compared with an alkane mix of standards and information registered in the literature. For mushroom aroma, 2-octanone was described as the most possible compound related to this aroma in the samples analysed. The aroma described as violet was associated to four different compounds: (Z)-linalool oxide, (E)-linalool oxide, nerol oxide and linalool oxide. The cooked potato-like aroma was related to methional, which was described for the first time in this experiment on Pinot noir juice. Linalool was associated with the aroma described as rose by the panellist, and β -ionone was related with the blackberry-like aroma.

Keywords: Pinot noir, reflective mulches, crushed glass, UV-B, UV-A, PAR, IR, aroma compounds, hexanal, 1-hexanol, methional, linalool, nerol, 2-octanone, β -ionone

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Table of Contents

Abstract	ii
Acknowledgements	iv
Table of Contents.....	v
List of Tables.....	vii
List of Figures	ix
Chapter 1 Introduction	1
Chapter 2 Literature review.....	3
2.1 Pinot noir statistics in New Zealand.....	3
2.2 Waste glass in New Zealand	3
2.3 The use of mulches in agriculture	4
2.4 Influence of light in the canopy environment	7
2.4.1 Photoreceptors	9
2.5 Gas chromatography-olfactometry (GC-O)	10
2.6 Pinot noir aroma compounds	11
Chapter 3 Materials and methods.....	14
3.1 Field experiment.....	14
3.2 Measurements of mulch reflection under field conditions.....	16
3.2.1 Statistical analysis	19
3.3 Model system	19
3.3.1 Statistical analysis	21
3.4 Harvest and sampling process	22
3.4.1 Statistical analysis	24
3.5 GC-O analysis and aroma identification	24
3.5.1 Aroma identification	26
3.5.2 Statistical analysis and analysis of data.....	27

Chapter 4 Results and discussion.....	30
4.1 Mulch reflectance under field conditions.....	30
4.2 Model system	37
4.3 Yield components and grape composition	41
4.3.1 Yield components.....	41
4.3.2 Grape composition	42
4.4 GC-O analysis and aroma identification	45
4.4.1 FID data from the GC-O device.....	46
4.4.2 Data from the panellist	49
4.4.3 Correlations between GC-O device sensibility and panellist's perception	52
4.4.4 Aroma identification	53
 Chapter 5 Conclusions	 60
 Chapter 6 Further research.....	 64
 References.....	 65

List of Tables

Table 3. 1 – Different ranges of PAR considered in the research.....	18
Table 4. 1 – Reflectance of the different treatments in specific ranges. Each mean was calculated from four replicates. Results expressed in miliwatts per square metre per nanometer ($\text{mW m}^{-2} \text{nm}^{-1}$). Values within columns followed by different letters indicate significant differences in a Student's t-test at $p < 0.05$	32
Table 4. 2 – Reflectance in the visible range divided in different colours. Each mean was calculated from four replicates. Results expressed in miliwatts per square metre per nanometer ($\text{mW m}^{-2} \text{nm}^{-1}$). Values within columns followed by different letters indicate significant differences in a Student's t-test at $p < 0.05$	36
Table 4. 3 – Reflection of the different mulches under controlled conditions. Each mean was calculated from four replicates. Results expressed in miliwatts per square metre per nanometer ($\text{mW m}^{-2} \text{nm}^{-1}$). Values within columns followed by different letters indicate significant differences in a Student's t-test at $p < 0.05$...	38
Table 4. 4 - Reflectance in the visible range divided in different colours under controlled conditions. Each mean was calculated from four replicates. Results expressed in miliwatts per square metre per nanometer ($\text{mW m}^{-2} \text{nm}^{-1}$). Values within columns followed by different letters indicate significant differences in a Student's t-test at $p < 0.05$	40
Table 4. 5 – Yield components of the fruit from the mulch trial. Each mean was calculated from four values. Fruit harvested in May 1, 2012. No significant differences were found	41
Table 4. 6 - Brix degrees ($^{\circ}\text{Brix}$), pH and titratable acidity (TA) from the different treatments of the trial. Each mean was calculated from four values. Fruit harvested in May 1, 2012. No significant differences were found.....	43
Table 4. 7 – Most frequent aromas described by the panellist during the experiment, and their retention times (minutes).....	45

Table 4. 8 – Area of the peak (divided by 1,000) obtained from the GC-O device. Each mean was calculated from eight values. Different letters in the same column indicate significant differences (ANOVA at $p < 0.05$, Fisher’s LSD test) between treatments.	46
Table 4. 9 - Height of the peak (divided by 1,000) obtained from the GC-O device. Each mean was calculated from eight values. Results expressed in microvolts (mv). Different letters in the same column indicate significant differences (ANOVA at $p < 0.05$, Fisher’s LSD test) between treatments.	47
Table 4. 10 – Frequencies of the different aroma descriptors in data collected from the device.	48
Table 4. 11 - Area of the peak obtained by the panellist. Each mean was calculated from eight values. Different letters in the same column indicate significant differences (ANOVA at $p < 0.05$, Fisher’s LSD test) between treatments.	49
Table 4. 12 - Height of the peak obtained by the panellist. Each mean was calculated from eight values. Different letters in the same column indicate significant differences (ANOVA at $p < 0.05$, Fisher’s LSD test) between treatments.	50
Table 4. 13 - Frequencies of the different aroma descriptors in data collected by the panellist.	51
Table 4. 14 – Correlations between FID readings and panellist’s perception for peak area data. Pearson’s correlation coefficient was calculated at $p < 0.05$ and $p < 0.01$ using a two-tailed test.	52
Table 4. 15 - Correlations between FID and panellist’s perception for peak height data. Pearson’s correlation coefficient was calculated at $p < 0.05$ and $p < 0.01$ using a two-tailed test.	53
Table 4. 16 – Aroma compounds identified and tentatively identified associated to each aroma descriptor used by the panellist. Cut grass and fresh pea aromas were confirmed by GC-MS. Mushroom, violet, cooked potato, rose and blackberry aromas were tentatively identified.	54

List of Figures

Figure 3. 1 – Different treatments. Clockwise from top left: undisturbed soil, clear glass, brown glass and mixed glass.	15
Figure 3. 2 – Clear glass applied in the field trial.	16
Figure 3. 3 – Bentham spectroradiometer reading reflection data	17
Figure 3. 4 – Model system set up in the growth chamber.....	20
Figure 3. 5 – Photometric data of the bulb used in the trial (MASTER SON-T PIA Plus 600W/220 E40 1SL).....	21
Figure 3. 6 – Addition of nitrogen gas	23
Figure 4. 1 – Energy reflected for the different treatments with respect to direct sunlight.....	30
Figure 4. 2 – Reflectance under field conditions.....	31
Figure 4. 3 – R:FR ratio under field conditions. Each mean was calculated from four values. Different letters above the columns indicate a statistically significant difference in an ANOVA at $p < 0.05$ using Tukey HSD test.....	35
Figure 4. 4 – Incident energy reflected for each treatment under controlled conditions.....	37
Figure 4. 5 – Reflectance under controlled environmental conditions.....	38
Figure 4. 6 – R:FR ratio under controlled conditions. Each mean was calculated from two values. Different letters above the columns indicate a statistically significant difference in an ANOVA at $p < 0.05$ using Tukey HSD test.....	39

Chapter 1

Introduction

Since the Packaging Accord was introduced in New Zealand in 2004 (Snow and Dickinson 2005), recycling has become a common practice in New Zealand. Although it is an eco-friendly activity, it has had some problems, especially in zones where people produce large amounts of waste glass. In the South Island, specifically in Otago and Christchurch (Snow and Dickinson 2005, Thomas 2005), the reduction in demand and a drop in prices paid for recycled glass have produced mountains of unused glass in the vicinity of crushing plants. Crushed glass can be used for multiple purposes such as fibre glass insulation, flat glass, construction and road aggregate, landfill cover and abrasives, to name a few (Thomas 2005). One alternative use for crushed glass was using it as reflective mulch.

Mulches have been evaluated around the world. The variety of materials used for this purpose and the number of crops they have been used for was large. For example, mulches have been used in apple orchards (Blanke 2008, Solomakhin and Blanke 2007), and in the production of peppers (Hutton and Handley 2007), summer squash (Brown et al. 1993), strawberries (Kasperbauer et al. 2001, Rhainds et al. 2001) and pumpkins (Brust 2000). Previous experiments also have shown the effectiveness of different materials used in vineyards. For example, those made from different waste streams (Agnew et al. 2002), composted mulch (Chan and Fahey 2011, Chan et al. 2010), geotextile mulches (Hostetler et al. 2007a), polyethylene (Jamshidian et al. 2010), aluminised polypropylene (Reynolds et al. 2008, Sandler et al. 2009) and crushed shells (Crawford 2007, Leal 2007, Sandler et al. 2009) have been evaluated for their potential use in vineyards. In addition, Ross (2010) evaluated the use of crushed glass (green and clear), establishing a new use for this product and found that using it as mulch in vineyards can affect the canopy environment, increasing the radiation levels and heat.

Several trials have demonstrated that the use of reflective mulches increased the amount of radiation received by the plants. Specifically, crushed glass was used as reflective mulch by Ross (2010) who found that clear glass enhanced the quantity of photosynthetically active radiation (PAR) and ultraviolet radiation (UV) received by the vineyard. Also, in that trial, the red:far red ratio (R:FR) registered significant differences in the mulched treatments. Phytochromes are affected by the red and far-red part of the spectrum, and changes in light quality can alter photosynthesis (Sharrock 2008, Taiz and Zeiger 2010). Although the

research conducted by Ross (2010) evaluated the amounts of radiation reflected within the canopy in a Pinot noir vineyard, the method needed to be refined, because it was necessary to investigate how the R:FR ratio, UV, PAR and infra red (IR) may change as a result of the mulch and the different colours of glass.

The hypotheses for this research proposal were: first, crushed glass mulches have reflective properties useful in a vineyard situation because they can reflect PAR, IR and UV radiation into the fruiting zone. The second hypothesis was that mulch made from different colours of glass will have different reflective properties. Finally, it was proposed that grape juice profiles will be affected by the colours of the different glass.

The objectives of this research proposal, therefore, were:

- To evaluate, in a model system and in the vineyard, the reflective properties of three colours of crushed glass.
- To measure the impact of these mulches on grape berry and grape juice composition.
- To formulate experimental questions for a subsequent, commercial field experiment.

Chapter 2

Literature review

2.1 Pinot noir statistics in New Zealand

The New Zealand Wine magazine (2011) noted in 2011 that wine was the ninth ranked export product in New Zealand, reaching \$1.1 billion in value. The main export market for New Zealand wine in this period was the UK, with 34% of exports, followed by Australia, with around 30%.

The total volume of grapes harvested in the country during the 2011 vintage was 328,000 tonnes, 62,000 tonnes greater than the 2010 vintage (New Zealand Wine 2011). Pinot noir represented around 10% of New Zealand wine production in 2011, with 31.2 thousand tonnes.

Currently, New Zealand has 4,800 hectares of Pinot noir (the second most planted variety in the country after Sauvignon blanc), which produced 31,160 tonnes of grapes in 2011. New Zealand Wine (2011) estimated that the area planted with this variety will reach 4,830 hectares in 2012.

2.2 Waste glass in New Zealand

The Glass Packaging Forum (2010) indicated that the amount of glass recycled in New Zealand reached 166,600 tonnes in 2010. This represented around 66% of the total amount of glass used in the country that year. Compared with the 90,000 tonnes recovered by the recycling industry in 2004 (Thomas 2005), it represented an increase of 85% over a six year period.

The NZ Packaging Accord helped to solve the environmental problem of the thousands of tonnes of waste that were deposited in landfills before 2004, but it created a problem for recycling plants because the glass recycled exceeded their capacity for using it, generating mountains of recycled glass around the plants, especially in the South Island. In addition, the only glass container manufacturer, ACI – OI in Auckland, dropped the price paid for clear glass cullet (crushed waste glass), increasing the problem (Snow and Dickinson 2005, Thomas 2005).

The glass industry has developed several uses for recycled glass. The most evident use for recycled glass was remanufacturing containers, but this was not the most frequent application.

For example, Metso Minerals (Matamata) Limited (2003) used 80% of recycled glass for sandblasting material and around 15% as water filter packing. Other significant possible uses included: construction aggregate, fibre glass insulation, foam glass insulation, flat glass, landfill cover, abrasives, asphalt, concrete (glasscrete), glassphalt, glass tiles, glass flooring and vineyard mulch (Metso Minerals (Matamata) Limited 2003, Snow and Dickinson 2005, Thomas 2005).

The costs involved in the crushing process were directly related to the use of heavy machinery or portable crushing equipment and the distance to Auckland, where the only bottle manufacturing plant was. Although the cost of crushing glass using heavy machinery (e.g. that used for road construction or construction excavation) was lower than using portable crushing equipment (around 90% less), the quality of the final product was not uniform and potentially dangerous for users (Snow and Dickinson 2005). Portable crushing equipment has the advantage that the final product has a higher quality, so can be used for many purposes. The main problem of recycling glass in the South Island was the transport costs involved in moving cullet glass to Auckland. Places such as Dunedin, Queenstown, Wanaka, Oamaru and Central Otago were located far away from Auckland. For example, the transport cost per tonne of glass from Christchurch to Auckland was around \$100 NZD, but from Timaru it rose to \$125 NZD. The transport cost from Invercargill can reach \$240 NZD per tonne, making it uneconomic to move it to the ACI – OI plant, and increasing the size of glass mountains every day (Snow and Dickinson 2005, Thomas 2005). Another important reason to consider the costs involved in glass recycling was that, at least in the Otago region, low landfill gate fees worked against it, because depositing the glass in a landfill was cheaper than finding a new use for it (Snow and Dickinson 2005).

The New Zealand glass recycling industry not only faced the problem of transport cost. As stated by Covec & Environmental Resources Management (2007) the glass collected by co – mingling systems, increasingly common in New Zealand, was of lower quality because of the presence of more contaminants. They also suggested the possibility of separating glass by colour, enhancing the possibilities of using it for new alternative uses, but the cost of the type of machinery required was higher.

2.3 The use of mulches in agriculture

Mulches were currently used for many purposes in agriculture. These can include: increasing yields, inhibition of weed growth, improving the quality of different products and the control

of pests and diseases. For example, red mulch was evaluated by Kasperbauer et al.(2001) for cultivating strawberries. They observed that the strawberry size was larger in plants that grew over red mulch and the concentration of fructose, glucose and sucrose in them was higher. Red mulch also affected the aroma of the strawberries, enhancing the organoleptic properties of this fruit. Moreover, Hutton and Handley (2007) found that in bell pepper production, reflective mulch (silver colour) slightly increased the soil temperature compared to the other treatments, but there were no significant differences in pepper yield.

Reflective mulches have also been used in tree fruit crops. For example, in Germany, apple orchards usually used hail nets to protect the fruit from damage caused by hailstorms. Although necessary, the netting had adverse effects on fruit quality, especially in the form of lower sugar content and colour (Solomakhin and Blanke 2007). Reflective mulches have been shown to improve the colour and sugar content of apples, especially in the lower canopy because of the extra light available to the fruit (Blanke 2008, Solomakhin and Blanke 2007). In another study, Layne et al. (2001) evaluated the effect of an aluminized mulch on the red skin colouration and advances in the maturity of peach. Results indicated that the light reflected by the film did not significantly affect fruit size compared with the control treatment, but the use of film improved the colour of the peaches during the two seasons of evaluation.

Mulches have also been used to control insects and, indirectly, diseases. A good example was the trial directed by Brown et al. (1993) who found that silver reflective plastic mulch delayed the onset of foliar mosaic virus symptoms by reducing the aphid vector's population. A similar experience showed that using reflective mulches in pumpkin cultivation reduced the population of aphids that transmit viruses. As a result, reflective mulch increased the pumpkin yield 2.2 times (Brust 2000). Rhainds et al. (2001) evaluated a reflective mulch controlling tarnished plant bugs and its influence on yield in strawberries. They found that the reflective mulch suppressed the incidence of damage from pest attack, leading to less direct damage to fruit and plants and increased productivity. Similar results were reported by Summers and Stapleton (2002) who found that reflective mulch reduced the population of *Bemisa argentifolii* (Homoptera: Aleyrodidae) in pumpkin, cucumber and zucchini squash. Reflective mulch also contributed with the increment of yield in these crops. Greer & Dole (2003) showed important data about increased yields and decreased insect-vectored viral diseases of vegetables using different mulches. In a review, they noted that aluminium foil mulches always enhanced yields and contributed to water conservation. This kind of mulch also repelled aphids and thrips and, as a consequence, reduced the incidence of viral diseases in many crops.

