# The effect of shading and crop load on flavour and aroma compounds in Sauvignon blanc grapes and wine

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# The effect of shading and crop load on flavour and aroma compounds in Sauvignon blanc grapes and wine

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The effects of crop load and berry exposure on the composition of Marlborough Sauvignon blanc grapes and wine from the Brancott vineyard, Blenheim, were explored.

Commercially grown, 2-cane and 4-cane Sauvignon blanc vines were used with a row orientation of north-south. Two exposure treatments were imposed in the following manner: complete leaf removal was undertaken in the fruit zone and 50% shade cloth was erected to give a uniform shading treatment to half the trial vines. Weekly thirty-berry and whole bunch samples were taken from each of the 32 plots with the exception of the veraison period when two samples per week were taken. Vine vigour was assessed using pruning and leaf area per vine data. Harvest occurred on different dates for 2-cane and 4-cane pruned vines so that fruit attained from both treatments had similar °Brix. Fruit was processed at the Lincoln University winery. Must analysis and wine analysis were undertaken.

As expected, 4-cane vines had almost double the yield of 2-cane vines. Higher crop load significantly reduced leaf area per shoot and shoot thickness. Lower leaf area to fruit ratio for 4-cane berries resulted in delayed onset of veraison and slowed the rate of sugar accumulation. Crop load, which limited leaf area to fruit ratio, appeared to be the dominant

factor in determining timing of grape physiological ripeness as expressed by °Brix over other factors such as fruit exposure. Malic acid, tartaric acid, IPMP (iso-propyl-methoxypyrazine) and IBMP (iso-butyl-methoxypyrazine) were lower at equivalent °Brix in 4-cane compared with 2-cane berries. Significantly higher concentrations of quercetin were found in exposed compared to shaded berries. Must analysis showed a significant influence of crop load on berry titratable acidity and pH, reflecting berry ripening results. Exposure significantly increased the concentrations of nitrogenous compounds in 4-cane must yet showed no influence on 2-cane must.

After wine processing lower malic acid concentrations in wines made from 100% exposed fruit became evident in lower wine titratable acidity but showed no influence on wine pH. Bentonite addition to wines had a small but statistically significant influence on wine by reducing pH, titratable acidity and alcohol. Bound sulphur concentrations were significantly higher in 4-cane versus 2-cane wines. At harvest, methoxypyrazine levels in grapes and wines were very low; IBMP concentrations where significantly lower than those normally found in Sauvignon blanc wines from Marlborough. This was attributed to the absence of basal leaves from the shoots of ripening berries.

The results suggest that leaf area to fruit ratio is a powerful determinant of grape and wine quality.

**Keywords:** Sauvignon blanc, crop load, exposure, leaf area, canopy structure, quercetin, methoxypyrazines, thiols, °Brix, organic acids, sulphur dioxide, bentonite.

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## Context of this study

This study is part of a larger programme of work currently work being undertaken on what differentiates Marlborough Sauvignon blanc from other wines of the same variety in New Zealand and around the world (Benkwitz and Nicolau, 2006). Studies are focused on measuring the key impact compounds from wines from different regions and countries and factors which control their variation.

This work is concerned with utilising common viticultural tools (pruning, fruit exposure and fruit-zone leaf removal) to modify the concentrations of these key impact compounds in Marlborough fruit and corresponding wines. Understanding how these viticultural tools can be used to modify the concentrations these important compounds will allow winegrowers to use pruning and fruit-zone leaf removal to combat seasonal variation and vineyard variation. In addition, innovation and the evolution of the Marlborough wine style can be thoughtfully directed.

The number of flavour and aroma compounds in wines is vast with over five hundred volatile constituents; the majority of these are as yet unidentified (Peynaud, 1987). The aroma of a wine is characteristic to grape variety or wine style (Peynaud, 1987). Red wines for example are often characterised by aromatic ester and phenolic contribution and Riesling and Gewürztraminer by terpenes (Jackson, 2000; Rapp, 1988). A relatively small number of known 'impact' compounds within Sauvignon blanc provide a spectrum of flavours and aromas which defines the Marlborough wine style (Parr et al., 2007). The

spectrum ranges from fruity and tropical through to vegetative and grassy, sometimes described as green pepper or asparagus.

The senses of smell and taste are separate, but compliment one another greatly. So important is the sense of smell on taste that if it were absent our taste would be greatly impaired (Brillat-Savarin, 1839). The sense of taste is crucial for assessing wine quality, the four basic tastes being sweet, sour, bitter and salty (Peynaud, 1987). The greatest contributors to sweetness in wine are residual sugars and alcohol, acids are responsible for the sourness and bitterness (albeit avoided in Sauvignon blanc wine production) is provided by phenolic compounds (Peynaud, 1987).

Four groups of compounds considered significant to Sauvignon blanc flavour and aroma are acids, sugars, methoxypyrazines and thiols.

Fruit ripeness has been found to influence the aromatic profile of Sauvignon blanc wines appreciably (Marais et al., 2001). The point of perfect berry ripeness is subjective. Rough guidelines of berry ripeness are given by measuring sugar concentration (actually the concentration of soluble solids) as °Brix and acid concentration by measuring titratable acidity and pH. Such measurements will give the viticulturist and winemaker an idea of the resulting alcohol concentration of the wine after fermentation and the possible sourness of the wine. However, such information is not sufficient to give a holistic impression of the resulting wine's quality. Objective measurement is further complicated by the significance of impact compounds many of which are only released during fermentation, during ageing or in the mouth of the consumer (Murat et al., 2001; Peynaud, 1987). Quick and inexpensive methods for the analysis of the key flavour and aroma compounds methoxypyrazines, thiols and quercetin are yet to be developed. That is why the most

common method for holistically assessing the ripeness of berries is for the trained palate of the viticulturist and winemaker to simply taste test fruit.

The vegetative aroma of cool climate Sauvignon blanc wines has been viewed by some as typical for quality wines of this cultivar (Marais et al., 1999). The vegetative, grassy aroma of Sauvignon blanc is attributed to methoxypyrazines. One compound in particular, 2-methoxy-3-isobutylpyrazine (IBMP), is considered by many to be a key indicator of Sauvignon blanc wine quality (Marais et al., 2001). The 'support structure' of a wine as described by Peynaud (1987) is the sweet-acid balance. Sweet and acid components provide the crispness and freshness, and when in conjunction with aroma compounds, a fruity flavour to the wine. One such important group of aroma compounds that add to the perception of fruitiness are thiols. Thiols impart the nuances of passionfruit, grapefruit and boxwood or 'cats pee' to Sauvignon blanc wines (Murat et al., 2001; Tominaga et al., 2000; Tominaga et al., 1998a). New research indicates that Marlborough Sauvignon blanc wines have significantly higher concentrations of some thiols which give these wines their unmistakeable Marlborough style (Benkwitz and Nicolau, 2006). Methoxypyrazines have also been found in higher concentration in Marlborough Sauvignon blanc wines compared to wines made from this variety grown in other regions (Lacey et al., 1991).

Trials undertaken by a number of different workers (Bureau et al., 2000; Downey et al., 2004; Marais et al., 1999; Naylor et al., 2000) imply that fruit exposure influences flavour and aroma precursors and compounds in grape berries. Increased berry exposure leads to the enhancement of fruity/tropical wine characteristics and a reduction in vegetative/grassy qualities. For example, Marais *et al.* (2001) found that exposed Sauvignon blanc fruit was lower in vegetative aromas than shaded fruit.