Moreover, a large range of materials have been evaluated in vineyards as mulch. They included bark, plastics, geotextile mats, composted vegetal materials, crushed shells and glass, and a mix of other waste stream products. Important research was conducted in New Zealand by Ross (2010), who investigated the effect of crushed glass and mussel shells used as mulch on the grapevine environment, vine performance, and juice and wine characteristics. The results indicated that the extra light reflected by mulches positively influenced Pinot noir grapes, juice and wine, by enhancing their aromatic and phenolic profiles, and sugar contents. Additionally, mulches in that trial showed having an effect on soil moisture content and nutrients in the canopy, which were higher in the mulched treatments compared with the un-mulched control. Microbial biomass was also higher for the mussel shells treatment. However, canopy temperature was not affected by the treatments. Leal (2007) found that mussel shell mulch altered the fruiting zone temperature and reflected greater amounts of UV-A, UV-B and PAR radiation. It also slightly raised the flavonoid concentration, but these differences were not statistically significant. The same effects were obtained in the United States by Hostetler et al. (2007a) using reflective geotextile mulches. Aluminised reflective films have also been evaluated in different countries like Iran and Canada reaching similar results in both cases. However, in the Canadian research the researchers described a significant deterioration of the film during the trial that affected the results (Coventry et al. 2005, Jamshidian et al. 2010). In a two season study, Sandler et al. (2009) studied the effect of a white reflective woven material, a silver aluminised reflective mulch and crushed quahog shells, and found that all the treatments reflected higher quantities of PAR light with respect to the control treatment; but only in Cabernet franc during the season 2006 were the concentrations of total anthocyanins, flavonols, and phenolics affected by the shells.

Previous experiments using organic materials as mulch have not been completely successful. Hostetler et al. (2007b) compared the influence of composted bark mulch, reflective (white) and black geotextile mulches, and mechanical soil cultivation in Pinot noir and found that the white geotextile mulch had the largest effect on yield. The only positive effect from bark mulch was a reduction in weed biomass when compared with soil cultivation as the control. Additionally, Chan and Fahey (2011) studied the effect of composted mulch application on the soil and wine grape potassium statuses. Even though using composted mulch significantly increased the extractable soil K, at the same time, it raised the pH in the grapes up to dangerous levels for the winemaking process. The optimum ranges of pH are between 3.1 and 3.4 for white wines and between 3.3 and 3.6 for the majority of red wines (Jackson 2008). A pH over these values exposes wines to microbiological contamination and also oxidation problems (Butzke 2010, Jackson 2008). Moreover, a high potassium concentration in wines

can interfere with the efficient uptake of amino acids, and also cause stuck fermentations. The combination of high pH and high potassium concentrations can lead to microbial instability, turning white wines brown and causing colour instability in red wines (Jackson 2008).

2.4 Influence of light in the canopy environment

Radiation is the most important environmental factor that regulates the major functions of plants. Not only does the amount of light influence plant performance but also the quality of sunlight is important. To understand how light affects plants it is helpful to divide the light into wavelengths. Plant leaves can make use of only a part of the sun's radiation, mainly in photosynthetically active radiation (PAR), which lies between 400 to 700 nm. Grapevines are also very sensitive to changes in the ratio of red light (660 nm) to far red light (730 nm) (Smart et al. 1988) and UV radiation, especially UV-B (280-315 nm), as it affects the synthesis of colour compounds (Smart and Robinson 1991).

Canopy management determines the light environment within the grapevine, as it is known that radiation in the "visible range" directly affects photosynthesis. Around 90% of PAR is absorbed by grapevine leaves, with the remainder either transmitted or reflected (Dokoozlian and Kliewer 1995a, Smart et al. 1988, Taiz and Zeiger 2010). PAR has a very important role in the accumulation of sugars and other compounds in the fruit (Medrano et al. 2003). For example, Dokoozlian and Kliewer (1995b) reported that fruit well exposed to sunlight usually had higher concentrations of sugars, anthocyanins and total phenolics, and lower levels of malic acid, potassium and juice pH compared with fruit that grew in a shaded canopy. Moreover, the way in which leaves are located in the canopy interior to capture sunlight is interesting to analyse. The frequency and duration of sunflecks (which happen when direct sunlight enters gaps in the canopy surface) are a significant aspect of the light environment of the canopy (Dokoozlian and Kliewer 1995a). The contribution of these to the total fluence rate of incident light available for the plants is very important (Bukhov 2004). Sunflecks also had a positive effect on the carbon economy of leaves located in the canopy interior (Dokoozlian and Kliewer 1995a, Taiz and Zeiger 2010).

Additionally, phytochromes have an important role as a photoreceptor for plants. Their main characteristic is the capacity to absorb light in the red/far-red part of the spectrum (Sharrock 2008); they can also transmit changes in the light environment within the plant cell (Jordan and Callow 1996). For this reason, phytochromes have an important role determining plant photomorphogenesis and controlling many aspects of plant development, such as flowering,

germination and morphology (Jordan and Callow 1996, Sharrock 2008) This is possible through the process called photoreversibility, which is the conversion/reconversion property of phytochromes because of changes in the light spectrum absorbed. Phytochromes pass from the red light-absorbing form to a far-red light-absorbing form, and far-red light can reverse the process (Jordan and Callow 1996, Taiz and Zeiger 2010). In research conducted by Smart et al. (1988), the authors indicated that the ratio of red to far-red radiation was important for phytochrome reactions, and that the ratio varied under shade, compared to sun conditions. When they used a supplementary source of red light, the berry colour and sugar content was significantly enhanced; the leaves of the canopy interior also had higher levels of $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$, and shade decreased the Ca^{2+} concentration, reduced berry weight, decreased the rate of net photosynthesis, stomatal conductance and delayed veraison. The same authors determined that light supplementation increased the red to far-red ratio 1.4 to 3.0 times above the natural light intensity. Similarly, Dokoozlian and Kliewer (1995a) reported that the R:FR in direct sunlight was between 1.1 to 1.2, while it fell to 0.1 or less within dense vine canopies. In the fruit zone, at berry set, the R:FR range was 0.58 to 0.40 in low density canopies and 0.2 or less in high density canopies, providing evidence of the importance of canopy density for this parameter (Dokoozlian and Kliewer 1995a).

Another important aspect that influenced plant performance was UV-B radiation. This was defined as radiation in the range of 280 – 315 nm (Jordan and Callow 1996). In general, UV radiation can have effects upon plants such as inhibition of photosynthesis, damage to lipids, nucleic acids and proteins in leaves, changes in leaf area, changes in assimilate partitioning, and alterations in pigment biosynthesis (Jordan and Callow 1996, Kolb et al. 2001). Several experiments have been carried out to determine the role of UV and UV-B on grape composition. For example, Koyama et al. (2012) showed the importance of UV radiation for flavonoid synthesis using bags of UV-proof film to exclude UV. Results indicated that UV exclusion directly affected flavonoid accumulation on Cabernet Sauvignon berries. Berries of clusters covered with UV-proof film remarkably decreased flavonol concentration to 19% of that of control berries. In a similar experience, Gregan et al. (2012) in a trial carried out at the Lincoln University vineyard, reported that UV-B radiation influenced the flavonol composition in the skins of Sauvignon blanc grapes. In Spain, Nuñez-Olivera et al. (2006) evaluated the physiological effects of solar UV-B exclusion on Tempranillo and Viura vines. They concluded that in Tempranillo, chlorophyll was reduced under solar UV-B while Viura did not show any change. UV-B was also involved in stress tolerance of grape leaves. It was demonstrated by Berli et al. (2010), who observed that UV-B not only increased flavonols and phenolic compounds, but also increased abscisic acid This was also confirmed by

Downey et al. (2006), who reported that in grapevines that abscisic acid has been shown to increase anthocyanin accumulation in grape berries of the Olympia, Kyoho, and Cabernet Sauvignon cultivars.

2.4.1 Photoreceptors

Photoreceptors are pigments able to absorb light in some specific ranges of the spectrum. For example, chlorophylls, which have a key role in photosynthesis (Hopkins 1999, Taiz and Zeiger 2010), absorb strongly in the blue (chlorophyll b) and red range (chlorophyll a), but they not in the green part of the spectrum (Hopkins 1999). As “green light” is reflected, it confers to the characteristic green colour to plants (Keller 2010).

Flavonoids also have the characteristic of absorbing UV radiation (Winkel-Shirley 2002). When plants were exposed to UV radiation, they rapidly increased the flavonoid concentration to reduce the potential for damage. The relationship between UV and flavonoid synthesis can be considered as evidence for the role of flavonoids in UV protection (Jordan and Callow 1996). The flavonoid group included flavones, flavonols and isoflavonoids. Other related phenolic compounds, such as sinapic acid esters were also accumulated after exposure of plants to UV-B radiation (Jordan and Callow 1996). Plants can also accumulate anthocyanins in response to UV-B radiation, although they absorbed maximally outside the UV range at about 530 nm at acidic pH (Downey et al. 2006, Jordan and Callow 1996). Additionally, Winkel-Shirley (2002) related the accumulation of anthocyanin pigments with plant stress, although further studies were needed here.

Carotenoids are another important group of pigments involved in light absorption. They are part of the orange and yellow family of pigments present in most of the photosynthetic organisms (Hopkins 1999). An absorption band in the 400 to 500 nm region gives carotenoids their characteristic orange colour (Taiz and Zeiger 2010). The most common carotenoid reported in higher plants was β carotene (Attridge 1990, Hopkins 1999, Taiz and Zeiger 2010), whose main physiological function was the protection of the plants from damage caused by light (Taiz and Zeiger 2010).

Pigments related with blue light absorption, however, have been less studied. Cryptochromes have been reported only in lower plants such as ferns, mosses and fungi (Hopkins 1999, Wada et al. 2005). Phototropins were recently discovered and described in higher plants (Christie 2007, Wada et al. 2005). They controlled a range of responses in plant such as phototropism, light-induced stomatal opening, leaf expansion, and chloroplast movements in response to changes in light intensity (Christie 2007, Takemiya et al. 2005, Wada et al. 2005). Their action spectra exhibited important activity in the “blue region” at about 450 nm, but have been reported to have an active response between 320 to 500 nm (Christie 2007, Hopkins 1999). The results obtained by Pontin et al. (2010) suggested that high UV-B caused a negative phototropic response of grapevine leaves as an escape response, regulated by phototropins, to protect leaf tissues against a potential damage produced by UV-B radiation. Little information is available about the role of phototropins in grapevines, makes this an interesting research area to discover their possible role in grapevine physiology

2.5 Gas chromatography-olfactometry (GC-O)

Gas chromatography-olfactometry is widely used in aroma research to determine the odour active compounds in foods (Van Ruth 2001). The quality of an alcoholic product is, in part, determined by the composition and content of the odour compounds contained in it. Thus, the aromatic profile of an alcoholic beverage is composed of a large number of chemical compounds of different concentrations (Plutowska and Wardencki 2008). GC-O is based on sensory evaluation of the effluent as it emerged from the chromatographic column aimed at discovering the active odour compounds. This technique was proposed by Fuller et al. in 1964 when they showed the importance of determining the odour-active compounds for complex mixtures (Fuller et al., 1964, as cited in Van Ruth, 2001).

Numerous studies have been carried out to determine the aromatic profile in wine since this technique was created. For example, Fang and Qian (2006) quantified the aroma-active compounds in Pinot noir. They indicated that the Pinot noir aroma comprised a complex formulation of many aroma compounds, and the characteristic aroma of this variety was not the responsibility of a single compound. In this study the authors also indicated that the majority of aroma-active compounds analysed were esters. Moreover, GC-O has been demonstrated as an effective way to determine the aromatic profile in wines with high alcohol contents. Thus, Campo et al. (2006) studied the aromatic profile of Madeira wines (18-20% of

alcohol) and found more than 90 odourants present, some in high concentrations, such as those that came directly from the wood the wine was aged in.

This method, however, has had some limitations in the past. For example, Culleré et al. (2004) concluded that although GC-O was a suitable way to do olfactometry studies, the difficulties of interpreting excessively complex olfactograms could be a limitation of this technique. Moreover, samples can be affected by oxygen altering the aroma composition. Thus, Reynolds (2010) reported that the direct exposition of the samples to air can produce polyphenol and aroma compound oxidation, likely affecting the GC-O analysis. To prevent this, Plutowska and Wardencki (2008) suggested dividing the sample into smaller subsamples to avoid changes in the composition of the wine as a consequence of repeatedly opening the container. Additionally, other authors suggested to store the wine in a carbon dioxide or nitrogen atmosphere to avoid oxidation (Le Fur et al. 2002). A combination of both techniques was used by Bernet et al. (2002) who stored the wine at +5°C under a CO₂ stream in the original bottles and then divided each sample into 50 vials and preserved them under CO₂ at -80°C.

2.6 Pinot noir aroma compounds

Pinot noir aromatic profiles have been widely studied in recent decades. The most common methods to determine the aroma compounds in this variety have been gas chromatography – olfactometry (GC-O) and gas chromatography mass – spectrometry (GC-MS). Research directed at determining the aromatic profile of Pinot noir have been focused on the evaluation of wines rather than must or juice. The only previous experience registered in the literature in which Pinot noir juice was analysed corresponds to the research conducted by Ross (2010) at Lincoln University. The methodology used in that experiment was adapted to be used in this trial.

GC-MS is a technique widely used in aromatic profile analysis of wine. Specifically, for Pinot noir, there are some examples of successful experiences in the past. Probably the first attempts to try to determine the Pinot noir aromatic profile were carried out by Schreier (1980) and Brander et al. (1980). The first author evaluated five Burgundy Pinot noir samples using GC-MS, finding eleven different aroma compounds in these wines. The second group of researchers, using the same analysis, reported nearly a dozen different components such as

alcohols, esters, carbonyls, acetals, hydrocarbons, terpenes and lactones. Allen et al. (1994) described methoxypyrazine for the first time in Pinot noir. Then, Hashizume and Samuta (1999) reported methoxypyrazine in berries of this variety as well. In another experiment, Aubry et al. (1997) focused their attention in four esters (ethyl dihydrocinnamate, ethyl cinnamate, methyl anthranilate and ethyl anthranilate). They found these four esters in thirty three Pinot noir wines analysed, but at low concentrations. Furthermore, β – damascenone and β – ionone, two aroma-active compounds, have been reported in Pinot noir by Fang and Qian (2006) and Pineau et al. (2007) who also used GC-MS. Moreover, in a recent study Tomasino (2011), at Lincoln University, used GC-MS to successfully characterize regional differences of New Zealand Pinot noir.

GC-O analysis, which needed a human assessor to sniff the effluent as it emerged from the GC column, has been used to determine the aroma compounds in Pinot noir wine and juice previously. Ross (2010) used GC-O to analyse juice samples of Pinot noir from a reflective mulch trial. This was the only experience registered in which grape juice was analysed using this technique before now at Lincoln University. In that experiment, the author identified eleven aroma compounds from juice evaluated by two panellists. Most of the analyses registered in the bibliography corresponded to data reported from Pinot noir wines. Miranda-Lopez et al. (1992) showed the results of an experiment in which they used GC-O to evaluate the aromatic profile of Pinot noir wine from different maturities. They noted that only a few of the peaks detected by the device were sniffed by the panellist, which was normal in this kind of analysis due to the sensory thresholds of the different aroma compounds and the sensitivity of the panellist to specific aromas (Polaskova et al. 2008). An important discovery was reported by Moio and Etievant (1995) who used this analytical tool to analyse four important odorants in Pinot noir wines from Burgundy. They described for the first time ethyl anthranilate, ethyl cinnamate, ethyl 2,3-dihydrocinnamate and methyl anthranilate in this kind of wine. Results were obtained using GC-MS and confirmed through GC-O analysis. Oregon Pinot noir wine was analysed by Fang and Qian (2005a) using this technique. They determined that Pinot noir wine aroma was determined by a complex combination of many compounds.

Chapter 3

Materials and methods

3.1 Field experiment

The field trial was carried out in row 50 of the Lincoln University vineyard, which had been planted in 1999 with Pinot noir 777 on rootstock 3309. It comprise 68 vines planted at 1.2 metre spacing and distributed in 14 bays with five plants each, minus the first and the last bays which had four plants each. The rows are orientated north – south. The vineyard has a drip irrigation system and the field management was conventional with application of agrochemicals to control insects, diseases and weeds. Canopy management included defoliation in the fruit zone after fruit set, by trimming and removal of secondary shoots to enhance canopy interior illumination.

The experiment comprised four treatments with four replicates each distributed in four blocks arranged in a randomized complete block design. The treatments were: undisturbed soil (CON), clear glass mulch (CG), brown glass mulch (BG) and mixed glass (which is primarily green and brown) mulch (MG) (Figure 3.1).

The glass particles used in this experiment had a diameter between 1 – 3 mm. The brown and clear glass were supplied by Naked Waste Ltd (Cromwell, New Zealand) and the mixed glass was donated by EcoCentral (Christchurch, New Zealand).

Season 2011-2012 was characterised by cool temperatures during the growing season and the low accumulation of Growing Degree Days (GGD) base 10 °C. Thus, the monthly average temperatures during season 2011-2012 were: October 2011, 11.2°C; November 2011, 12.6 °C; December 2011, 14.9 °C; January 2012, 15.6 °C; February 2012, 15.6 °C; March 2012, 13.5 °C; and April 2012, 12.2 °C. The accumulation of GDD base 10 °C was 776 for this season (G. L. Creasy, personal communication).



Figure 3. 1 – Different treatments. Clockwise from top left: undisturbed soil, clear glass, brown glass and mixed glass.

The trial was established on January 24th 2012. Each treatment, except for the control, consisted in putting a piece of non-woven weedmat of 90 cm width and 2.4 m length underneath the vines. Two cuts with scissors were made on the weedmat from one side up to the centre, exactly in front of each plant position, such that the mat covered the space between the plants, plus a buffer zone. Approximately 12.5 kg of crushed glass were then poured over each section to make a layer of glass as continuous as possible (Figure 3.2). It is important to note that the weedmat was essentially covered by the mulch, and because of its porosity did not interfere the infiltration of water into the ground. Therefore, there was no reason to expect an effect of the weedmat on the final results and no weedmat control was included. A stone was put on each corner of the mat to prevent wind from lifting up the mat.

Once a week until harvest, leaves and weeds that had fallen on the mulch were removed from the surface of the glass to try to keep them clean and avoid possible disturbances of treatment effects because of a decrease in reflection.



Figure 3. 2 – Clear glass applied in the field trial.

3.2 Measurements of mulch reflection under field conditions

Reflection from mulches and undisturbed soil was measured between 3:45 and 4:45 pm during the afternoon of the days 9th, 23th and 29th of March 2012, which were three of the few clear sunny days in that season in Lincoln.

The device used in this trial for measuring the reflection of the mulches was a Bentham DM 150BC Double Monochromator spectroradiometer with motorised 1800 gratings, end window photomultiplier tube detector and cosine corrected Teflon diffuser (Bentham Instruments Limited, Reading, UK), which has a range of measurement between 250 and 800 nm. It was mounted on a table specially adapted to support the different parts of the spectroradiometer

(Figure 3.3). Two extension cords of approximately 50 m each were used to energize the device.



Figure 3. 3 – Bentham spectroradiometer reading reflection data

To take the measurements, the sensor was pointed down towards the mulch at fruiting wire height (approximately 90 cm from the ground) to measure the amount of reflected light received by the bunches on a clear sunny day. The orientation of the rows (north – south) did not allow recording of the data for more than one hour per day, because of the shade of the row located on the west side of the row 50 came quickly over the mulches affecting the reflection. For this reason, two blocks were measured the first day, one during the second day and one the last day. Each measurement took around seven minutes and the spectroradiometer was programmed to measure at 3 nm intervals. Data were collected by a laptop using the software BenWin+ Version 2.1.6.0 DLL v2.2.39.0 (32 bit). Finalising each block (four replications) a measurement from the direct sunlight was taken to compare it with the light reflected for the mulches. Data collected were recorded in miliwatts per square metre per nanometer ($\text{mW m}^{-2} \text{nm}^{-1}$)

A series of analyses was carried out to understand the different reflective properties of the mulches and their possible effect on Pinot noir vine performance. The first step was determining the different ranges of the spectrum to be evaluated. Specific points of the spectrum were analysed separately because of their known effect on different physiological processes of the plants. UV-B (280-315 nm), which affected the synthesis of colour compounds (Smart and Robinson 1991), PAR (400-700 nm), which was directly related with photosynthesis (Taiz and Zeiger 2010) and R:FR ratio for its involvement with phytochrome response (Taiz and Zeiger 2010) was analysed from the data.

Because of the 3 nm resolution of the Bentham data, UV-B was considered as values between 280 and 316 nm, and the range between 319 and 397 nm was taken as UV-A. To determine PAR, values between 400 and 700 nm were taken, and to calculate the R:FR ratio the equation proposed by Taiz and Zeiger (2010) was adapted to:

$$R/FR = \frac{\text{Average of photon fluence rate between 655 nm and 667 nm}}{\text{Average of photon fluence rate between 724 nm and 736 nm}}$$

In addition, the range between 703 – 799 nm was considered IR.

Additionally, PAR was divided in sub-groups to evaluate the differences amongst different ranges of the visible spectrum. The different PAR ranges analysed for their effects in this research are listed in Table 3.1:

Table 3. 1 – Different ranges of PAR considered in the research

<i>Colour</i>	<i>Range</i>
Violet	379 – 448 nm
Blue	451 – 475 nm
Cyan	478 – 493 nm
Green	496 – 568 nm
Yellow	571 – 589 nm
Orange	592 – 619 nm
Red	622 – 748 nm

The percentage of incident energy was calculated using, as a reference, the readings taken directly from the sun. To calculate this, a simple equation was used for each datum of the reading:

$$\text{Percentage of incident energy} = (\text{Data from mulch} * 100) / \text{Data from sun light}$$

3.2.1 Statistical analysis

Data of the field experiment were analysed using one sample Student's t-test with a confidence level of 95% to test the equality of means between the control group and the treatments in the different levels. To do this, IBM SPSS Statistics version 19 (SPSS Inc., an IBM Company ©) was used.

The R:FR ratio was analysed using analysis of variance (ANOVA) with IBM SPSS Statistics version 19 (SPSS Inc., an IBM Company ©). Significant differences were calculated at the 95% confidence interval using Tukey HSD (Honestly Significant Difference) test.

3.3 Model system

Reflection from the different treatments was evaluated under controlled conditions: the “model system”. It was set up in a Conviron GR48 walk-in plant growth chamber (Conviron, Canada), located in the Hilgendorf building of Lincoln University. This chamber provided the dark space necessary to implement the experiment. The experiment was carried out on 28th of June 2012.

About 3 kg of glass from each treatment of the field trial was collected to be evaluated in the model system. This glass was cleaned using hot water and caustic soda at 2% to remove the dust and some fungus that grew up over the glass during the winter. Soil was also collected to be used as a control. Soil and glass were put into trays of 21 cm width, 30 cm length and 4 cm depth which were covered with a piece of the same non-woven weedmat used in the field trial in the bottom to evaluate the reflection in similar conditions of the experiment at the vineyard. A cardboard box was constructed for this experiment that consisted of pieces of cardboard painted with black matt paint to avoid any external reflection which could alter the results.

The box was assembled using the frame of a table as support structure (Figure 3.4). An extra steel frame was used to support the whole structure.

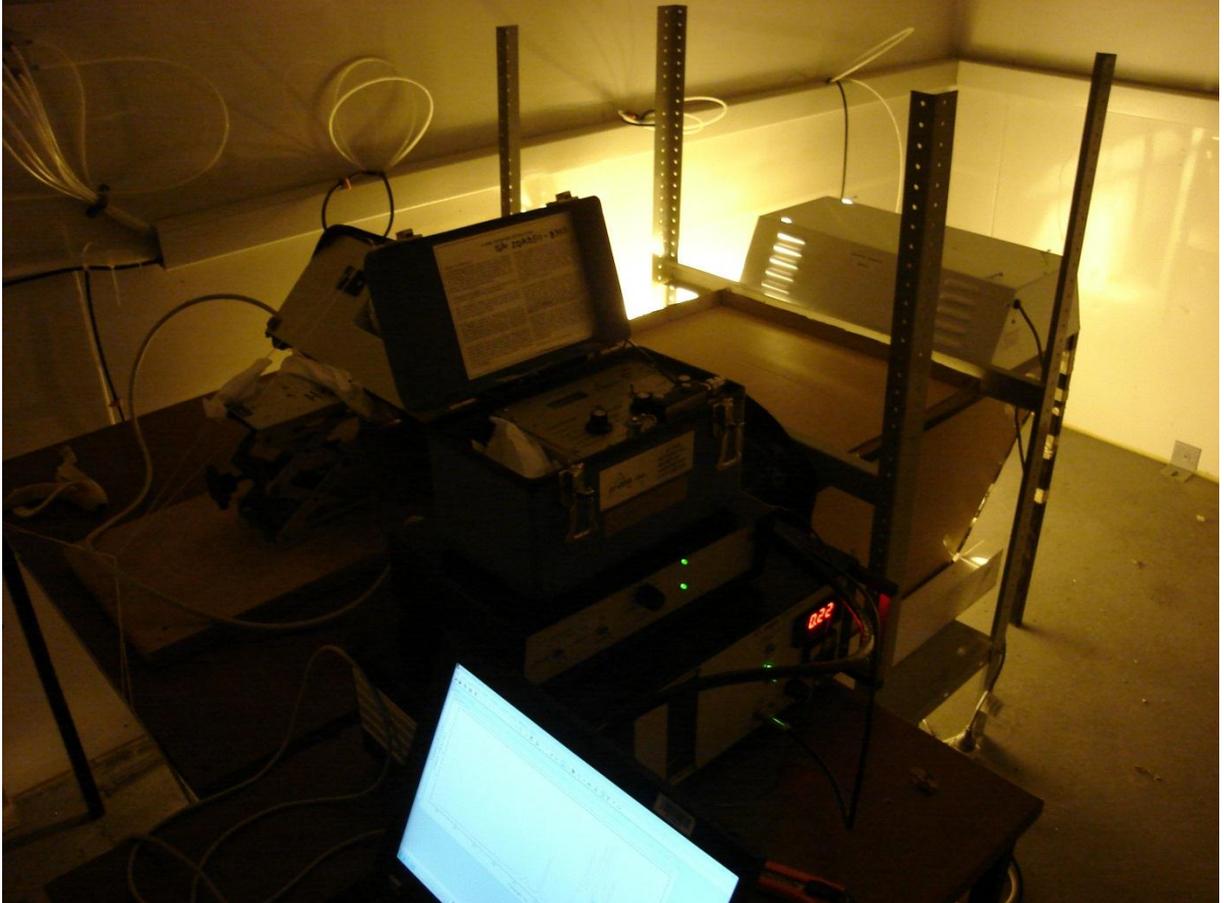


Figure 3. 4 – Model system set up in the growth chamber

The same Bentham spectroradiometer that was used in the field trial was set up on one side of the box with the sensor pointing down in a 45 degrees angle. At the opposite end of the box a high pressure sodium lamp, model Master SON-T PIA Plus 600W/220 E40 1SL (Philips, The Netherlands) was installed as the source of light. This lamp is able to generate light in the range between 400 – 700 nm (Figure 3.5) (Philips n.d).

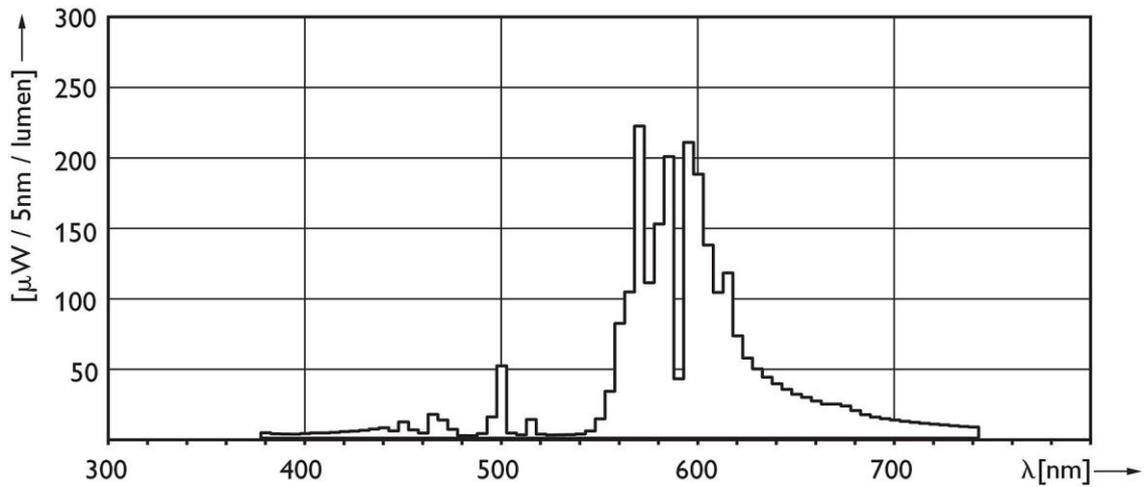


Figure 3. 5 – Photometric data of the bulb used in the trial (MASTER SON-T PIA Plus 600W/220 E40 1SL)

Prior to the measurements, a portable radiometer LI 188B Integrating quantum/radiometer/photometer (Li – Cor Inc., USA) was used to check the bulb performance in real time. Once the reading in this device was stable, the reflection of the different treatments was measured.

First, the total radiation generated for the lamp was evaluated using a mirror (40 cm X 30 cm), which was put in the bottom of the cardboard box and the measurement from it used as a reference to evaluate the incident energy reflected as in the field trial. Each tray was put in the bottom of the cardboard box twice (the mirror as well). As the same 3 nm scale was used, the measurements took the same time of the field trial (about seven minutes each). A piece of painted cardboard was put in the top of the structure to minimize any light leakage from the system.

Because of the bulb capacity generating light just in the PAR range and the first part of the IR range, PAR was considered as the range between 400 – 700nm and IR between 703 – 799 nm. Moreover, as in the field trial, PAR was separated in different sub-ranges using exactly the same parameters.

3.3.1 Statistical analysis

Data from the model system were analysed using one sample Student’s t-test with a confidence level of 95% to test the equality of means between the control group and the

treatments in different levels. To do this, IBM SPSS Statistics version 19 (SPSS Inc., an IBM Company ©) was used.

The R:FR ratio was analysed using analysis of variance (ANOVA) with IBM SPSS Statistics version 19 (SPSS Inc., an IBM Company ©). Significant differences were calculated at the 95% confidence interval using Tukey HSD (Honestly Significant Difference) test.

3.4 Harvest and sampling process

Grapes were harvested on May 1st 2012. For harvest criteria, a cluster was defined as having 25 or more berries. Clusters with fewer than 25 berries or those damaged by birds were harvested, but separated from those used to obtain the juice samples. The number of clusters in each treatment was also recorded. Fruit was collected from the space of approximately 1.2 m between two plants, immediately above the mulch treatment.

Samples were processed in the Lincoln University winery on the same day as harvest. First, grapes were weighed to determine the average cluster weight. The potential crop was calculated by multiplying the average weight of cluster per replicate by the total number of clusters per replicate, including those rejected. The fruit were then crushed by hand in the same buckets they were harvested into and stems were separated, leaving only pulp, seeds and skins. One kilogram of grapes per replication was used to obtain the juice samples. This mix was introduced in a one litre bottle for a cold maceration with the aim of extraction aroma compounds from the skins. Sulphur dioxide (5 % w/v) (0.525 ml), was added to each bottle to bring the concentration to 35 ppm of SO₂ in the juice, to avoid fermentation or oxidation. Nitrogen gas was added into the headspace of the bottles to help prevent oxidation (Figure 3.6).