Vine canopy modification results in changes to fruit exposure that alters berry microclimatic factors such as ambient and individual berry temperature and exposure to u.v. radiation. Fully exposed berries in some cases exceed ambient air temperature quite significantly (Bergqvist et al., 2001; Smart and Sinclair, 1976). Increased temperatures may have an impact on grape cell metabolism through a rise in transpiration rates and cellular respiration (Bergqvist et al., 2001; Crippen and Morrison, 1986). Sauvignon blanc wines made from grapes grown in warmer climates have been found to have washed out flavours or have a neutral character (Marais et al., 1999). Marais *et al.* (1999) argues that a model for prediction of Sauvignon blanc wine quality can be based on microclimatic data. The same researcher found that increased grape exposure led to enhanced levels of monoterpenes, but a decrease in IBMP in grapes and wines. Other grape varieties show similar trends. Gewürztraminer berries have been found higher in potentially volatile terpenes from exposed treatments in comparison with partially exposed or shaded berries (Reynolds and Wardle, 1989). A study showing the linear relationship between exposure and quercetin levels in Pinot noir grapes (Price et al., 1995) support the theory that flavonol concentration is a good indicator of berry exposure (Downey et al., 2004).

However, Hashizume & Samuta (1999) found light exposure had two opposite effects on the concentration of methoxypyrazines. Prior to veraison, IBMP concentration within berries was enhanced by berry light exposure; after veraison IPMP concentration decreased with exposure. They suggested that the production of methoxypyrazines might be closely related to the developmental stage of grapes, such that in the early stages the amount formed biologically exceed that degraded but in the later stages decomposition exceeded formation. Grape berry development is known to be strongly influenced by crop load, so this factor is also likely to be important in controlling IBMP concentrations in grapes at harvest. Workers (Bennett and Trought, 2004; Bravdo et al., 1984) have found that fruit

development is slowed by higher crop load. In these studies the onset of veraison was found to be delayed and rate of sugar accumulation slowed. Bravdo *et al.* (1984) found at harvest berry total acid concentration decreased at higher crop levels, differences related to lower malate content in berries.

Potential thiol concentration in grape must can be assessed by measuring cysteinylated precursor compounds (Peyrot des Gachons et al., 2000). It is not until after liberation during yeast fermentation that the concentration of the volatile thiols can be assessed in wine as only a fraction of the precursor is transferred to the wine (Peyrot des Gachons et al., 2000). Both the cysteinylated precursor of 3-mercaptohexan-1-ol (3MH) and S-3-(Hexan-1-ol) –glutathione has been found in Sauvignon blanc must (Peyrot des Gachons, 2002). S-glutathione conjugates are normally involved in toxin removal in living systems (Peyrot des Gachons, 2002). Marrs (1995) found that as a response to quercetin toxicity anthocyanins were conjugated to glutathione, transported to cell vacuoles and metabolised. It is not known yet how crop load or fruit exposure influence thiol precursor concentrations in grape berries. However, grapevine water status and nitrogen deficiency is known to influence the concentrations of cysteinylated precursors significantly (Peyrot des Gachons, 2002). Severe water stress is known to decrease the concentration of precursors whereas moderate water stress increases precursor concentration (Peyrot des Gachons, 2002).

## Grape berry development and composition

Grape berry development begins with flowering. After fertilisation, the flower starts to grow and develop into a berry (Jackson, 2000). As the berry develops it follows a double sigmoid curve in growth trend (Coombe, 1980). The curve is commonly divided up into

three stages of maturation. Each stage is important for the production or degradation of IBMP (Hashizume and Samuta, 1999; Marais, 1994; Roujou de Boubee, 2003), organic acids (Gutierrez-Granda and Morrison, 1992; Ruffner, 1982), quercetin (Downey et al., 2004) and sugars (Coombe, 1992). If the proposed biosynthetic pathway for the 3MH precursor proposed by Peyrot des Gachons (2002) is correct, then it is possible that cysteinylated and gluthionylated 3MH thiol precursor concentrations within the grapes could be related to the concentration of glutathione, which is known to increase during grape ripening (Adams and Liyanage, 1993).

Stage I is characterised by rapid growth, followed by cell enlargement and endosperm development (Jackson, 2000). This phase usually lasts between six weeks and 2 months. Marlborough Sauvignon blanc berries at this stage are small (reaching approximately 8.6 mm (Dryden et al., 2005)), green and hard; the fruit is low in sugar and high in acid concentration (Coombe, 1992). At the onset, tartaric acid is rapidly synthesised in the ovary, then both tartaric and malic acids increase slowly during the latter part of this phase (Jackson, 2000). Tartaric acid is found mainly as a free acid but this slowly changes during ripening, with an increasing amount found as $K^+$ salts (Jackson, 2000). Malic acid on the other hand, remains as a free acid (Jackson, 2000).

Stage II (or the lag phase) is characterised by a slowing of berry growth and seed development. This period, according to Jackson (2000), is the most variable in length being 1-6 weeks. It is during this period that IBMP is thought to reach its maximum concentration (Hashizume and Samuta, 1999; Sala et al., 2004). Berries are still small (approximately 9.9 mm (Dryden et al., 2005)), green and hard and remain high in acid and low in sugar.

Veraison, apparent by berry softening and a colour change in red grape varieties and a semi transparent appearance of green grapes, signifies the beginning of stage III. The berries enlarge reaching approximately 12.3 mm in size (Dryden et al., 2005) and continue to soften, sugars increase and berry acid levels decline (Coombe, 1980; Coombe, 1992). Malic acid accumulates in grapes up until veraison, declining during stage III (Gutierrez-Granda and Morrison, 1992). Ruffner and Hawker (1977) have inferred that the accretion of malic acid may act as a bank of "reducing power" to be used in biochemical processes during maturation. Glutathione content over the veraison period varies between berries (Adams and Liyanage, 1993). An increase in concentration coincides with the onset of sugar accumulation and ranges from 42 nmoles/g fresh weight in immature berries to 116 nmoles/g fresh weight in large green berries (Adams and Liyanage, 1993). The glutathione to cysteine ratio is approximately 20 in grape berries (Adams and Liyanage, 1993) so that glutathione content is a good indicator of cysteine concentration also.

The third stage in development is particularly important for the accumulation of phenolic compounds (Pirie and Mullins, 1977). Flavonols accumulate in grape berry skins during maturation (Downey et al., 2004; Kolb et al., 2003). These compounds work to screen u.v. radiation; u.v.-A (325-400 nm) is shielded primarily by quercetin in epidermal tissue in the grape skins (Price et al., 1995).

Hernandez Orte *et al.* (1999) found that the total concentration of amino acids increase during stage I, II and III. However the same authors also contend that the concentrations of individual amino acids vary, some increasing and some decreasing, during berry development. Also, these researchers found that there were changes in the concentration during development from year to year. Adams and Liyanage (1993) discovered during their study that glutathione increase initiated at veraison continues during ripening.

**Crop load: effects on composition**

Crop load is defined as the ratio between vine fruit yield and vine above ground vegetative yield in a season (Bledsoe et al., 1988; Howell, 2001) and is highly correlated ($r^2$=0.86) with leaf area to fruit ratio (Naor et al., 2002).

It appears that crop load is a decisive factor in quality grape and wine production. Important compounds found in lower concentrations from vines with high yield to pruning weight ratios include acids, sugars and amino acids (Bravdo et al., 1984; Edson et al., 1995). High crop load appears to slow down development of grapes. In a trial undertaken by Bennett and Trought (2004), the grapes on 4-cane vertical shoot positioned Sauvignon blanc vines grown in Marlborough were about three weeks behind in level of sugar concentration compared with grapes on 2-cane vines. However, these authors observed that the rate at which sugars accumulated during stage III was the same, but the onset of veraison that was later. Slowed rates of sugar accumulation noted by other researchers (Bravdo et al., 1984) indirectly influences acid levels in grapes. This is due to the longer hang time necessary to achieve equivalent °Brix ripeness in fruit. In addition to sugars and acids, methoxypyrazines are also influenced by crop load. Cabernet Sauvignon grapes from highly cropped vines have lower concentrations of IBMP compared with those from low cropped treatments (Bravdo et al., 1984).

**Fruit exposure: effects on composition**

It is difficult to separate the effects of light exposure from those of temperature on fruit composition, particularly under field conditions. Bergqvist *et al.* (2001) found that there was

a linear relationship between exposure to photosynthetically active radiation (PAR) and berry temperature. This supports earlier research by Smart and Sinclair (1976) which found that the two most important environmental factors influencing berry temperature are solar radiation and wind speed. The highest skin temperatures achieved by exposed green berries in field trials were 12°C above ambient temperature (Smart and Sinclair, 1976). In addition berries in clusters that were tightly packed (as is often the case with Marlborough Sauvignon blanc) heated more than berries from loose bunches (Smart and Sinclair, 1976). Naylor *et al.* (2000), in New Zealand, found that north facing fruit received more light (80% ambient) than south (23% ambient) facing fruit. North facing fruit, it was argued, achieve higher temperatures in Marlborough. Light levels within north-south rows showed more uniformity than in east-west rows and, therefore, more uniform berry temperatures. These same workers discovered a wider spectrum of flavours within the wines from grapes grown on east-west rows, which indicated a higher degree of variability within this fruit population.

Bergqvist *et al.* (2001) believe that when the differences in fruit temperatures are considered throughout berry development, it is a major factor in the compositional variation within a fruit population. It appears that both temperature and light exposure affect berry development and flavour and aroma compound accumulation. Spayd (2002) found that reducing the temperature of sun exposed berries increased total anthocyanin concentrations in berries. Temperature had little influence on quercetin concentration; however u.v. exposure significantly increased quercetin (Spayd et al., 2002). However, berry temperature impacts berry acid composition; after veraison increased berry temperature results in lower malic acid concentration (Jackson and Lombard, 1993).

Bergqvist *et al.* (2001) reported that a gradual reduction in berry size in exposed fruit may be due to affected cellular elongation and cell division, and increased transpiration rates due to higher temperatures. This results in stunted growth, berry dehydration and shrinking. The water loss from berries during exposure to high temperatures was found to concentrate berry constituents such as acids and sugars.

A study that differentiated the effects of temperature and light exposure on berries was undertaken by Dokoozlian and Kliewer (1996). In their study they examined the influence of light only on different stages of berry development (I, II and III). They found that berry light exposure during the primary stages of berry growth had greatest impact on berry size. The findings indicated that berries grown without light, with clusters placed in white aluminium lined bags, during stages I and II and without light during stages I, II and III had significantly smaller berry diameters and berry mass when compared to those grown with light throughout development. In addition, veraison and fruit softening were delayed in fruit grown in the absence of light. These authors accepted that it was light exposure during the initial stages of berry development (I and II) that was important to trigger veraison in berries.

Flavonoids, specifically quercetin and anthocyanin, are affected differently by light exposure and temperature (Spayd et al., 2002). Anthocyanin content in grape berries is increased when berries are exposed to higher temperatures, up to 26°C (Pirie, 1977), but shading or sun exposure has little influence (Price et al., 1995). In contrast quercetin levels are determined by u.v. exposure (Spayd et al., 2002).

The cysteinylated 3MH precursor is predominantly located in the Sauvignon blanc skin cells (Peyrot des Gachons et al., 2002b). The presence of 3MH conjugated to cysteine and

glutathione in grape must indicates that the cysteinylated precursor takes part in catabolising the glutathione conjugate during cell detoxification (Peyrot des Gachons et al., 2002b). Internally or externally produced toxins are conjugated to glutathione by *S*-glutathione transferase (EC 2.5.1.18) then the product is broken down successively by γ-glutamyltranspeptidase (EC 2.3.2.2) and a carboxypeptidase, eliminating glutamic acid then glycine respectively, resulting in the formation of the *S*-cysteine conjugate (Jakoby et al., 1984).

Detoxification involves the removal of quercetin and anthocyanin compounds via gluthione conjugation, transport to berry vacuoles where the compounds are metabolised (Peyrot des Gachons et al., 2002b). Increased quercetin and anthocyanin concentrations in grape berries due to elevated berry u.v. radiation and temperature may result in an increase in the concentration of thiol precursor compounds in grape berry skin.

**Canopy manipulation**

Factors such as temperature, radiant light levels, time of radiant light exposure and humidity are controlled by grape vine canopy structure and row orientation. Generally, the canopy density in the fruiting zone dictates fruit exposure. In addition, the canopy leaf area and density change throughout the season at different stages of fruit development (Dokoozlian and Kleiwer, 1995a; Dokoozlian and Kleiwer, 1995b).

To manipulate the vine canopy a viticulturist can employ a number of approaches including: water and nutrient management, canopy training and shoot positioning, hedging and leaf thinning. Such approaches are used to increase light levels within the canopy and in the fruiting zone specifically.

There have been a number of studies investigating shading and canopy manipulation on grape quality. Hunter *et al.* (2004) makes an interesting point about canopy manipulation (leaf removal, topping) in the pre-veraison period. These authors contend that such procedures increase leaf photosynthetic activity and increase carbohydrate levels. However, leaf removal in the fruiting zone has also been found to reduce whole vine photosynthesis (Petrie et al., 2003). In the Petrie *et al.* (2003) study a quarter of vine canopy was removed through basal leaf removal. Results indicated that individual leaf increase in photosynthetic activity was not enough to compensate for the unit area of photsynthetic loss due to leaf removal (Petrie et al., 2003).

Berry microclimate changes due to basal leaf removal practices have also been found to boost berry metabolic rate and transpiration rates (Hunter et al., 2004). Hunter *et al.* (2004) believe that this favours the development of berries higher in precursor, flavour and aroma compounds.

In cool climates, sugar concentration or °Brix is often the key indicator of grape maturity. Grapes are frequently picked at a given °Brix; therefore the rate at which sugars are accumulated could have an influence on the concentration of other "impact" compounds in Sauvignon blanc fruit. Two important factors limiting the rate of sugar accumulation in berries appears to be leaf area to fruit ratio (Edson et al., 1995) and leaf position on the shoot (Petrie et al., 2003). Leaf to fruit ratio can be manipulated by changing crop load, shoot trimming or leaf removal practices.