Bottles were maintained at 2°C in a cool room for about three days. They were shaken two times per day with the aim of enhancing the contact between juice and skins and, thus, aroma compound extraction. On May 4th, free run juice was obtained from the bottles using a strainer to separate solids from the liquid. Juice was put in 30 mL capacity vials and then frozen in a -20°C freezer. One sample (approximately 20 ml) per replication was maintained at around 2°C for more two days for chemical analysis.



Figure 3. 6 – Addition of nitrogen gas

On May 7th, after cold maceration, sugar content (Brix degrees), pH and titratable acidity (TA) were determined.

Total soluble solids, measured as degrees Brix ($^{\circ}$ Brix), were determined using an Atago Pocket refractometer PAL – 1 (Atago Inc., U.S.A) whose measurement range is from 0 to 53%. The procedure consisted in putting three drops (around 0.3 ml) onto the prism surface using a disposable pipette.

The pH was measured using a Suntex pH/mV/temperature meter SP-701 (Suntext, Taiwan) with a Eutech Instruments probe (Eutech Instruments Pte Ltd, Singapore). Before analysis, the pH meter was calibrated using two standard buffer solutions of pH 4.0 and 7.0. To measure pH of the juice, the electrode was immersed in the juice until the pH value was stabilised on the digital display. Finalising each measurement, the electrode was rinsed with distilled water and dried with a tissue.

Titrateable acidity (TA) was measured using the method described by Iland et al. (2000), titrating to pH 8.2.. The titrateable acidity was determined using this equation:

$$\text{Titrateable acidity (g/L as H}_2\text{T)} = 0.75 \times \text{Titre value (mL)}$$

3.4.1 Statistical analysis

The results of the grape harvest were analysed using analysis of variance (ANOVA) with IBM SPSS Statistics version 19 (SPSS Inc., an IBM Company ©). Significant differences were calculated at the 95% confidence interval using Tukey HSD (Honestly Significant Difference) test.

3.5 GC-O analysis and aroma identification

Juice samples were analysed using a Gas chromatography – olfactometry (GC-O) analysis to identify the aroma compounds present in each sample. This analysis was carried out between June 16th and July 16th 2012. To do this, the method used by Ross (2010) was followed. Analysis was via a Shimadzu GC-2010 (Shimadzu Corporation Kyoto, Japan) gas chromatograph equipped with an Rtx-Wax 30 m x 0.32 mm i.d x 1 µm 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 analysis time was 74.33 minutes using helium as a carrier gas at a flow rate of 24.6 cm/sec in the linear velocity mode. The olfactory port and FID ratio were programmed to split the GC effluent 1:1. Data of each run was registered by Shimadzu's GC Solution software Version 2.41.

The temperature of the injector and the FID detector were set at 250°C. The GC column oven temperature started at 40°C for three minutes. It was then raised to 118°C at 5°C/min. After that, temperature increased to 148°C at 2°C/min and, finally, it was increased to 240°C at 5°C/min. In the final part of the run, the temperature was maintained at 240°C to complete the 74.33 minutes runtime. The olfactory port was held at 200°C and humidified air was added to the nose cone to improve the comfort of the person responsible for the aroma identification.

Samples were taken out of the freezer and defrosted at room temperature for at least 12 hours. In the morning of the run day, the samples were prepared using the following protocol: 15 ml of juice were pipetted and put into a 40 ml screw cap amber vial with PTFE/Silicone septa (Supelco Bellefonte PA, USA, through Sigma – Aldrich, Australia). Then, 3 g of sodium chloride (NaCl) was added and the vials tightly capped. The vials were left at room temperature (20°C) for about 1 hour and 45 minutes prior to analysis. The samples were then put in a water bath at 50°C for 10 minutes to allow for temperature equilibration. Following this, a 2 cm long Stableflex DVB/CAR/PDMS combination SPME fibre of 50/30 µm thickness, 24 gauge (Supelco Bellefonte PA, USA, through Sigma – Aldrich, Australia) was introduced in the vial and the fibre was exposed to the headspace of the vial for a period of 40 minutes at 50°C. Having completed the time, the fibre was retracted and carried to the GC-O device where it was put in the injection port to start the analysis. A fibre conditioning run was done every day prior to the analysis to clean the fibre of any external aroma, which could alter the results. The fibre was retracted back into its holder and removed from the injection port about ten minutes after the run had started.

The person responsible for sniffing the aromas coming through the olfactory port of the device was the author of this thesis. Results were recorded in two ways. The intensity of the aroma and the duration were registered using a Velleman K8055 USB Interface Board with a potentiometer attached to a desktop computer. This device has a dial attached to the potentiometer, which the panellist turned to express how intense the aroma seemed in real time. The software K855TWusb 2.4 (<http://www.wenzlaff.de/twusb.html>) was used to register the data. The software was programmed to register data each 200 milliseconds. Aroma descriptors were recorded using a USB Logitech microphone attached to the GC-O computer. Voice records were registered with Audacity 2.0.1 (<http://audacity.sourceforge.net/>), which is open source software.

The peak height and peak area from the data obtained by the panellist, and peak area from the FID were not registered in any specific measurement unit, because they were expressed only in relative numbers to enable a comparison to be made between them. Only height peak from the FID was registered in microvolts (mv).

Samples were randomised using a three-numbered code. The panellist sniffed the samples in duplicate (32 samples total). He did not know what treatment he was sniffing until the end of the experiment.

3.5.1 Aroma identification

The aromas that came through the olfactory port were sniffed by the panellist for each sample. At the moment when each aroma was detected, the panellist linked the time with the known aroma. The intensity and the duration of the aroma were recorded using the devices described in the section before. Simultaneously, the GC-O registered FID data to be analysed later.

Once all the samples were sniffed by the panellist, two kinds of analyses were conducted to try to identify each of the aroma compound detected on the GC-O runs. One gas chromatography mass-spectrometry (GC-MS) run and two GC-O runs were carried out using a mix of standards and alkane mix.

The GC-MS analysis was performed with the aim of identifying the aroma compound identified on the GC-O runs more precisely. It was done on a Shimadzu GC-MS-QP2010 gas chromatograph-mass spectrometer (Shimadzu Corporation Kyoto, Japan) equipped with a CTC Combi-Pal autosampler (CTC-Analytics AG, Switzerland) using Version 2.50 of Shimadzu's GC-MS solution data acquisition software. The method was the same as that Tomasino (2011) used to analyse samples of Pinot noir wine, except for the amount of liquid used to fill the vial, which, in this case, was 7.5 ml of juice and 3 g of NaCl. As in the GC-O runs, the sample was held at room temperature (20°C) for about 1 hour and 45 minutes prior to analysis to emulate the same conditions as the rest of the runs.

The second part of this method consisted in two GC-O runs to try to establish a relationship between the retention time on the juice run and the retention time of a specific peak in the run using standards. The first of them was a run performed in exactly the same conditions of the juice runs, but using just three drops of an alkane standard solution C₈ – C₂₀ (Sigma-Aldrich Co., St Louis, USA) (J. Breitmeyer, personal communication). The chromatogram of this analysis was used as a reference to identify possible odorant related with the aroma sniffed by the panellist. The alkane mix contained standards which were measured with the GC-O device, using the same method of the grape juice runs, indicating the retention time of all the compounds contained in the solution. Each compound of the mix, in this case 11, has a known

Kovats index of between 800 and 1800. These indices, after the analysis, allowed the rejection or acceptance of the possible relationship between the descriptor and the aroma compounds consulted in the flavornet database (J. Breitmeyer, personal communication).

The second run was carried out to examine if some of the aromas sniffed during the runs had the same aroma characteristics and retention time compared with the standards. The run was undertaken under the same conditions as the juice runs. The standard solution contained the next standards: 0.0052 g of β – ionone, 0.0058 g of phenylethyl alcohol, 0.0055 g of cis – 3 – hexen – 1 – ol, 0.0053 g of benzaldehyde and 0.0049 g of hexanal. These were diluted in a solution of 90% of deionised water and 10% of HPLC-grade ethanol to reach 5 ppm. Fifteen ml of this solution were used for a GC-O run using the same protocol as the juice samples and the GC effluent was sniffed by the panellist to establish a possible relationship between the juice aromas and the standards.

3.5.2 Statistical analysis and analysis of data

The criterion used to select which peak corresponds to a specific aroma was as follows: The panellist made an association between the perception during the run of closest relationship between this aroma and a known aroma. Under this criterion, seven basic aromas were chosen as the most repeated during the runs. Thus, cut grass, mushrooms, fresh peas, violets, cooked potato, roses and blackberry were the descriptors used to identify each aroma sniffed by the panellist.

First, the data were divided in four different groups to perform the statistical analyses. The peak area registered by the FID was the first group. The second group was the area of the peak recorded by the panellist, which was calculated as the sum of all the values registered during the period of time that the aroma was sniffed. The third group was formed for the height of the peak recorded from the FID and the last group was composed for the height of the peak registered by the panellist.

The results of each group of data were analysed using analysis of variance (ANOVA) with IBM SPSS Statistics version 19 (SPSS Inc., an IBM Company ©). Significant differences were calculated at the 95% confidence interval using Fisher LSD (Least Significant Difference) test. After that the correlation between the FID data and the panellist were analysed. Data for peak areas were statistically analysed by calculating a Pearson's correlation coefficient between these two sets. The same analysis was used to calculate the correlation

coefficient between the peak heights from the FID and panellist. IBM SPSS Statistics version 19 (SPSS Inc., an IBM Company ©) was used to do these statistical analysis. A two-tailed test of significance was used to establish statistically significant differences between the groups.

Finally, using the results of the GC-MS run, the alkane standard solution analysed on the GC-O and the analysis using the mix of aroma standards, possible relationships between the panellist's perception and the most likely odorant were made. This was based in information extracted from Flavornet Web page (Acree and Arn n.d) and the LRI and odour database (Mottram n.d).

Chapter 4

Results and discussion

4.1 Mulch reflectance under field conditions

Data collected on 9th, 23th and 29th of March 2012 were analysed in different ways to try to establish differences between the treatments. The first analysis was to calculate the percentage of incident energy reflected for the mulches with respect to the sunlight (Figure 4.1). The graph represents the averages of each treatment compared with direct sunlight.

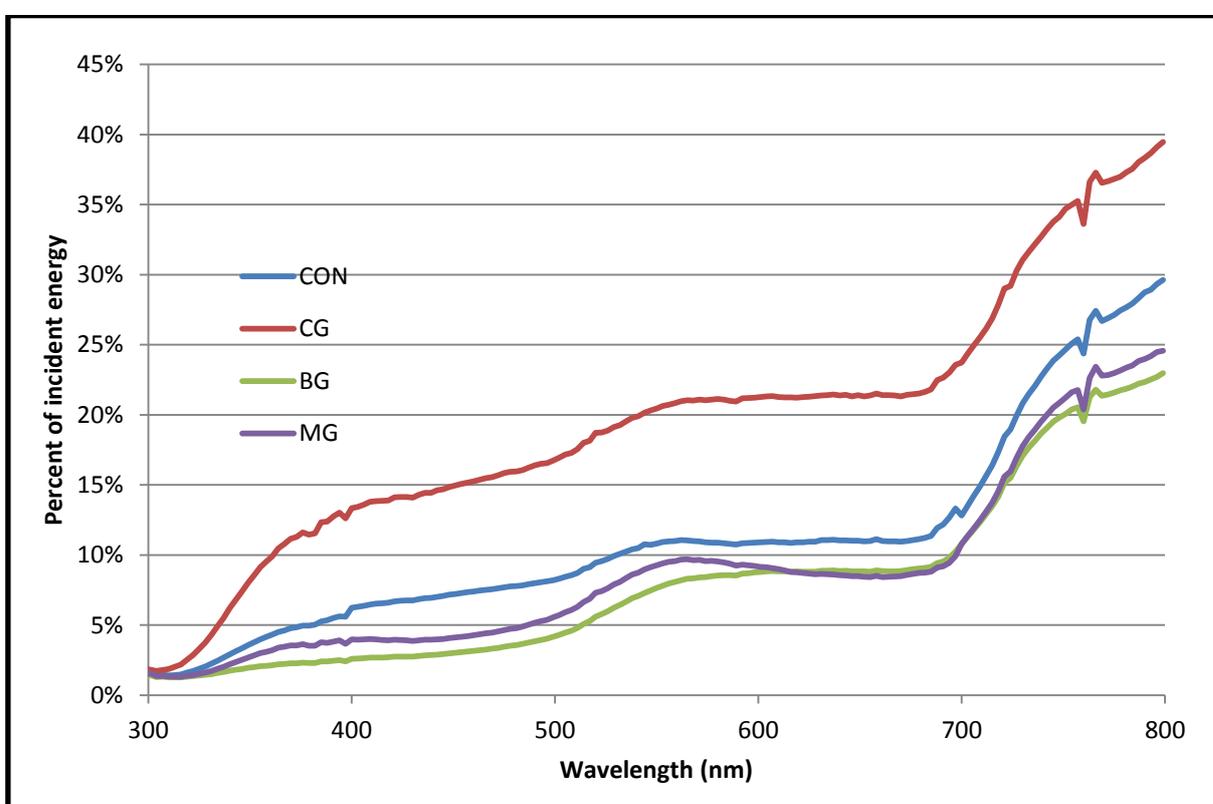


Figure 4. 1 – Energy reflected for the different treatments respect to the direct sunlight.

Overall, clear glass (CG) reflected more of the sun's radiation compared with the other treatments over the whole range measured. The percentage of incident energy always increased when the wavelength rose, except in the range between 550 and 700 nm, which was relatively stable. Although the control treatment (CON) reflected more of the sun's radiation than mixed (MG) and brown glass (BG), all have a similar performance.

A similar experience was reported by Leal (2007) who evaluated the incident energy reflected from mussel shells used as a reflective mulch compared with a bare soil control. In that experiment, the researcher reported more incident energy reflected for mussel shells compared with bare soil in UV-A, UV-B and PAR ranges.

Reflectance was also evaluated for each treatment itself. To show the differences amongst the treatments, a graph was made using the data from Block Number 1 in the field trial measurements (Figure 4.2)

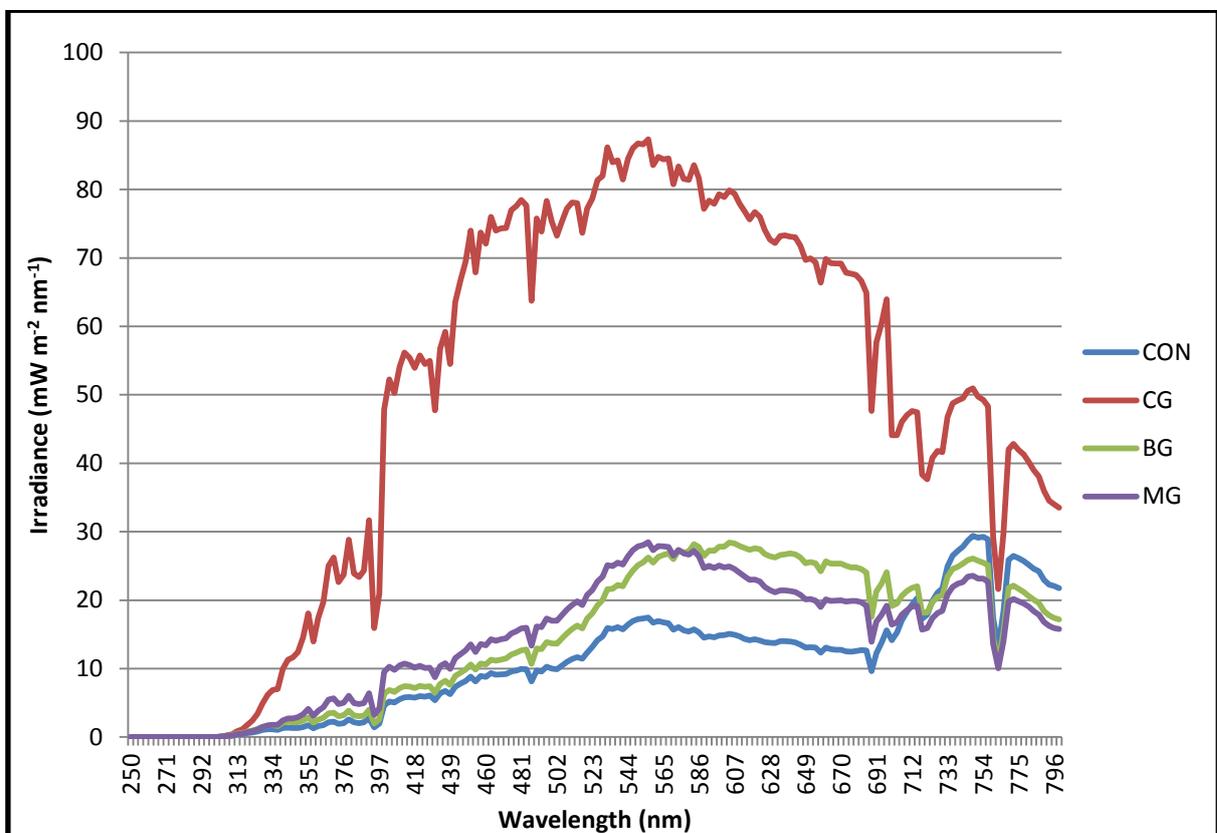


Figure 4. 2 – Reflectance under field conditions.