In grape vines high levels of fruit production appear to limit the allocation of carbon resources to other vine organs e.g. shoots and leaves (Edson et al., 1995). A reduction in

the rate of sugar accumulation occurs with overcropping (Bravdo et al., 1984) in addition to delaying stages of berry development, e.g. veraison (Bennett and Trought, 2004), and maturation (Bennett and Trought, 2004; Edson et al., 1995).

Petrie *et al.* (2003) compared the effects of leaf removal and whole vine topping on whole vine photosynthesis. Comparisons were made between short and tall vines, short vines having had the top 30 cm of canopy removed by trimming. Petrie *et al.* (2003) found that basal leaf removal had a greater impact on whole vine photosynthesis than vine topping. Removing leaves from the bottom 30 cm of the canopy decreased whole vine photosynthesis on a per unit leaf area basis. This was greater in short vines where leaf removal resulted in a 20% loss in canopy surface area. The reduction of whole vine photosynthesis in short vines with leaves removed was around 50% compared with a loss of 35% in tall vines. Treatments were imposed during the lag phase of berry development when the basal leaves were approximately 3 months old, and although possibly in decline, still contributed more to vine photosynthesis than immature (<1 month old) leaves (Petrie et al., 2003). From this information, these researchers concluded that the basal portion of the canopy contributes more to the entire vines photosynthetic capacity than the upper portion.

## Important components of Marlborough Sauvignon blanc wines

Grapes are harvested at a determined maturation point, usually at a given °Brix level. Sauvignon blanc in Marlborough is usually picked at around 22 °Brix (Dr J. Bennett, *pers. comm.*). Berries that are under this target will be higher in unripe, herbaceous flavour and aroma compounds such as IBMP, high in malic acid concentration and low in sugar (and

hence alcohol) concentration. However, berries well over the target may not exhibit the "green" qualities desired in the Marlborough wine style.

**Sugars**

Glucose and fructose are the most important contributors to sweetness in wines (Thorngate, 1997). Sugars also appear to enhance flavours, lifting the fruitiness of wines (Bonnans and Noble, 1993). Terms given to describe sweetness include: supple, sweet, and luscious (Peynaud, 1987). Sauvignon blanc wines, which are generally fermented to near dryness, are not regarded for their sweetness, as with some other wine styles. However, sugars are an important factor for the expression of fruity characters.

Yeasts convert sugars to alcohol during fermentation. Higher concentrations of alcohol also give character to a wine's profile. Descriptors for low alcohol wines include spineless, watery and thin; for moderate alcohol, warm, generous; and for high alcohol, hot, heady, and powerful (Media, 2002). Marlborough Sauvignon blanc wines are moderate to high in alcohol, typically between 13-14% v/v. In addition, alcohol is known to impart a sweet flavour to wine (Peynaud, 1987).

Photosynthesis is responsible for the production of carbon-based compounds within the vine: the most important include sugars and acids. Sucrose is an important compound utilised in different ways within the berry at different stages during development. During the primary and secondary phases sugars are produced in the photosynthesising berry and metabolised during cell production (Jackson, 2000). Both leaf and berry photosynthesis reaches a peak at around four weeks after berry set (Pandey and Farmahan, 1977). After veraison, chlorophyll and starch content is lost from berry plastids (Jackson, 2000). The

role of berry photosynthesis on berry development is not completely clear; some research suggests that berry produced photosynthates contribute very little to berry development (Pandey and Farmahan, 1977) while others have found smaller berry weights, delay in veraison and higher malate concentrations in berries grown in the absence of light (Dokoozlian and Kleiwer, 1996). During the third stage, berry ripening, sucrose is transported to berries and stored. Sucrose produced in vine leaves is transported via the phloem to the berries and hydrolysed to form glucose and fructose (Jackson, 2000). These two hexoses are the most important solutes that accumulate in berries during ripening (Coombe, 1992).

The leaf area to fruit ratio appears to be key in determining rate of sugar accumulation and the timing of initiating rapid sugar accumulation (veraison) (Bennett and Trought, 2004; Edson et al., 1995). Exposed leaf surface area available for $CO_2$ fixation limits the rate of carbohydrate production by vines. Vines are known to compensate for leaf area loss by increasing photosynthetic efficiency (Petrie et al., 2003). The level of compensation is limited, however, and significant leaf loss has a large impact on whole vine photosynthesis and berry sugar accumulation (Petrie et al., 2003). Sugar accumulation in grapes is slower in shaded grapes than exposed grapes (Marais, 1996). In the field however, these differences become less pronounced closer to maturity (Marais, 1996). In contrast, under phytotron conditions, temperature controlled berries grown in the absence of light through all stages of berry development are lower in sugar concentrations compared with those grown in light exposed conditions (Dokoozlian and Kleiwer, 1996).

**Organic Acids**

The presence and concentration of acids can give wines refreshing, piquant, fresh, racy, zesty, sharp, tart and sour flavours (Media, 2002). To produce wine enjoyed for a fresh, zesty and lively style, adequate acid levels are very important. The two most important acids in grape leaves, berries and wine are malic acid and tartaric acid (Jackson, 2000; Ruffner, 1982). Acceptable concentrations of acid in wine are between 5.5-8.5 g/L titratable acidity as tartaric acid, with white wines at the higher end of the range (Jackson, 2000). The perception threshold for most people of tartaric acid in water is between 0.05-0.2 g/L (Peynaud, 1987).

Tartaric acid is considered a secondary metabolite (Ruffner, 1982); malic acid on the other-hand plays a pivotal role in anabolic reactions such as dark fixation of $CO_2$ in addition to acid catabolism during ripening (Ruffner, 1982). Photosynthesis is responsible for the development of both berry acids (Jackson and Lombard, 1993). Both the leaves and berries are thought to have the ability to produce malic acid and tartaric acid (Botha, 2000). The green berry could be responsible for up to 50% of tartaric acid and malic acid synthesis *in situ* (Botha, 2000). Malic acid concentration declines in berries during ripening; therefore, tartaric acid is thought to contribute most of the sour flavour of grapes and wine. Studies (Noble et al., 1986; Thorngate, 1997) have shown that there is no major difference in perceived sourness of the different acids found in grapes.

Tartaric acid is synthesised for the most part from sugars and malic acid from pyruvates or phosphoenolpyruvates (Fig. 1).

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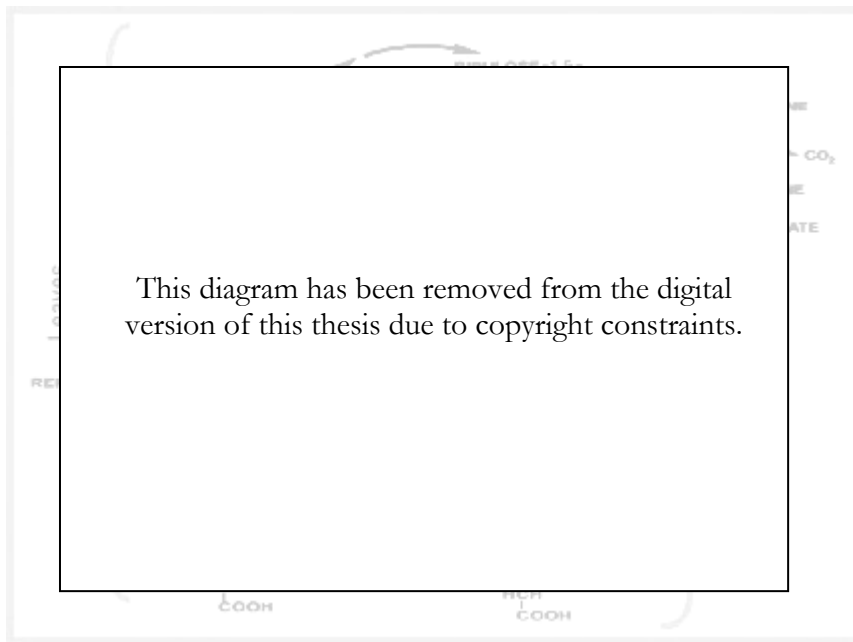
**Figure 1.** A flow diagram representation of the formation of tartaric acid and malic acid in leaves and berries from Botha (2000).