Reflectance of clear glass was always greater than the other treatments. The bigger difference was in the visible range (PAR), which in some parts of the range reflected more than four times the radiation reflected for the other treatments.

Ross (2010) tested clear and green glass, and mussel shells as reflective mulches. She obtained similar results to this experiment in which clear glass reflected high amounts of UV, PAR and IR compared with the other treatments.

Measurements taken from the field trial were divided in different ranges (UV-B, UV-A, PAR and IR) to try to understand its possible effect on grapevine physiology (Table 4.1). Results were also statistically analysed using one sample Student's t-test.

Table 4. 1 – Reflectance of the different treatments in specific ranges. Each mean was calculated from four replicates. Results expressed in miliwatts per square metre per nanometer ($\text{mW m}^{-2} \text{nm}^{-1}$). Values within columns followed by different letters indicate significant differences in a Student's t-test at $p < 0.05$

<i>Treatment</i>	<i>UV-B</i>	<i>UV-A</i>	<i>PAR</i>	<i>IR</i>
CON	0.08 b	1.69 d	12.32 d	21.08 b
CG	0.16 a	12.73 a	60.98 a	39.84 a
BG	0.09 b	2.04 c	16.78 c	20.35 b
MG	0.09 b	3.24 b	18.83 b	20.63 b

There were statistical differences amongst the treatments for UV-B reflection. Clear glass reflected about the double the UV-B compared with the undisturbed soil (Table 4.1). UV-B may affect directly grape characteristics such as flavonoid and anthocyanin accumulation, and it may also modify plant responses to fungal diseases (Núñez-Olivera et al. 2006). UV-B can also induce changes in gene expression, despite the fact that no photoreceptor molecule that can perceive the UV-B signal has been identified (Jordan 2002). It was difficult to find specific information about UV-B reflection in reflective mulch experiments in the past. As mentioned earlier, Leal (2007) reported large amounts of UV-B reflected for mussel shells used as a reflective mulch. Ross (2010) also concluded that clear glass, in a reflective mulches trial, reflected 83% more UV than the control. Moreover, Coventry et al., (2005) indicated that an aluminised polyethylene sheeting reflected well in the UV range while bare soil did not. However, Keller (2010) stated that too much UV-B radiation can inhibit

anthocyanin production or induce degradation. For this reason, extra amounts of UV-B reflected into the fruit zone must be treated carefully. Jordan and Callow (1996) also reported that relatively high levels of UV-B radiation can have specific effects upon plants such as: inhibition of photosynthesis, changes in assimilate partitioning and effects upon flowering and reproduction.

When the UV-A range was analysed, clear glass showed statistically significant differences with respect to the other treatments. Thus, UV-A was about four times higher than in the mixed glass (the second most reflective) and about seven times more than the control. Although UV-A has been less studied, Price et al. (1995) and Castillo-Muñoz et al. (2007) indicated that flavonoid compounds have a strong absorbance in UV-A and this range of the spectrum was involved in their accumulation on grape skins. Leal (2007) also reported results specifically for UV-A. He indicated that mussel shells reflected 25 times more UV-A compared with the control. Downey et al. (2006) indicated that exposure to UV was shown to increase flavonol glucosides in plants. For this reason, extra amounts of UV-A radiation reflected by the mulches could have increased the flavonoid concentration in the grapes of this trial, but this was not investigated. As a reference, the extra amount of UV reflected by mussel shells in the experiment conducted by Leal (2007) showed no consistent differences in flavonoids concentration.

PAR was also analysed separately. In this case, as before, clear glass reflected the highest amount of light in this part of the spectrum. The bigger, almost 5-fold difference was between clear glass and the control. Although brown and green glasses were statistically different than the undisturbed soil, clear glass reflected about four times more PAR than either of them. Plant leaves are able to absorb only a part of the sunlight, mainly in the “visible spectrum” (Smart and Robinson 1991). This part of the spectrum is important for photosynthesis (Coventry et al. 2005, Keller 2010). There are many experiences reporting extra amounts of PAR from the use of reflective mulches. For example, in an experiment using an aluminised polyethylene sheeting, it reflected 40% of PAR in comparison with the non-mulched plot which reflected less than 10% (Coventry et al. 2005, Reynolds et al. 2008). Furthermore, Hostetler et al. (2007a) showed that a white geotextile mulch reflected significantly more sunlight than a black geotextile and the control treatment. In Iran, an aluminium reflective film was used as a reflective mulch in table grapes, with the results indicating that it increased

light intensity considerably (Jamshidian et al. 2010). Additionally, Sandler et al. (2009) in a trial which evaluated a silver aluminised reflective mulch and a thin layer of crushed quahog shells, both reflected more PAR into the fruiting zone compared with the control. Thus, extra PAR reflected by the mulches in this trial may have an effect on grapevine physiology. For example, PAR reflected into the fruit zone may influence cane maturation (Jackson 2008), inflorescence initiation (Jackson 2008, Keller 2010, Smart and Robinson 1991), grape maturation and the aromatic attributes of the fruit (Jackson 2008, Keller 2010). In this experiment, only the two last aspects were evaluated, showing the results that extra PAR reflected by the mulches did not affect °Brix, TA and pH, but did influence the aromatic profile of the grapes.

Analysing IR as a separate group indicated that there were significant differences between clear glass and the rest of the treatments. Clear glass reflected about two times more IR compared with the other treatments. The importance of IR is mainly focused on 730 nm, which is related with the R:FR ratio (Dokoozlian and Kliewer 1995a, Dokoozlian and Kliewer 1995b, Smart and Robinson 1991, Smart et al. 1988). The R:FR ratio has been evaluated in the past in different canopy manipulation systems with the aim of enhancing canopy interior illumination (Dokoozlian and Kliewer 1995a, Dokoozlian and Kliewer 1995b, Smart et al. 1988), but not for evaluating reflective mulches. There are few reports regarding R:FR ratio or IR evaluation. Thus, Witbooi (2008) tested the effect of soil surface colour and its possible effect on grape physiology. The author concluded that R:FR ratio was affected by soil surface colour, and reported that the grey treatment had the highest R:FR ratio, followed by the red and black treatments. Research conducted by Coventry et al. (2005), reported that the R:FR ratio was higher in the reflective mulch, but not statistically significantly so. Ross (2010) also obtained high values of R:FR ratio from the reflective mulches compared with the control. Clear glass and mussel shells reflected more than 60% of this parameter, compared with the control. Extra radiation reflected by the mulches in this trial, especially in 730 nm, may have had an influence on flowering (Jordan and Callow 1996), nitrate reductase activity (NRA), which regulated must K and pH (Smart et al. 1988), and yield (Taiz and Zeiger 2010), but only this last aspect was evaluated in this trial, showing that this was not influenced by the extra amounts of sun radiation reflected into the fruit zone. It may be necessary to undertake a long term experiment to check the real effect of this parameter on grapevine physiology.

The red:far red ratio was calculated separately using the formula proposed by Taiz and Zeiger (2010), adapted for this research. Results were illustrated in Figure 4.3.

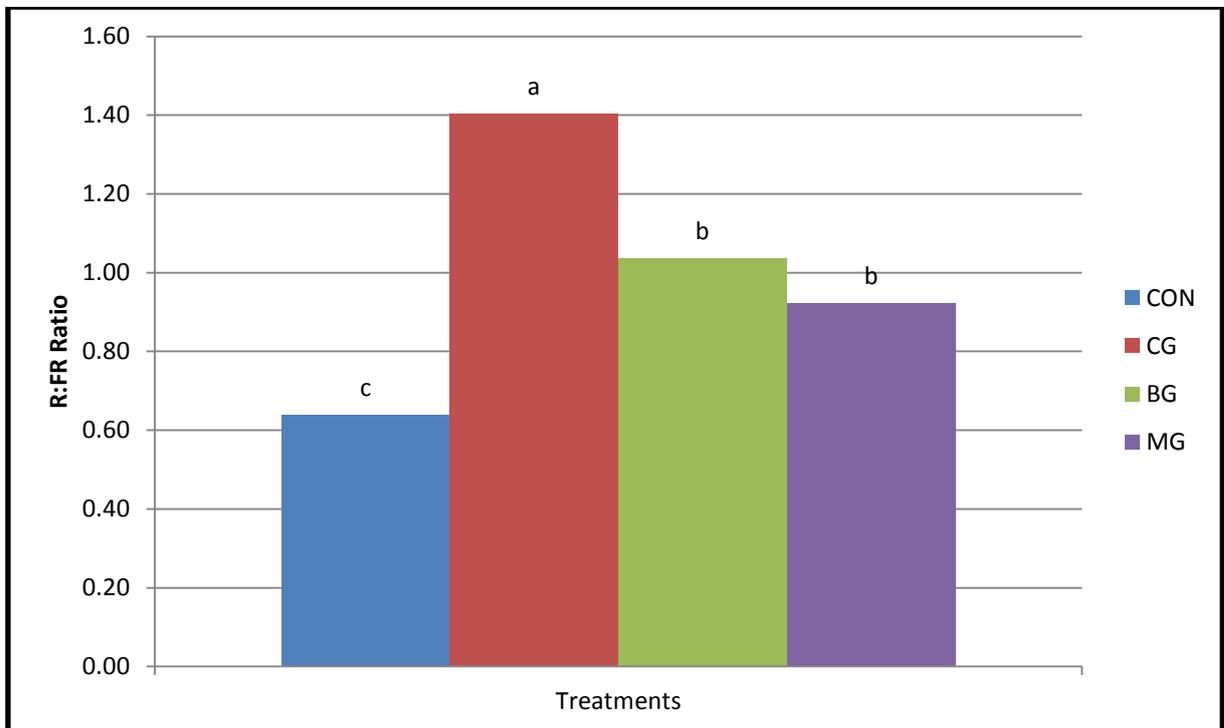


Figure 4. 3 – R:FR ratio under field conditions. Each mean was calculated from four values. Different letters above the columns indicate a statistically significant difference in an ANOVA at $p < 0.05$ using Tukey HSD test.

There were statistical differences among the treatments when Red:Far ratio was analysed. Clear glass reflected statistically more Red:Far ratio than the other treatments, followed by brown and mixed glass, which reflected statistically the same amount of radiation. These agreed with the results reported by Ross (2010), who concluded that clear glass reflected statistically more radiation than the control, but not statistically different than mussel shells treatment.

Additionally, the range between 380 and 750 nm was divided in different colour ranges (Table 4.2). Results were statistically analysed using a one sample Student's t-test. No similar analyses were found when conducting the literature review for this study.

Table 4. 2 – Reflectance in the visible range divided in different colours. Each mean was calculated from four replicates. Results expressed in miliwatts per square metre per nanometer ($\text{mW m}^{-2} \text{nm}^{-1}$). Values within columns followed by different letters indicate significant differences in a Student's t-test at $p < 0.05$

<i>Treatment</i>	<i>Violet</i>	<i>Blue</i>	<i>Cyan</i>	<i>Green</i>	<i>Yellow</i>	<i>Orange</i>	<i>Red</i>
CON	5.25 c	9.43 c	9.95 d	14.56 d	15.74 d	15.05 c	16.18 d
CG	38.47 a	61.42 a	62.37 a	69.29 a	69.60 a	66.75 a	52.31 a
BG	5.35 c	9.48 c	10.64 c	18.04 c	23.63 c	24.00 b	21.33 b
MG	8.44 b	13.50 b	14.99 b	23.02 b	25.96 b	23.71 b	20.05 c

Overall, clear glass was statistically more reflective than the other treatments in all the ranges studied. When the ranges were analysed individually, there were differences in magnitudes.

Thus, the reflectance in the violet range, which is absorbed by flavonoids (Jordan and Callow 1996), was about seven times more than the control in the clear glass treatment. For the blue range, which is mainly absorbed by chlorophyll b (Keller 2010, Taiz and Zeiger 2010), this difference was around six times. The same tendency was followed in the cyan range, where the radiation reflected by the clear glass was six times higher. These differences were less marked for green light, which is reflected by chlorophyll (Hopkins 1999), where clear glass was almost five times more reflective, but only about three times more compared with mixed glass. Differences among treatments for yellow light, which together with orange light are absorbed by carotenoids (Hopkins 1999, Taiz and Zeiger 2010), were registered in the same magnitude as green light. Orange light was reflected about four times more for clear glass compared with the control but, in this case, brown glass and mixed glass reflected statistically the same amount of light in this range. Finally, red light, which is absorbed by chlorophyll b (Keller 2010, Taiz and Zeiger 2010) and phytochromes (Jordan and Callow 1996, Sharrock 2008), was more reflected by clear glass, registering more than double with respect to brown glass, the second most reflective.

4.2 Model system

The experiment was carried out on 28th of June 2012. In this trial reflection of the different mulches were evaluated under controlled conditions. First, percentage of incident light was calculated using as a reference the radiation reflected by a mirror, which was considered to be 100% (Figure 4.4).

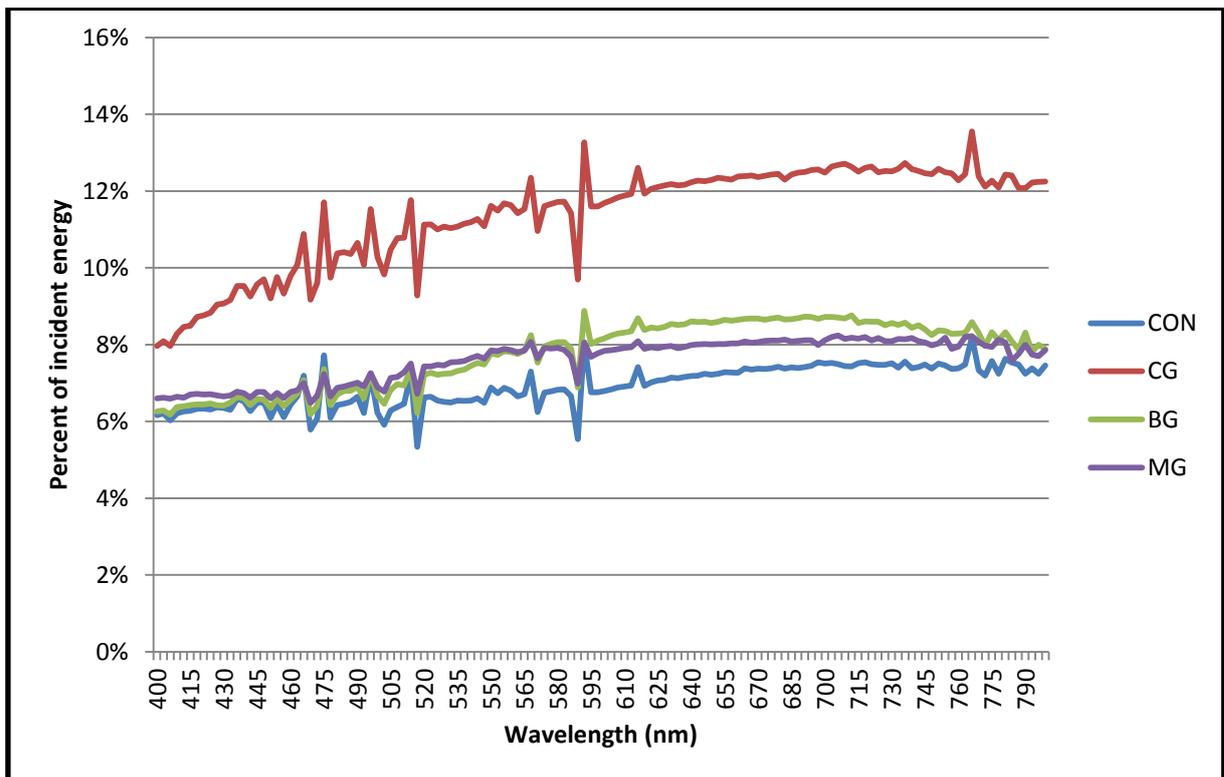


Figure 4. 4 – Incident energy reflected for each treatment under controlled conditions.