A slower rate of sugar accumulation in highly cropped vines means that fruit must be left for longer periods on the vine to achieve set levels of sugar ripeness for harvest. Extra hang time results in significantly lower acid concentrations at comparable °Brix (Bravdo et al., 1984).

Of the two acids, it appears that malic acid is most affected by over cropping; delayed maturation results in greater tartaric to malic acid ratio in highly cropped vine fruit. Bravdo et al. (1984) showed that slower rates of berry sugar accumulation (longer hang time) in highly cropped vines were responsible for lower acid to sugar ratios and lower malic acid content at harvest.

Lower temperatures favour malic acid accumulation in berries (Lakso and Kleiwer, 1978; Lakso and Kliewer, 1975; Ruffner, 1982). The maximum rate of accumulation for malic

acid prior to veraison appears to occur at temperatures close to 20°C (Ruffner, 1982). Simultaneous degradation and accumulation of malic acid occurs in immature grape berries (Lakso and Kliewer, 1975). However, at temperatures in the range 20-25°C the activities of malic acid-producing (PEP carboxylase and malic dehydrogenase) and malic acid-degrading enzymes (malic enzyme) favour malic acid accumulation (Lakso and Kliewer, 1975). Research indicates that temperature is not the defining factor in regulating the mechanism of malic enzyme activity prior to veraison (Lakso and Kleiwer, 1978). This is because malic acid is isolated from the malic enzyme in immature berries (Lakso and Kleiwer, 1978). After veraison, malic acid becomes accessible for respiration and gluconeogenesis (Lakso and Kleiwer, 1978). During this phase of development the reduction of malic acid concentration during ripening has been directly linked to temperature (Jackson and Lombard, 1993). After veraison the activity of the malic enzyme increases (Gutierrez-Granda and Morrison, 1992) and temperature influences on the rate of berry respiration determine malic acid levels in fruit during maturation (Lakso and Kleiwer, 1978; Ruffner, 1982).

Berry exposure has been found to have an important influence on both acid accumulation and degradation during maturation. An early study undertaken by Kliewer *et al.* (1967b) showed that fruit grown under 30% of the normal sun exposure had 20% higher total acidity and 13% higher malate at maturity compared to fruit of normal exposure. More recent work (Dokoozlian and Kleiwer, 1996) supports this observation and found the same pattern at maturity. Berries shaded pre-veraison had lower malic acid concentration at veraison than exposed berries.

Water content in berries was found to be a decisive factor in acid concentration in a study by Crippen and Morrison (1986). Significant differences in the concentrations of both

malic and tartaric acids in shaded and exposed berries can be due to higher water content in shaded berries (Crippen and Morrison, 1986). These results support findings by other researchers (Buttrose et al., 1971; Dokoozlian and Kleiwer, 1996; Morrison and Noble, 1990) which show exposed berries significantly higher in tartrate and malate.

Generally it is agreed that the rate of malic acid degradation post veraison is affected more by temperature than light exposure (Kliewer, 1977a; Kliewer, 1977b). In a trial undertaken by Lakso and Kliewer (1978) it was found that the malic acid pool size decreased with higher temperatures. The same workers suggest that increased enzymatic action is partly responsible for this phenomenon. Viticulturists who grow grapes in regions with very high temperatures have found the consequences of this. Growers face the problem of acids "dropping out" quickly during ripening. Some wine makers are forced to add acid to must during wine making to compensate for acid loss in berries. Acid loss is not usually the issue in New Zealand's cooler regions; getting the fruit to ripen adequately is of greater concern.

Variety appears to have some bearing on the acid composition of grapes (Soyer et al., 2003). One study found that within ten *Vitis vinifera* cultivars from the same vineyard and growing season there were significant differences in acid composition; among cultivars the range of malic acid concentrations was 1.87-3.40 g/L (Soyer et al., 2003). A study by Kliewer et al. (1967a) divided grape varieties into groups based on acid composition. The categories given were: high malate, moderately high malate, intermediate malate and low malate with tartaric to malic ratios were <1.2, 1.21 to 1.75, 1.76 to 2.5 and >2.5 respectively. Sauvignon blanc grown in California is thought be an "intermediate malate variety" not overly high in malic acid with a tartrate to malate ration of 2.3 (Kliewer et al., 1967a). Other intermediate malate varieties include Merlot, Semillon and Cabernet Sauvignon; moderately high malate varieties include Granache, Chardonnay and Pinot Gris.

**Flavour and aroma compounds**

*Methoxypyrazines*

2-Methoxy-3-isobutylpyrazine (IBMP) is regarded as the most important contributor of the grassy, green pepper, asparagus, herbaceous aroma of Sauvignon blanc wines (Marais, 1994). This feature is typical of cool climate Sauvignon blanc wines; the detection threshold of IBMP is around 2 ng/L in water and up to 15 ng/L in red wines (Roujou de Boubee et al., 2000). Typical IBMP concentrations for New Zealand Sauvignon blanc wines are 10-35 ng/L making it an important aromatic compound in a characteristic Marlborough Sauvignon blanc (Lacey et al., 1991).

There are different trains of thought on where IBMP is synthesised and its storage within the berry. It is thought that IBMP is produced in both berries and leaves. Roujou de Boubee (2003) suggests that the compound is translocated mainly from the basal canopy leaves to the berries. Therefore the role of the leaves may be twofold; synthesis of IBMP and shading of berries reducing photo-degradation of the compound after veraison.

IBMP production has been found to occur between fruit-set and two to three weeks before veraison (Hashizume and Samuta, 1999; Roujou de Boubee, 2003). During this phase a considerable proportion of IBMP is found in the stems. In the berry, IBMP is found mostly in the skin (72%) and seeds (23.8%) (Roujou de Boubee, 2003).

It is generally believed that shading facilitates methoxypyrazine (MP) retention in grapes. Marais *et al.* (1999) undertook a study by increasing shading on fruiting shoots naturally by positioning one year old canes during winter pruning from adjacent vines to the middle of

the cordon of the treatment vines. They found over two seasons that shaded grapes were always higher in MP concentration. Lower 3-alkyl-2-methoxypyrazine concentrations in wine were found due to shading fruit artificially (sack cloth) during a study by Sala *et al.* (2004). This is in contrast to other literature. These workers contend that the degradation and formation of methoxypyrazines are influenced by several factors, not just levels of shading.

Research undertaken by Hashizume and Samuta (1999) showed that berries in the absence of light pre-veraison had lower levels of IBMP prior to veraison. However, in the absence of light after veraison, berries had higher levels of methoxypyrazines. These studies indicate that MP formation is enhanced by light exposure before berry softening and veraison. It is understood that photo-degradation rates of MP exceed production after berry softening and veraison (Hashizume and Samuta, 1999). Canopy manipulation (shoot positioning, shoot removal and leaf thinning) to increase light exposure in the fruit zone pre-veraison (berry set and pea size berries) increased MP concentration in ripe grapes (Hunter et al., 2004). It is thought that MP formation was maximised during the early stages of berry development due to a change in source to sink ratio between leaves and berries. Hunter *et al.* (2004) argue that the amount formed during this period is greater than the quantity degraded during the later ripening phase. The two studies Hashizume and Samuta (1999) and Hunter (2004) highlight different influences pertaining to berry IBMP concentrations e.g. light exposure pre-veraison vs source sink relationships between berries and leaves. Similarities between the studies make note of the importance of pre-veraison berry light exposure and IBMP concentrations.

Research has found that prior to veraison shading reduces the concentration of MP in berries (Hashizume and Samuta, 1999). Grapes used in the Hashizume and Samuta (1999)

trial were removed from clusters and placed in glass jars and exposed to artificial flourescent light. After veraison the opposite was found; shading slowed the reduction of MP in berries (Hashizume and Samuta, 1999). It was postulated by Hashizume and Samuta (1999) that light acts as a positive factor for the formation of methoxypyrazines in the early stages of berry development although no explanation for this phenomenon was given. The presence of leaves in the fruit zone serves to shade the berries from light exposure and may slow photo-degradation of methoxypyrazines in fruit.

Vegetative growth determines how much IBMP is synthesised and translocated to the berries (Roujou de Boubee, 2003). High levels of vegetative growth prior to veraison results in high concentrations within the berry; significant rainfall events (and possibly irrigation) show similar results due to resumed canopy growth (Roujou de Boubee, 2003). Roujou de Boubee (2003) found that vigorous vines that continue vegetative growth until late in the season produce fruit with high IBMP levels. Leaf area, particularly the 3-4 leaves closest to the base (Roujou de Boubee, 2003), to fruit ratio is thought to be a decisive factor in IBMP concentrations in fruit. The basal leaves show considerably greater (at least 4.6 times) concentrations of IBMP compared to bunches and other leaves (Roujou de Boubee, 2003). Roujou de Boubee (2003) concluded that clusters are the main sink for IBMP when exported from the basal leaves.

Crop load appears to have a significant effect on IBMP concentrations in fruit (Chapman et al., 2004). Chapman *et al.* (2004) found that IBMP concentrations had an inverse relationship with number of shoots. For example, wines made from vines pruned to 12 and 48 buds resulting in yields ranging from 6 to 22.2 tonne/ha. IBMP concentrations were approximately 7 and 2 ng/L for 12 and 48 buds per vine, respectively; showing a decreasing IBMP concentration with increasing crop load.

## *Quercetin and other flavonoids*

Quercetin is a flavonoid compound most often found in the vacuoles of grapes in the epidermal tissue (Stafford, 1990); in white grapes quercetin is restricted to the hypodermal layer (Jackson, 2000). This localisation is probably associated with quercetin being the most important compound involved in u.v. screening in grape berries (Price et al., 1995).

The main factor that influences the concentration of quercetin in berries appears to be berry exposure to sunlight (Price et al., 1995). Increased levels of u.v. exposure increase the concentrations of quercetin in berries which makes this compound an excellent indicator of light exposure level (Price et al., 1995). A study by Price *et al.* (1995) showed that shaded, moderately exposed and highly exposed grape berries had increasing levels of quercetin glycosides in the resulting wines (4.5, 14.8 and 33.7 mg/L, respectively).

Accumulation of some flavonoids has also been found to be significantly affected by temperature (Kliewer, 1977b). Kliewer and Torres (1972) established that maximum anthocyanin production in grape berries occurs at an optimum temperature of 17-26 °C. The degree to which flavonoid accretion was influenced by temperature was seen to be variety dependent. In this study, high temperatures inhibited accumulation in some varieties, such as Tokay. Sauvignon blanc was not part of this study, but its relative Cabernet Sauvignon was, and this variety was deemed to be highly temperature tolerant meaning that anthocyanin accumulation was not inhibited at temperatures over 26 °C (Kliewer and Torres, 1972).

The flavour of quercetin has been characterised as generally being bitter and astringent (Vaia and McDaniels, 1996). Other terms used by the panellists in the same study were: sweet, sour, bitter, metallic, musty/dirty, viscosity, burn/alcohol, mouth coating, numbness/tingling, astringency and throat tightness. Participants in this study were able to detect quercetin in model wine at 5 mg/L. One interesting conclusion made by Vaia (1996) was that the addition of low amounts of quercetin made a Chardonnay wine "watery and thinner", and left a smooth mouth coating. Price *et al.* (1995) believe that quercetin can have powerful effects on red wine quality through its ability to co-pigment with other wine constituents and possess the potential to change, boost and stabilise anthocyanins. Price *et al.* (1995) also suggest that quercetin may also have an effect on wine colour in its own right. They argue that a quercetin solution of 30 mg/L, i.e. the same concentration found in wine made from exposed fruit, is visibly yellow in colour.

### Thiols

Thiol precursors are present in grape musts before fermentation (Howell et al., 2004). During fermentation the S-cysteine conjugates are metabolised by yeasts to produce volatile thiols (Peyrot des Gachons et al., 2002b). However only small proportion of the precursor compounds (Table 1) are transferred from precursor into the wine as free volatile thiol these fractions being: 1.4% of P-4MMP, 3% of P-4MMPOH and 4.2% of 3MH (Peyrot des Gachons et al., 2002b). Thiols present in Sauvignon blanc wines and berries have been discovered in other plants and fruit. For example, 4MMP was found in box tree (Tominaga and Dubourdieu, 1997) and 3MH precursor has also been found in passionfruit juice (Tominaga and Dubourdieu, 2000).

In Marlborough Sauvingnon blanc wines have been found to have concentrations between 1400 and 18000 ng/L of 3MH (Benkwitz and Nicolau, 2006). There appears to be a strong relationship between the concentration of amino acids in grape berries and the resulting aromatic thiols in wine (Guitart et al., 1999). A study by Peyrot des Gachons (Peyrot des Gachons et al., 2002a) explored thiol precursor development in Sauvignon blanc berries and volatile thiol composition of Sauvignon blanc wines. In berries the majority of thiol precursor compounds for 4MMP and 4MMPOH are found in the juice (≈80%) the remainder is localised in the skin. However P-3MH is distributed evenly between juice and skins, subsequently juice skin contact increased 3MH thiol aroma potential of must.

**Table 1.** Some known thiol precursors in Sauvignon blanc grapes and thiols in wine.

| Precursor in grape | Volatile thiol in wine | Aroma descriptor | Perception threshold in wine (ng/L) |
|---|---|---|---|
| S-4-(4-methylpentan-2-one)-cysteine | 4-mercapto-4-methylpentan-2-one (4MMP) | Boxwood Cats' pee Eucalyptus | 0.8 |
| S-3-(hexan-1-ol) cysteine | 3-mercaptohexan-1-ol (3MH) | Grapefruit Passionfruit | 60 |
| S-4-(4-methylpentan-2-ol)-cysteine | 4-mercapto-4-methylpentan-2-ol (4MMPOH) | Citrus zest Passionfruit | 4.2 |

See Benkwitz and Nicolau, 2006; Peyrot des Gachons et al., 2002a; Peyrot des Gachons et al., 2002b; Tominaga et al., 1998a).