As in the field trial, clear glass reflected the higher amount of incident energy. All other treatments gave a similar performance and did not show any remarkable differences at all.

The reflectance of each treatment itself was also compared amongst the treatments (Figure 4.5). The graph shows the tendency of clear glass being the most reflective treatment, especially in the PAR part of the spectrum. Differences between the other treatments were imperceptible from the graph.

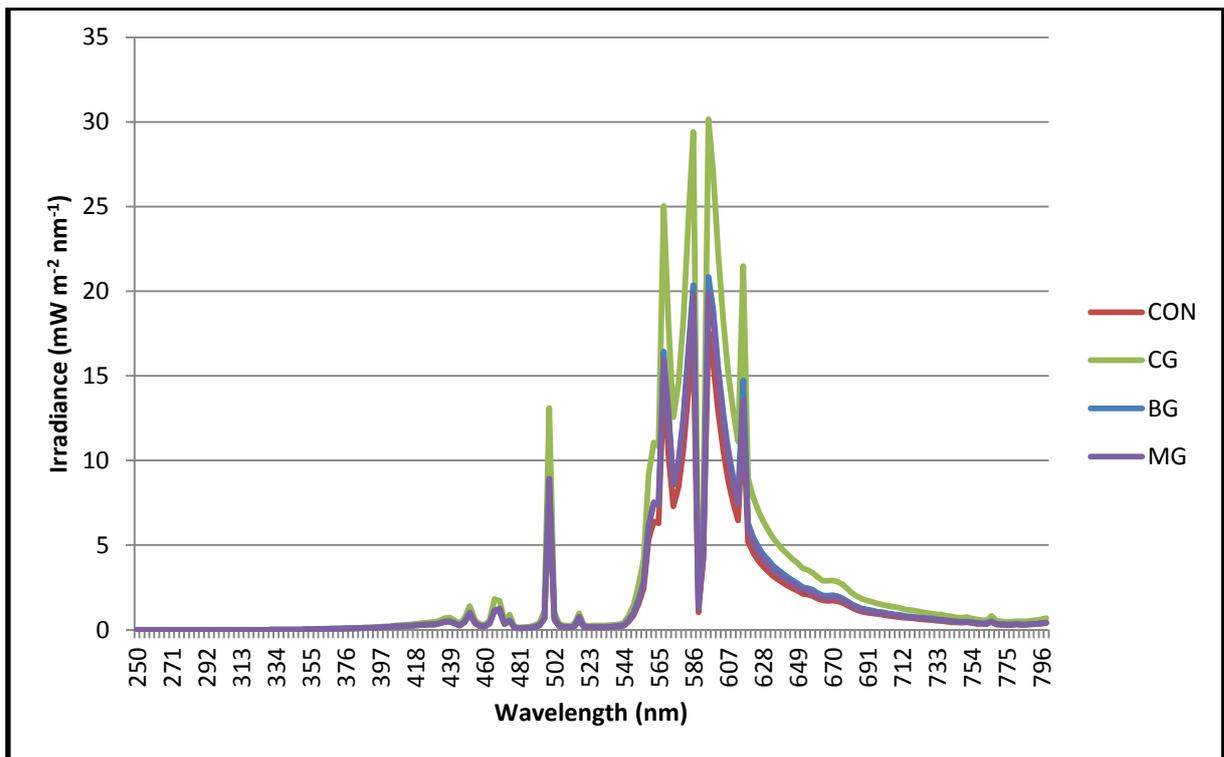


Figure 4. 5 – Reflectance under controlled environmental conditions

Reflection was also statistically analysed. In this case, just two ranges of wavelengths were evaluated, PAR and IR, because the technical characteristics of the lamp used as a source of light was able to generate light only in these ranges (Table 4.3).

Table 4. 3 – Reflection of the different mulches under controlled conditions. Each mean was calculated from four replicates. Results expressed in miliwatts per square metre per nanometer ($\text{mW m}^{-2} \text{nm}^{-1}$). Values within columns followed by different letters indicate significant differences in a Student’s t-test at $p < 0.05$

<i>Treatment</i>	<i>PAR</i>	<i>IR</i>
CON	2.86 b	0.48 c
CG	4.86 a	0.81 a
BG	3.35 b	0.54 b
MG	3.24 b	0.52 c

As in the case of the measurements from the field trial, clear glass was the statistically more reflective mulch. Nevertheless, in the PAR part of the spectrum, control, brown glass, and mixed glass, reflected statistically the same amount of radiation. For the IR, there were no statistical differences between the control and mixed glass.

The red:far red ratio was also calculated under controlled conditions. The results were illustrated in Figure 4.6.

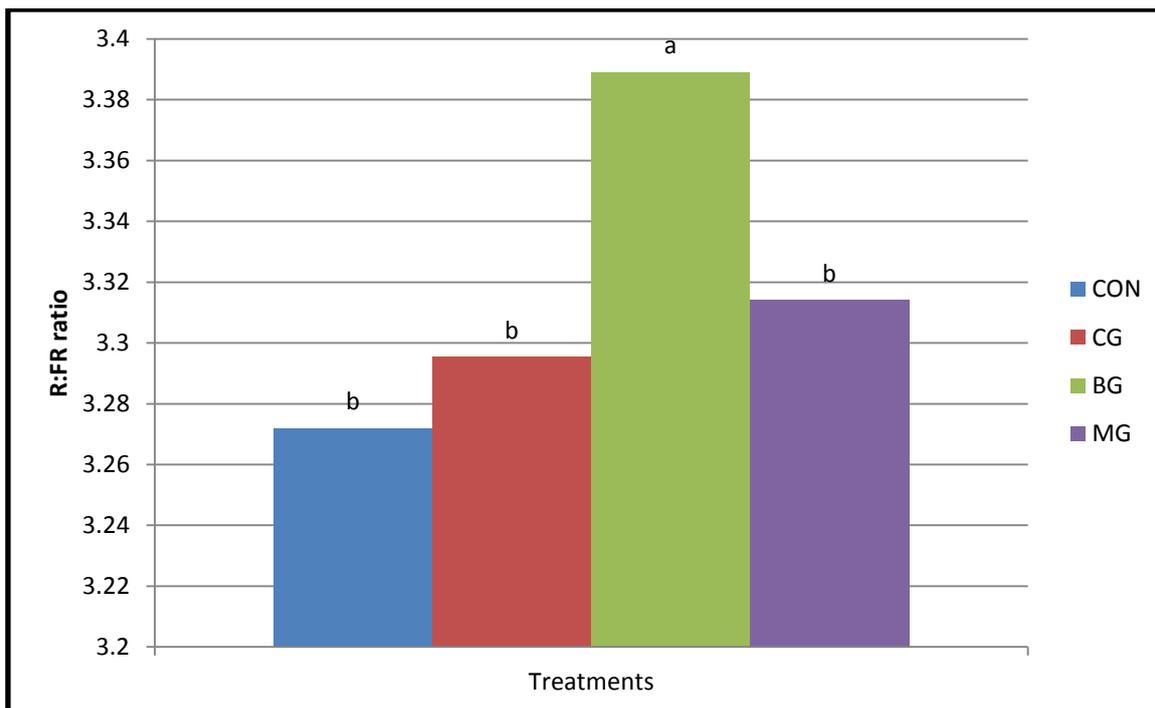


Figure 4. 6 – R:FR ratio under controlled conditions. Each mean was calculated from two values. Different letters above the columns indicate a statistically significant difference in an ANOVA at $p < 0.05$ using Tukey HSD test.

In this case, the red:far red ratio reflected by brown glass was the highest registered under controlled conditions. The rest of the treatments showed a similar performance among them. Although there were statistical differences among the treatments, the differences between the highest value and the lowest value was only 0.12, which may suggest an influence from the intensity of the light generated by the lamp used in this part of the experiment on the results obtained for this parameter. No similar experience was found in the literature so comparisons could not be made.

The range between 380 and 750 nm was divided in different colour ranges, following the same method as the field trial (Table 4.4).

Table 4. 4 - Reflectance in the visible range divided in different colours under controlled conditions. Each mean was calculated from four replicates. Results expressed in miliwatts per square metre per nanometer ($\text{mW m}^{-2} \text{nm}^{-1}$). Values within columns followed by different letters indicate significant differences in a Student's t-test at $p < 0.05$

<i>Treatment</i>	<i>Violet</i>	<i>Blue</i>	<i>Cyan</i>	<i>Green</i>	<i>Yellow</i>	<i>Orange</i>	<i>Red</i>
CON	0.24 b	0.59 b	0.14 b	1.97 b	9.95 b	10.07 b	1.58 c
CG	0.34 a	0.90 a	0.23 a	3.34 a	17.14 a	17.26 a	2.67 a
BG	0.25 b	0.60 b	0.15 b	2.23 b	11.81 b	12.01 b	1.86 b
MG	0.26 b	0.62 b	0.15 b	2.24 b	11.64 b	11.42 b	1.74 c

Overall, as in the case of the field trial, the clear glass treatment reflected more radiation in all the ranges. However, under controlled conditions, in all the ranges except for red light, the control, brown glass, and mixed glass were statistically equal.

These results were different from those found in the field trial. Under field conditions, clear glass showed a higher reflection in all the ranges, as in this experiment, but in the model system brown and mixed glass were statistically equal with respect to the control. This demonstrated that there are external factors such as presence of weeds, shadows of the other rows, soil moisture, stones or any other factor which can affect the reflection of the mulches under field conditions. For example, weeds inter-rows, because of the chlorophyll capacity of reflecting green light (Hopkins 1999, Taiz and Zeiger 2010), clearly affected the amount of light registered in the field trial in this part of the spectrum. Shadows from the other rows and also the shadow of the row itself had an influence in the reflection of the mulches. This was increased because of the orientation north-south of this vineyard, which allowed the mulches to reflect sun radiation only for short periods during the day. In the morning, the shadow of the row located to the east of the row used in this trial shaded the mulches. Then, near to noon, the own row shadow affected the reflection of the mulches, and in the afternoon, the shadow of the row located to the west of this row did the same. For the control, the soil moisture changed the soil colour. Thus, wet soil usually has a black or brown colour, and dry

soil commonly presents a clear-brown to white colour, likely changing its reflection capacity. All of these factors could have had an influence on the results of this experiment. For example, because of the difference of 20 days between the first and the last measurements of radiation under field conditions, the conditions changed over this period. Soil moisture was different on the three days when radiation was measured. The same happened with the weed presence, which especially in March, under warm conditions, was higher during the last day of the measurements. The angle of the sun also changed over the 20 days with respect to the horizon line. This angle is lower close to the autumn, affecting the period when the mulches were shaded by the other rows, and by the mulched row itself.

4.3 Yield components and grape composition

4.3.1 Yield components

The fruit was harvested on May 1, 2012 and number of clusters, cluster weight and potential crop were determined at that time. There were no statistically different results, however (Table 4.5). It is important to note that mulches had no important effects on these parameters, likely because they were established after fruit set during 2011 - 2012 season. For this reason, mulches did not have any influence in the induction phase, which happened approximately 18 months before the harvest date of this trial.

Table 4. 5 – Yield components of the fruit from the mulch trial. Each mean was calculated from four values. Fruit harvested in May 1, 2012. No significant differences were found

Treatments	Number of clusters	Cluster weight (kg)	Potential crop (kg/replicate)
CON	25.8	0.064	1.610
CG	30.3	0.059	1.806
BG	28.5	0.059	1.685
MG	30.3	0.059	1.795

There were no statistical differences among the treatments for number of clusters. Ross (2010) obtained similar results evaluating crushed glass and mussel shells as reflective mulches. Moreover, Agnew et al. (2002) found similar results in an trial conducted in Marlborough, where the researchers did not find consistent cluster number effects among the different mulch treatments. In that experiment there were also no statistical differences on juice °Brix, titratable acidity and pH. The first experiment was established in December 2005 and it was carried out for one season. The second trial was set up in January 1999 and it consisted of three seasons work.

Cluster weight was not affected by mulches. Agnew et al. (2002) reported slight differences on yield in a three year trial using mulches, but it could be attributed to the use of animal manure in the mulch mix rather than the radiation reflected from them. Also, Hostetler et al. (2007b) evaluated the effect of composted bark mulch, two reflective (white and black) geotextile mulches and soil cultivation and their effects on Pinot noir yield components. They found that although white geotextile mulch yielded more total crop per vine than the other treatments, it was not statistically different. Similar results were obtained by Reynolds et al. (2008) who tested the effect of reflective mulches on Riesling yield components. They also reported no statistical differences among the treatments.

The potential crop was also not influenced by the use of reflective mulches. Among the documents checked to write this thesis, only Ross (2010) reported potential crop results. In this experiment, the author concluded that despite there being differences among the treatments when this parameter was evaluated, they were not statistically different.

4.3.2 Grape composition

The grapes harvested on May 1, 2012, were chemically analysed for pH and titratable acidity. The total soluble solids content (measured as °Brix) was also determined. Data were statistically analysed using an ANOVA. Overall, just small differences were found in total soluble solids concentration, pH and titratable acidity amongst the treatments, providing evidence of the small effect mulches had on these parameters under the conditions of this trial (Table 4.6).

Table 4. 6 - Brix degrees (°Brix), pH and titratable acidity (TA) from the different treatments of the trial. Each mean was calculated from four values. Fruit harvested in May 1, 2012. No significant differences were found.

Treatments	°Brix	pH	TA (g/L)
CON	24.1	3.6	9.6
CG	24.0	3.6	9.4
BG	23.5	3.5	9.8
MG	24.2	3.6	9.9

The fact that there were no differences among the treatments in these parameters, could suggest that the extra light reflected into the canopy for the mulches was not enough to produce significant changes in the metabolic pathways related with sugar accumulation, pH and acids synthesis (photosynthesis and respiration, mainly) (Keller 2010, Taiz and Zeiger 2010). It was important to highlight that this was a particularly cool season, which was characterised by the low accumulation of GDD (776, base 10°C). This had a direct impact on grape ripening, as evidenced in the later date of harvest in respect to the normal date for the zone (data not shown). Although this was a later harvest, the appearance and turgor of the berries showed no evidence of dehydration or other problems. However, the late harvest could have had an effect on the aromatic profile of the grapes when compared to other studies. Previous research has demonstrated the effect of grape maturity on the concentration of aroma compounds in Pinot noir (Fang and Qian 2006). This highlights the influence of the harvest date on the aromatic profile of this variety. This could be investigated in a further research.