No research as yet has been done on the influence of fruit exposure of crop load on thiol precursor concetrations in Sauvignon blanc grapes. Peyrot des Gachons (Peyrot des Gachons et al., 2002a) has explored the influence of vine water stress on thiol precursor

concentrations in Sauvignon blanc berries. This researcher found that mild water stress favoured precursor development but severe water stress led to a reduction in precursor levels.

## The use of bentonite and its effect on wine composition

In Sauvignon blanc wine production the main use of bentonite is for wine clarification and the removal of unstable proteins. However, some researchers (Zoecklein et al., 1999) have found that the excessive addition of bentonite ($>0.5$ g/L) can cause stripping of wine body, flavour and in some red wines, colour.

Bentonite is a 2:1 layer aluminosilicate clay which carries a negative charge. This charge is balanced by cations, often $Na^+$ and $Ca^{2+}$, which can be hydrated to varying extents. The $Na^+$ form of bentonite can be fully dispersed resulting in a very large exposed aluminosilicate surface area; one gram of bentonite gives a potential absorbing area of approximately 750 $m^2$ (Rankine, 2004). Absorption occurs primarily through electrostatic attraction.

Bentonite is most commonly used in white wines for protein stability but can also be effective in reducing browning (Main and Morris, 1994) and pesticide removal (Ruediger et al., 2004). In a study by Puig-Deu *et al.* (1996) wines fined with bentonite (0.5 g/L) had 45% less protein than control wines. However additions at 0.18 g/L showed no reduction in protein content. Post-ferment bentonite tests are undertaken to determine the least amount needed to give protein stability. A bentonite test is done by adding a given amount of bentonite to a wine (e.g. 0, 0.2, 0.4, 0.6 g/L), filtering, then heating to 80 °C for 6 hours; samples are then checked for clarity to find appropriate dosage level (Rankine, 2004).

Important studies on unstable proteins in wines by Hsu & Heatherbell (1987) found that the most important proteins contributing to protein instability were of a low MW (molecular weight) of 12,600 and 20,000-30,000 and of a low pI, 4.1-5.8, and included glycoproteins which contribute a large proportion of grape proteins. The same study found that Sauvignon blanc wines are high in MW 25,000 proteins, which fall outside the parameters of those most readily removed by bentonite e.g. intermediate MW 32,000-45,000 with a higher pI of 5.8-8.0. The Hsu & Heatherbell (1987) study gives insight into why Sauvignon blanc wines have a predilection to protein instability (Dr. D. Heatherbell, *pers. comm.*)

When bentonite is added pre-ferment it is thought to have a less serious impact on wine quality compared with post-fermentation addition (Wehrung, 1996). This is in contradiction with other findings that show that bentonite added pre-fermentation led to the greatest loss of volatiles (Puig-Deu et al., 1996). Another pre-fermentation advantage is that bentonite acts to promote yeast growth encouraging ferment completion and quicker ferments (Groat and Ough, 1978). When used as a settling agent, bentonite was found to produce wines that were lowest in concentrations of volatile compounds compared with potassium caseinate (Puig-Deu et al., 1996). In addition, it is the most likely fining agent to have a negative effect on wine quality (Zoecklein et al., 1999).

## Materials and Methods

### Site

The experiment was located in a commercial vineyard (Booker, operated by Pernod Ricard NZ Ltd.) in the southern part of the Wairau Valley, near Blenheim, New Zealand (41°34′ South, 173°51' East) (Fig. 2). The site was selected on the basis of uniformity with respect to soil type, vine age, vine size and training system.



**Figure 2.** View of the trial site facing south towards Wither Hills, Marlborough, New Zealand (courtesy of Dr. J. Bennett).

The soils are described as "shallow (< 45 cm) and stony ... with silt loam A and Bw horizons over lying C horizons of stony sand" (Rae and Tozer, 1990). Topography is flat to gently undulating (0-3°). Vineyard rows have a north-south orientation planted with 4-year old Sauvignon blanc vines grafted onto SO4 rootstock. The training system is Guyot

otherwise known as Vertical Shoot Positioned (VSP). Vine spacing is at 1.8 m with 2.4 m between rows.

Except for treatments applied in this trial, vines were managed according to local commercial practice, including grass ground-cover cropping, spraying and row wire lifting.

## Experiment design

The trial was a split plot factorial design with crop load (2-cane and 4-cane pruning) as main plot and fruit exposure (50% and 100%) as split plot treatments, respectively. The layout is shown in Figure 3. All leaves were removed from the fruiting zone from the bottom cane to the first foliage wire, approximately four weeks prior to veraison and shade treatments imposed by applying green, 50% shade-cloth on both sides of the row (i.e. east and west) to the fruiting zone only on the 50% exposure split plots (Fig. 4). The shade-cloth which covered the fruiting zone of the vine was clipped to the first foliage wire. Green shade-cloth was chosen to prevent excess heating of canopy anticipated with black cloth and high light reflection into the canopy anticipated with white cloth.

Shading treatments were replicated at both ends of each row, resulting in eight replicates of the four treatments. Each replicate comprised four bays of vines, each bay consisting of four vines. Bays were initially assessed for vine uniformity. Only bays where there were no younger, smaller or missing vines were selected. Pruning treatments (2-cane, 4-cane) were randomly assigned to rows.

Vineyard row (#) and pruning treatment (2- or 4-cane)

| #778 | #781 | | #784 | #786 | | #835 | #837 | | #839 | #841 |
|------|------|--|------|------|--|------|------|--|------|------|
| 4 | 2 | | 4 | 2 | | 4 | 2 | | 4 | 2 |

Bay no.

#1

BLOCK 1

BLOCK 3

BLOCK 5

BLOCK 7

#5

Plot 5
50%

Plot 13
50%

Plot 21
100%

Plot 29
50%

Plot 18
100%

#9

Plot 1
100%

Plot 9
100%

Plot 23
50%

Plot 25
100%

Plot 30
100%

#13

Plot 2
50%

Plot 6
100%

Plot 10
50%

Plot 14
100%

Plot 17
50%

Plot 26
50%

#17

#21

BLOCK 2

BLOCK 4

BLOCK 6

BLOCK 8

#25

Plot 3
50%

Plot 7
50%

Plot 19
100%

Plot 22
100%

Plot 27
100%

#29

Plot 12
100%

Plot 15
50%

Plot 31
50%

#33

Plot 4
100%

Plot 11
50%

Plot 24
50%

Plot 28
50%

Plot 32
100%

#37

Plot 8
100%

Plot 16
100%

Plot 20
50%

#41

**Figure 3.** Trial layout with plot numbers and exposure (50 or 100%) treatments. Northern end at Bay # 1.

**Figure 4.** View of 4-cane 100% exposure (left) and 4-cane 50% exposure (right) plots (courtesy of Dr. J. Bennett).

During harvest and vinification, fruit from the same exposure treatment on each row were combined. Thus, there were 4 replicates for each treatment for the wine assessments, rather than eight for canopy, fruit composition and pruning weight assessments.