In this experiment, there were no statistically significant differences in Brix. This was similar to other studies; for example, Sandler et al. (2009), who tested a white reflective woven material, crushed quahog shells and a silver aluminised reflective mulch; they also did not find differences amongst the treatments when they measured °Brix, pH and TA in different grape varieties. Agnew et al. (2002) did not record significant effects from using different waste stream components as mulch in Sauvignon blanc. Nevertheless, Coventry et al. (2005) reported that aluminised polyethylene sheeting enhanced the °Brix in Cabernet franc compared with the control. Also, in a research conducted by Jamshidian et al. (2010), an

aluminium reflective film resulted in significant differences in Brix from berries treated by this film compared with the control treatment.

The pH of the juice did not vary among the treatments. It was important to know that pH was measured from the juice after cold maceration, which influenced the final values in this experiment. The contact between juice and skins increased the proton concentration (especially K) of the juice, raising the pH values in all the treatments with respect to a value of 2.9 measured from a berry sample the week before the harvest (data not shown).

The literature indicated that the use of mulches in viticulture did not alter the pH of the grapes in some cases and other authors showed differences. Thus, Hostetler et al. (2007b) reported that in an experiment in which they used composted bark, reflective (white and black) geotextile mulches and mechanical soil cultivation to evaluate their effect on Pinot noir, there were negligible differences in pH among the treatments. The same group tested the same mulches in Cabernet franc and found similar results. Additionally, in New Zealand, different mulches have been evaluated that showed no influences on the pH of the grapes. Thus, Leal (2007) and Ross (2010) concluded that in the first case, mussel shells, and in the second one mussel shells and crushed glass used as mulches did not affect the pH of the grapes. However, Chan et al. (2010) reported significant increments in pH of grapes harvested in an experiment in which the authors evaluated the use of composted mulch in Australia. In a similar experience, Chan and Fahey (2011) tested the effect of composted mulch application on soil and wine potassium status. They found that as a consequence of the high levels of potassium in the soil as a result of mulch application, the pH of the grapes increased significantly. These effects were not observed in grapes used in this experiment, because crushed glass, being an inorganic material, was not able to supply nutrients to the grapevines.

Mulches also did not have an effect on TA. In the past, results from different experiments showed similar results. Data reported by Reynolds et al. (2008) indicated that there were no differences in TA between mulched and non-mulched treatments. Furthermore, Witbooi (2008) indicated that plastic mulches painted black, red and grey did not affect TA in a trial conducted in South Africa. Similar results have been obtained in investigating different materials as a reflective mulch around the world, demonstrating that TA was definitely not

related to the material used for this purpose (Agnew et al. 2002, Hostetler et al. 2007b, Leal 2007, Ross 2010, Sandler et al. 2009). Grape acidity is brought about mainly by tartaric acid (about 90%), malic acid and other organic acids (Dai et al. 2011, Keller 2010). Because the synthesis of most of them was directly related with the amount and the quality of the light received by the plants (mainly PAR) (Dai et al. 2011), this factor may have an effect on acid accumulation, but data obtained in this trial indicated that light reflected by the mulches did not have an effect on this parameter. Maybe in a long term trial, or in different climatic conditions, this would be different.

4.4 GC-O analysis and aroma identification

The experiment was carried out between June 16th and July 16th 2012. Many aromas were detected by the panellist during the runs in all the 32 samples analysed, but only the seven most frequent aromas were selected to do the analysis (Table 4.7). Retention time of each aroma was based on results from the clear glass treatment, replicate 3.

Table 4. 7 – Most frequent aromas described by the panellist during the experiment, and their retention times (minutes)

<i>Retention time</i>	<i>Descriptor</i>
12.92	Cut grass
17.56	Mushrooms
21.65	Fresh peas
23.62	Violets
25.65	Cooked potato
29.01	Roses
41.26	Blackberry

It is important to note that each descriptor was based on the panellist's perception at the moment he sniffed them, and it just corresponded to an association between the aroma and a known aroma.

Data from each of these peaks were analysed in three different ways. First, area of the peaks was compared amongst the treatments separately. Then, the heights of the peaks were analysed in the same way and, finally, the frequencies that each peak was reported during each run was also ranked. Data obtained from the device and the panellist were analysed separately.

As was indicated in the materials and methods section, the peak height and peak area from the data obtained by the panellist, and peak area from the FID were not registered in any specific measurement unit, because they were expressed only in relative numbers to enable a comparison to be made between them. Only height peak from the FID was registered in microvolts (mv).

4.4.1 FID data from the GC-O device

The area of the peaks registered for the device were statistically analysed using ANOVA (Table 4.8). Results indicated that there were statistical differences among the treatments only in the aroma described as cooked potato.

Table 4. 8 – Area of the peak (divided by 1,000) obtained from the GC-O device. Each mean was calculated from eight values. Different letters in the same column indicate significant differences (ANOVA at $p < 0.05$, Fisher's LSD test) between treatments.

<i>Treatment</i>	<i>Cut grass</i>	<i>Mushrooms</i>	<i>Fresh peas</i>	<i>Violets</i>	<i>Cooked potato</i>	<i>Roses</i>	<i>Blackberry</i>
CON	1,956 a	1,215 a	5,957 a	1,321 a	51 a	410 a	194 a
CG	2,127 a	995 a	5,592 a	1,239 a	5 b	459 a	222 a
BG	3,265 a	1,242 a	6,193 a	1,388 a	16 ab	590 a	266 a
MG	1,743 a	955 a	7,220 a	1,363 a	22 ab	434 a	280 a

In this case, the area of this peak was about ten times less in the clear glass treatment compared with the control. Peaks of this aroma were also smaller than the control for brown and mixed glass, evidence of the effect mulches had on this specific aroma compound.

Another way in which data from the GC-O device were analysed was a comparison of the peaks height between treatments (Table 4.9). For this parameter, aromas described as cut grass and cooked potato showed statistical differences among the treatments.

Table 4. 9 - Height of the peak (divided by 1,000) obtained from the GC-O device. Each mean was calculated from eight values. Results expressed in microvolts (mv). Different letters in the same column indicate significant differences (ANOVA at $p < 0.05$, Fisher's LSD test) between treatments.

<i>Treatment</i>	<i>Cut grass</i>	<i>Mushrooms</i>	<i>Fresh peas</i>	<i>Violets</i>	<i>Cooked potato</i>	<i>Roses</i>	<i>Blackberry</i>
CON	106 b	82 a	382 a	82 a	3 a	23 a	14 a
CG	110 b	65 a	362 a	77 a	0.6 b	25 a	17 a
BG	170 a	83 a	406 a	88 a	1.6 ab	33 a	20 a
MG	90 c	62 a	465 a	84 a	2.3 ab	24 a	22 a

The highest value for cut grass height peak was obtained from the brown glass treatment. It was almost double that of the mixed glass, the smallest value. The control and clear glass showed values that were statistically equal but greater than for the mixed glass.

For the cooked potato aroma, the tendency was exactly the same for peak area, where the lower value was obtained from clear glass, and the greater from the control. Brown and mixed glass also obtained smaller values compared with the control, showing once again the influence of the treatment on this parameter.

Additionally, frequencies which each aroma descriptor was present in the runs were registered (Table 4.10). Overall, cut grass, mushrooms, fresh peas, and violets were detected 100% of

the time for the device, while cooked potato, roses, and blackberry were not present in 100% of the runs.

Table 4. 10 – Frequencies of the different aroma descriptors in data collected from the device.

<i>Treatment</i>	<i>Cut grass</i>	<i>Mushrooms</i>	<i>Fresh peas</i>	<i>Violets</i>	<i>Cooked potato</i>	<i>Roses</i>	<i>Blackberry</i>
CON	8	8	8	8	5	7	7
CG	8	8	8	8	2	8	7
BG	8	8	8	8	5	8	8
MG	8	8	8	8	5	8	8

Cooked potato was present only in the 25% of the samples analysed from the clear glass treatment. For the other three treatments, it was present in the 62.5% of the cases. Moreover, the device was able to detect the rose aroma only in one sample from the control treatment. Furthermore, the aroma described as blackberry was not detected in two samples, one in the control and one in the clear glass treatment, while it was present in the 100% of the samples of brown and mixed glass.

Using this method to express the result is an interesting form to compare the FID capacity of detection with the panellist's perception, because sometimes the FID is able to detect a compound present in the samples, but the panellist was not able to sniff this. The opposite situation was also likely. This point will be discussed in the next section. Also, this could be a good way to define the aroma complexity of the samples, as aromas are important themselves, but the combination of them is even more important, especially in wine production. This must be investigated in the future.

4.4.2 Data from the panellist

Data registered by the panellist were analysed in the same way as data from the FID. Thus, the area of the peak of each descriptor was statistically analysed using ANOVA (Table 4.11). For this parameter, statistical differences were found in the rose and blackberry aromas. The rest of the aroma descriptors did not show statistical differences.

Table 4. 11 - Area of the peak obtained by the panellist. Each mean was calculated from eight values. Different letters in the same column indicate significant differences (ANOVA at $p < 0.05$, Fisher's LSD test) between treatments.

<i>Treatment</i>	<i>Cut grass</i>	<i>Mushrooms</i>	<i>Fresh peas</i>	<i>Violets</i>	<i>Cooked potato</i>	<i>Roses</i>	<i>Blackberry</i>
CON	5,501 a	727 a	350 a	133 a	2,685 a	970 b	853 c
CG	4,492 a	569 a	866 a	878 a	1,832 a	1,973 a	2,369 a
BG	4,964 a	939 a	1576 a	438 a	1,696 a	750 c	1,291 b
MG	3,892 a	625 a	703 a	291 a	3,057 a	677 c	851 c

In the case of the aroma described as roses, a larger area was obtained in the clear glass treatment, which was almost double that of the control treatment. Interestingly, the lower value was not obtained in the control treatment, but in mixed glass. Blackberry aroma showed the maximum value in the clear glass treatment, which was almost three times more than the control treatment. Mixed glass had statistically the same area as the control.

The peak heights were also statistically analysed (Table 4.12), with significant differences found for violet, rose and blackberry aromas.

Table 4. 12 - Height of the peak obtained by the panellist. Each mean was calculated from eight values. Different letters in the same column indicate significant differences (ANOVA at $p < 0.05$, Fisher's LSD test) between treatments.

<i>Treatment</i>	<i>Cut grass</i>	<i>Mushrooms</i>	<i>Fresh peas</i>	<i>Violets</i>	<i>Cooked potato</i>	<i>Roses</i>	<i>Blackberry</i>
CON	56.3 a	19.4 a	9 a	3 b	33.9 a	26.3 b	16.7 b
CG	52.3 a	16 a	20 a	17.7 a	32.3 a	36.5 a	32.5 a
BG	48.5 a	20.3 a	18.8 a	4.3 ab	27.8 a	18.5 c	23.7 ab
MG	48.1 a	17 a	18.3 a	9.8 ab	35.3 a	19 c	19.7 b

Thus, the largest height peak was from clear glass treatment for violet, rose, and blackberry aromas, whose differences were significantly different. The violet aroma was almost six times more intense in samples from the clear glass treatment, compared with the control. For rose aroma, it was about two times more than the lowest one, brown glass. Also, for blackberry the difference between the lower and the higher treatments was almost a factor of two.

It was important to know that the averages in Table 4.12 corresponded only to the averages of the maximum values (one per peak) obtained during the runs, and it may be not the most accurate point of comparison between treatments. This was because in this way of analysing the data, the attention is focused only in one number per peak (the biggest one), and this number may be not be representative of the real peak intensity perceived by the panellist.

As in the case of data collected from the GC-O FID, frequencies were analysed for the GC-O data (Table 4.13). Data from this table were important themselves, but they were more important when they were compared with the frequencies registered from the FID, which demonstrated the important differences between panellist's perception and the FID detection capacity.

Table 4. 13 - Frequencies of the different aroma descriptors in data collected by the panellist.

<i>Treatment</i>	<i>Cut grass</i>	<i>Mushrooms</i>	<i>Fresh peas</i>	<i>Violets</i>	<i>Cooked potato</i>	<i>Roses</i>	<i>Blackberry</i>
CON	8	6	3	1	8	8	6
CG	8	6	5	5	8	8	8
BG	8	7	3	1	8	7	8
MG	8	5	5	3	8	7	8

Thus, the only aroma detected in 100% of the samples by both for the FID and the panellist was cut grass. Mushroom aroma was detected in all the samples by the FID, but was detected by the panellist only in some cases. For fresh peas there was the same tendency, but in this case, although this aroma was detected in all the samples from the FID, it was registered just three out of eight times by the panellist in the control and brown glass treatments. This demonstrated the importance of the threshold of perception, which was not always related with the detection capacity of the device via FID. For violets, it was even more extreme. This aroma was likely the most difficult to detect by the panellist, but it registered in the 100% of the samples by FID. In samples from the control and brown glass treatments, it was sniffed just one out of eight times by the panellist, followed for mixed glass (three times) and clear glass with five times. The opposite phenomenon happened with cooked potato aroma. For example, in clear glass treatment this aroma was detected just two times by FID, but it was sniffed in 100% of the samples by the panellist. In the other treatments it was reported five times by FID and eight times by the panellist. Rose and blackberry aromas, however, did not show big differences at all. This highlights the importance of the human nose in this analysis, which in some cases is more sensitive to specific aromas than the device.

4.4.3 Correlations between GC-O device sensibility and panellist's perception

Results of the device and the panellist were correlated using Pearson's correlation. First, the area of the peak was correlated between data obtained from the GC-O and the panellist (Table 4.14).

Table 4. 14 – Correlations between FID readings and panellist's perception for peak area data. Pearson's correlation coefficient was calculated at $p<0.05$ and $p<0.01$ using a two-tailed test.

<i>Descriptor</i>	<i>Correlation (r)</i>	<i>Significance</i>
Cut grass	0.370	0.037
Mushrooms	0.469	0.007
Fresh peas	0.135	0.462
Violets	-0.007	0.972
Cooked potato	0.571	0.001
Roses	0.109	0.553
Blackberry	0.310	0.085

The area of the peak for cut grass was significantly correlated ($p<0.05$) between the FID and panellist results. Data for the mushroom and cooked potato aromas were also significantly correlated ($p<0.01$). There were no significant correlations for any of the other aromas. Similar information was not found in the literature so a comparison was not able to be made with other experiences.

Correlations between the peak height by FID height and the panellist were also undertaken. Results showed that in this case, only the mushroom aroma had a statistically significant correlation ($p<0.01$) (Table 4.15).

Table 4. 15 - Correlations between FID and panellist's perception for peak height data. Pearson's correlation coefficient was calculated at $p < 0.05$ and $p < 0.01$ using a two-tailed test.

<i>Descriptor</i>	<i>Correlation (r)</i>	<i>Significance</i>
Cut grass	0.307	0.087
Mushrooms	0.494	0.004
Fresh peas	0.207	0.255
Violets	0.005	0.977
Cooked potato	0.291	0.106
Roses	0.005	0.976
Blackberry	0.152	0.407

4.4.4 Aroma identification

Three different methods were used to try to determine each specific aroma compound present in the juice samples used in this trial: one GC-MS run, one GC-O run using an alkane mix, and one GC-O run using standards.

Using these three techniques, only the cut grass and fresh pea descriptors were successfully identified. The rest were tentatively identified, with additional analyses being necessary to check if they really corresponded to the compounds described in Table 4.16. For some of them, more than one possible aroma compound was associated with the descriptor.

Table 4. 16 – Aroma compounds identified and tentatively identified associated to each aroma descriptor used by the panellist. Cut grass and fresh pea aromas were confirmed by GC-MS. Mushroom, violet, cooked potato, rose and blackberry aromas were tentatively identified.

<i>Descriptor used by panellist</i>	<i>Aroma compound identified using GC-MS</i>	<i>Aroma compound tentatively identified using GC-O</i>
Cut grass	hexanal	
Mushrooms		2-octanone
Fresh peas	1-hexanol	
Violets		(Z)-linalool oxide (E)-linalool oxide nerol oxide linalool oxide
Cooked potato		methional
Roses		linalool
Blackberry		β -ionone

Hexanal, which was described as grass aroma in the Flavornet database (Acree and Arn n.d), was checked in two ways, and in both cases the results were successful. First, the GC-MS analysis indicated the presence of hexanal at retention time 13.657 min, which was close to the 12.927 min registered for the aroma described as cut grass by the panellist. Also, the mix of standards used for a GC-O run contained hexanal, which was sniffed by the panellist to check for any correlation between the standards and the aroma perceived during the runs. The panellist reported that hexanal used in the standards smelled similar to the aroma described as cut grass from the samples.

A fresh peas aroma was related to the aroma compound called 1-hexanol, which was described as a green aroma in the Flavornet database (Acree and Arn n.d). This was also detected in the GC-MS analysis at a retention time 21.51 min, which was very close to 21.654 min registered during the GC-O runs. In previous experiences, Kemp (2010) and Ross (2010) reported hexanal and hexanol in Pinot noir wine and juice, respectively. Data obtained by Kemp (2010) indicated a similar retention time for both compounds with respect to the result in this trial. Hexanals and hexenols are formed by enzymatic cleavage of linoleic and linolenic acids, respectively (Montedoro and Bertuccioli, 1986, as cited in Dumont 1994). Aldehydes of C-6 chains are formed by enzymes during breaking of the grape cell (Dumont 1994), and they are rapidly converted to hexanol, a process that was accelerated in the presence of yeast (Hashizume and Samuta 1997). This indicated that the presence of both compounds (hexanal and 1-hexanol) in the juice samples of this trial could be not a direct effect of the mulches on the grape composition, but mulches may have a direct effect on the linoleic and linolenic acids, which were the substrate for hexanal and hexanol synthesis. This needed to be investigated more deeply.

There were many reasons why the retention time of an eluting compound can change run by run. For example, FID indicated as the retention time the top of the peak, which can vary depending on the amount of the compound registered. Thus, large peaks displayed a later retention time than a small peak of the same compound. Both peaks started eluting at the same time. However, the larger peak continued to rise while the smaller peak had already reached its peak maximum and was returning to the baseline. The top of a peak was deemed to be the retention time. Also, the “human” part of the process is important as well. The injection used with the GC-O is manual, meaning the introduction of the SPME needle is immediately followed by a press of the START button. Any delays here, or differences in technique, will affect the retention time. Furthermore, the injection septa seals the injection port off from the open air and is punctured each time the SPME needle is inserted (injected). There is a limit to how many injections can be made before the septa begins to produce a noticeable leak. Manual injections are harder on the injection septa compared to an autosampler which tends to use the same punctured hole repeatedly. Small leaks cause a slight slowing in the column flow which results in a retention time change (longer times reported). The GC column also has an important role in retention time changes. Changes to the GC column occur when it is installed, removed and then installed again. Each time it was installed a small section was cut

off each end to make a new connection (nut and ferrule). This was performed in order to remove contamination left by caps used to seal the column ends when it was not in use. Changes in GC column length shorten retention times due to subtle changes in the column flow and pressure. Retention times can also shorten when GC columns deteriorate with age and use. Wax columns (as the used in this experiment) bleed their stationary phase and, over time, have a reduced capacity to hold onto eluting compounds. This reduction in performance results in peak broadening and shorter retention times (J. Breitmeyer, personal communication).

The method used to try and identify the rest of the aroma compounds was a little different, because GC-MS was not able to give precise results. The GC-O run using standards did help to confirm the presence of β -ionone, because it was included in the mix and it smelled similar to the aroma described from the grape juice. The GC-O run using an alkane mix solution was important to determine what compounds may correspond to each of the descriptors used by the panellist. The retention time of each alkane was related with the retention time of the aromas described by the panellist during the runs, and the Kovats index of each of them were considered as well. Thus, a combination between retention times, Kovats indices, and literature review were used to do the selection of the most possible aroma compounds related with each of the descriptors used by the panellist.

For the aroma described as mushroom by the panellist, octanone was selected as the most likely class of aroma compounds related to it. Octanone was described by Canuti et al. (2009) in Cabernet Sauvignon grapes. 3-octanone and 2-octanone, which are reported as having a mushroom and musty aroma, respectively (Mottram n.d), were the two most likely individual aroma compounds related with the aroma. Analysing the retention time reported by Canuti et al. (2009) using GC-MS, 3-octanone was registered at 14.13 min and 2-octanone at 17.39 min, the latter of which is very close to the 17.915 min retention time of the mushroom aroma described by the panellist. Both analyses were carried out using a wax column, which allowed the determining of a relationship between the retention time in both experiments. Despite this Ross (2010) concluded that the most possible aroma compound related with mushrooms aroma in that study was (E)-2-penten-1-ol, although it was not checked with additional analysis. The retention time indicated for this descriptor (24.29 min) by that experiment did not correspond with the retention time obtained for the aroma described as mushroom in this experiment. For this reason, (E)-2-penten-1-ol was rejected as the compound related to this aroma.

For the aromas described as rose and violet, linalool and its oxidised forms (*cis* and *trans*) were determined as the most likely aroma compounds related with the panellist's response during the runs. Nerol was also included in this group. Linalool, linalool oxide and nerol have been described by some authors in the past. Fang and Qian (2006) reported four monoterpene alcohols in Pinot noir wine from different grape maturities. Linalool, geraniol, nerol, and citronellol were quantified in that experiment. The same authors suggested that these compounds have been reported as being responsible for the characteristic floral aromas in grapes and wines. Moreover, linalool, trans-linalool oxide, and cis-linalool oxide were identified by Camara et al. (2006) in Malvazia wines. These compounds and nerol were also identified in red table grape cv. Muscat de Hambourg by Aubert et al. (2005). Terpenes are synthesized from acetyl-CoA or its glycolytic intermediates through the mevalonic acid pathway (Hopkins 1999, Taiz and Zeiger 2010). Their direct relationship with the respiration and photosynthesis process (Hopkins 1999), may suggest a direct influence of the extra radiation reflected from the mulches on their synthesis for the grapevines.

The aroma described as cooked potato could have come from two possible compounds: methionol and/or methional. Methionol was described by Fang and Qian (2005b) in Pinot noir wine. This compound that has been described as having a potato-like aroma (Acree and Arn n.d) was rejected as a possibility because, according to the Kovats index and the alkane mix run results, this compound should have been smelled after minute 35 during the run, which was not coincident with minute 25.659 when the panellist detected it. This time was when methional was expected to come out of the column. Also, methional was described precisely as cooked potato in the literature (Acree and Arn n.d, Falcão et al. 2008). For these reasons, the cooked potato-like aroma sniffed by the panellist was thought to be related to this compound. Note that this was the first time that methional has been suggested as occurring in Pinot noir juice. It has been described in other varieties in the past, however. For example, Augustyn et al. (1982) were the first to describe methional in Sauvignon blanc. In Brazil, Falcão et al. (2008) reported methional in Cabernet Sauvignon wines using GC-O, with the aroma of methional being described as like cooked potato, too. Methional is a derivative of methionine, which is related to ethylene production (Augustyn et al. 1982). Additional studies are necessary to establish the possible relationship between the use of reflective mulches and this aroma compound in Pinot noir grapes.

β – ionone was identified as being the most likely compound related with the blackberry aroma described by the panellist. Fang and Qian (2006) and, more recently, Kemp (2010) have described this as occurring in Pinot noir wines. Although it has not been related directly with blackberry aroma, it was described as berry, violet, and raspberry aroma (Acree and Arn n.d). For this reason, and also for the results obtained in the alkane mix run, the results indicated that this compound was the most likely odorant related to the blackberry aroma detected in the juices. Analysing the retention time of this compound during the runs, it was reported at 41.26 min. This was based in the alkane mix run results and it was the same as a compound with a Kovats index bigger than 1,800, which is the case with β -ionone (Kovats index 1920 in Flavornet). The retention time of this compound in the GC-O runs was also very close to the peaks registered during the standards mix run at 41.908, 42.481, and 43.468 min, which were described by the panellist as similar to the aroma sniffed during the juice runs. Unfortunately, it was impossible to determine which of the three peaks correspond to β -ionone, because phenylethyl alcohol came out from the FID almost together with β -ionone. This made it very difficult for the panellist to discriminate about which peak may correspond to each compound. Another run with only β -ionone would be necessary to determine the exact retention time of this compound under these experimental conditions. C₁₃-norisoprenoids, like β – ionone, are the product of chemical and enzymatic reactions and these breakdown products of carotenoids including compounds with the megastigmane structure *i.e.* the ionone and damascenone families, with oxygen at different positions as in β -ionone with the 20 alpha-keto group at C-9 and β -damascenone at C-7 (Kemp 2010, Mendes-Pinto 2009). β -Ionone was considered to be the primary product of β -carotene made by carotenoid cleavage dioxygenase activity (Mendes-Pinto 2009). The influence of light on carotenoid synthesis has been explained in the previous sections. This suggested a direct relationship between the extra radiation reflected by the mulches and the concentration of this aroma compound in the grapes used in this trial.

Chapter 5

Conclusions

Under field conditions, clear glass reflected more radiation than the other treatments in all the ranges evaluated. These differences in reflection were analysed for specific parts of the spectrum. Thus, clear glass reflected double the UV-B compared with the control. The reflection in UV-A was more than four times that of mixed glass and about seven times more than undisturbed soil. The readings for PAR were almost five times more than the control and, for IR, were almost double. When R:FR ratio was evaluated under field conditions, the ratio for clear glass was two times more than undisturbed soil for this parameter. Furthermore, PAR was divided in different “colours” to be evaluated separately. Violet, blue, cyan, green, yellow, orange, and red light were statistically analysed from data collected from the mulches. In all of these ranges, clear glass was more reflective compared with the other treatments. The differences in all the ranges were between five and seven times greater in clear glass compared with the control system, except for the range of red light, where the reflection of clear glass was about double in relation to brown glass.

The reflection of the mulches was also evaluated under controlled conditions, using growth chamber lamps as a source of PAR and IR. As was found in the field trial, clear glass was the most reflective treatment, but there were no statistical differences between brown and mixed glass compared with the undisturbed soil treatment. When PAR was analysed in the different ranges of colour, as in the field trial, clear glass maintained the same tendency, being the most reflective mulch in all the ranges evaluated.

Mulches did not influence yield components or traditional harvest parameters. The number of clusters, cluster weight, and potential crop were not altered by the use of reflective mulches. Also, parameters like °Brix, pH, and TA were not affected, being statistically equal in all the treatments.

Reflective mulches, however, had an influence on aromatic profiles of the grapes harvested in this experiment. Seven descriptors were selected for the comparisons between treatments

according to the panellist's perception during the analyses. The descriptors selected were: cut grass, mushroom, fresh peas, violet, cooked potato, rose, and blackberry. Data from the FID showed that clear glass influenced the intensity of the aroma described as cooked potato, which had a peak area almost ten times smaller than this aroma in the control treatment. Also, the peak height of this aroma was about five times lower in the clear glass treatment, compared with the control. The cut grass aroma was almost double in brown glass with respect to mixed glass. This reflected the influence of the different treatments on the aromatic profile measured by GC-O. Moreover, the panellist's perception reported statistical differences among the treatments. Thus, a greater peak area was registered in the clear glass treatment for the aroma described as rose, while this peak was smaller in mixed glass. For blackberry aroma, clear glass also registered a bigger peak area among the treatments. This treatment also influenced the peak height in the data registered by the panellist. Violet, rose, and blackberry aroma under clear glass showed the statistically largest peak height according with the panellist's perception. A Pearson's correlation was used to examine a relationship between the panellist's perception and data registered by the FID. There was a high correlation between both sets of data in the peak area for cut grass, mushroom, and cooked potato aroma. For peak height, only the mushroom aroma obtained a high correlation. The frequencies that each aroma registered by the FID and the panellist data were also analysed. This indicated the importance of the human nose in this analysis, because sometimes the GC-FID detected a specific aroma but the panellist was not able to. The opposite situation was also reported. For example, the aroma described as cooked potato in clear glass treatment was detected just two times by FID, but it was sniffed in 100% of the samples by the panellist.

The attempt to try to identify which aroma compound corresponded to each aroma described by the panellist was successful in two instances. Hexanal was the compound related to the aroma described as cut grass, and 1-hexanol to the aroma described as fresh peas. Both compounds were successfully identified using GC-MS analysis. The rest of the aromas were tentatively identified, with more specific analyses to check these results being necessary. Thus, for mushroom aroma, 2-octanone was described as the most likely compound related with this aroma in the samples analysed. The aroma described as violet by the panellist was associated to four different compounds: (Z)-linalool oxide, (E)-linalool oxide, nerol oxide, and linalool oxide. Cooked potato-like aroma was related to methional, which was described for the first time in Pinot noir juice. Linalool was associated with the aroma described as rose

by the panellist, and β -ionone was related to a blackberry-like aroma. These compounds need to be confirmed using specific analyses for each of them.

Despite reflective mulches, including crushed glass, being evaluated in the past in viticulture, this experiment contributed by increasing the understanding of the effect of the mulches on Pinot noir vine performance. The evaluation of the mulch reflection under field conditions was carried out using the Bentham spectroradiometer, which constituted a change in respect to the previous trials. Also, the analysis of the radiation reflected by the mulches separated in different ranges (UV-B, UV-A, PAR, and IR), and the separation of PAR in different ranges of colour, established a new way of analysing this kind of data. This allowed determining a relationship between different ranges of radiation and its possible effect on grapevine physiology. Another important contribution of this trial was the use of a lamp as source of light in the model system. Despite the limitations of this to generate light in the UV part of the spectrum, this demonstrated a good way to evaluate reflection under controlled conditions, because by using this method the reflection of the mulches could be evaluated independent of the weather conditions. In the laboratory work, this experiment checked new ways to analyse the data obtained by the panellist and the FID using GC-O analysis. The use of the frequencies that the aromas were perceived at by the panellist and the FID demonstrated the importance of the human nose in this analysis. The use of an alkane mix, a mix of standards, and a GC-MS run to try to determine which aroma compounds corresponded to each descriptor, also constituted a change in respect to trials in the past. Although all the technical resources necessary to determine each aroma compound were not available at the time when this experiment was carried out, this method was demonstrated as being a useful tool to determine the compounds responsible of each aroma that the panellist sniffed during the GC-O runs. A very important point to highlight was the fact that this trial was based on the analysis of grape juice. This method was useful to understand the real effect of extra amounts of radiation reflected by the mulches on the aromatic profile of the grapes. This was because when analysing the grape juice, the interference caused by compounds generated during the fermentation when wine was used to evaluate the effect of the mulches in this parameter, can be avoided. This also may constitute the base for further research to study the effect of reflective mulches on wine, which could contribute to understanding the real significance of the aroma differences in the juice. Finally, the use of reflective mulches in vineyards has been demonstrated as having an effect on soil and canopy environment (Leal 2007, Ross 2010), parameters that may be incorporated in further trials, which will help the understanding of the real effect of reflective mulches on wine quality.

Chapter 6

Further research

Although this experiment contributed important data for understanding reflective mulches in viticulture, many aspects of this needed to be investigated more deeply. Some aspects of the methods followed by Leal (2007) and Ross (2010) could be repeated in the future. For example, these authors evaluated the leaf chlorophyll content using a SPAD. Also, in these experiments aspects like soil temperature, soil moisture, soil microbiology, soil nutrient levels, photosynthesis rates, pruning weights, etc were considered. All of them could be included in new research. Moreover, from the juice and wine it was necessary to incorporate the HPLC analysis to determine the flavonoid and acid composition.

Another important point to be considered in the future is the evaluation of new materials as mulch. New products from recycling plants or natural products with reflection capacity could be tested in further research. The recycling plan implemented by the New Zealand Government and the availability of natural resources in this country, make New Zealand the ideal place to innovate in this area.

A long term trial of three to five seasons was necessary to evaluate the real effects of using reflective mulches in viticulture. This would diminish the effect of the variations that happen season by season, contributing to understanding the influence of mulches on grapevine physiology, which would be not beneficial in all cases.

Finally, the influence of reflective mulches on aromatic profiles of grapes and wine obtained in future trials, may be investigated more deeply incorporating the use of GC-MS and other techniques available to determine the effects of reflective mulches. Also, the number of standards used as reference of specific aromas should be larger, to provide tools to identify precisely each aroma compound present in the samples, especially in the GC-O runs.

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