**Bird damage**

Birds commonly damage fruit during ripening. This can lead to reduced sample availability, disease susceptibility, changes in berry composition and a smaller harvest. To prevent bird damage, white bird netting was applied to vines at the time that the exposure treatments were imposed (i.e. approximately 4 weeks prior to veraison). The use of bird netting is common practice in Marlborough.

**Sunburn**

Because all the leaves in the fruit zone were to be removed, it was thought that sunburn damage due to increased u.v. exposure might occur. To explore this possibility a sunburn trial was undertaken on four vines. Prior to veraison, on 23rd January 2005 all leaves on a

small sample of vines were removed in the fruit-zone to expose berries completely. Vines were observed four days later (27th January 2005). The berries on clusters looked green and healthy no berry damage was noted from increased cluster exposure.

**Light reduction value of shade-cloth and bird netting**

The Donaghys Hortshade green 50% knitted shade-cloth used in this trial was marketed as providing 50% light reduction. A lux meter (Extech, Model 401025) was used to measure differences in light intensity under shade cloth held at right angles to the sun. Measurements took place on 26 January, 2005 from 11.20-11.50 a.m. The weather was sunny with occasional cloud cover. It was found that the reduction in light intensity by the shade cloth at 90° angle to the sun was 50%. Readings were taken in full sunlight on two occasions gave an average reading of 131,325 lux compared to 63,750 lux behind the shade-cloth. Although not assessed, it was assumed that shading imposed by the cloth would have been greater at other sun angles during the day.

## Field measurements

### Weather data

Weather data was captured by a weather station located in the Brancott vineyard, Booker Block (SBLK) using a data logger (CR10, Campbell Scientific, Utah, USA) connected to a tipping bucket rain gauge (Ogawa Seiki Co., Japan), humidity sensor (50Y Humitter, Vaisala, Finland) and anemometer (A101, Vector Instruments, Clwyd, Wales). In addition, temperature was monitored with a TinyTag Ultra logger (Gemini Data Loggers UK Ltd., West Sussex, U.K.) installed inside a radiation screen in row 805 (see Fig. 3). Weather data were supplied courtesy of Mr. R. Agnew (HortReseach, Blenheim).

**Canopy Assessments**

*Canopy structure*

After leaf removal Point Quadrat measurements (Smart and Robinson, 1991) were undertaken, after a full canopy was achieved, on 24 March 2005, at two heights (130 cm and 190 cm above the ground surface) above the first fruiting wire, below which 100% of leaves has been removed. The method involved using a rod pushed into the canopy along a horizontal plane at the given height at 10 cm intervals from one end of vine canopy to the other. The data were collected and entered into a spreadsheet designed by Dr Mark Greven (HortResearch, Blenheim). The results were used to calculate the difference between 2-cane, 4-cane, 50% and 100% exposure treatments in leaf layer and canopy density.

*Leaf area*

Canopy leaf area was measured after fruit harvest. Five randomly selected sample vines were used from both 2-cane and 4-cane vines, five were from exposed and five were from shaded treatment plots, ten vines in all. All leaves were removed from the selected vines and a 10% fresh weight sample of each vine's leaves was measured to calculate leaf area using a Licor L1-3100 leaf area meter (Licor Inc., Lincoln, Nebraska, USA). These data were used to calculate average leaf area to weight ratio, total vine leaf area and leaf area per shoot. The impact of lateral leaves was not assessed, visible laterals were removed from vines throughout the season.

*Fruit environment*

Fruit zone incident light intensity, berry temperature, under vine soil temperature and ambient canopy measurements was supplied by Mr. R. Titheridge, visiting fellow, Marlborough Wine Research Centre, Blenheim.

Incident light was measured using a lux meter (Extech, Model 401025) to obtain data on differences between 50% and 100% exposure treatments. The lux meter was placed in the fruiting zone of 100% exposed and under the shade cloth in the fruiting zone in 50% exposure treatments, respectively. Ambient canopy temperature was measured using a TFA® digital Thermo-Hygrometer placed in the canopy. Individual berry and soil temperatures were taken with an infrared thermometer (MiniTemp, Raytek Corp., California, USA) pointed at an individual berry or the soil underneath vines at 600 mm range taken every 4 metres along row 778. These parameters were measured on the 22 March 2005 with a series of readings commencing at 8:00, 10:00, 12:00 14:00 and 16:00. Each series consisted of eight measurements of berry temperature and incident light for individual berries oriented north, south, east and west; a measurement of ambient canopy and soil temperature, and, a measurement of incident light intensity. For each time, 4 series of measurements (2 each for 50% and 100% exposure treatments) were carried out over a period of 25 to 50 minutes.

## Yield

Yield was assessed from two or three of the four bays of each plot to ensure that the total yield from vine plots was enough to fill the plastic 68 L containers for winemaking. Fruit weight and bunch numbers were recorded on a per bay basis. The proportion of reject fruit (> 5% botrytis infection) was also recorded.

**Pruning weight**

A procedure developed by Bennett (2005) was used to collect pruning weight data. Data (cane number, count nodes, count shoots, shoot size, total shoot number, new and old cane weight) were collected from one bay per plot. Vines were pruned to provide replacement canes for the following year (i.e. two for 2-cane and four for 4-cane treatments, respectively) and a maximum of two 2 node spurs under the fruiting wire and from the side of the head if practicable.

## Berry sampling

Both whole cluster and thirty berry samples were taken and stored for analysis. After vine leaf removal treatments were imposed, clusters were randomly pre-selected, tagged and individually coded for subsequent sampling. Shoots for whole-bunch sampling were in the mid-region of each cane (shoot numbers 3 to 8, numbered from the vine head) and were from the lower canes for the 4-cane vines, each sample having one apical and one basal bunch. It was estimated that the crop yield reduction due to whole-bunch sampling would have been approximately 9% of 2-cane vine and 6% of 4-cane vine yield. It is believed that crop reduction < 10% would not have a significant effect on fruit composition (Dr. M. Trought, *pers. comm.*).

From each plot a weekly (bi-weekly during veraison) thirty-berry sample was obtained from non-tagged clusters randomly from within the cluster. Non-tagged clusters were used to ensure that the berry sample did not compromise subsequent whole-bunch sampling which had been pre-tagged.

Sampling occurred from 16 February 2005 until harvest (4 April for 2-cane and 12 April for 4-cane treatments).

**Thirty-berry sample collection and treatment**

Individual berries were pulled by hand from clusters and placed in coded plastic bags sealed using twist ties. After collection samples were stored in cool 'chilly-bins' with ice packs for transport. Berries were processed and analysed within 24 hours for °Brix, pH and titratable acidity.

**Whole-bunch sample collection and treatment**

Whole bunch samples were cut from the shoot using secateurs, placed in coded plastic bags and sealed using twist ties. Timing of sampling, storage and transport conditions were the same as for the thirty-berry samples. However, whole bunches were frozen (-20 °C) within 4 hours of sampling.

# Harvest and winemaking

### Fruit

Separate harvest dates were selected for the 2-cane and 4-cane treatments in order that the grapes could be harvested at the same (or very similar) ripeness (*c*. 21.5 °Brix) levels. The harvest dates were 7 April and 13 April, 2005, for 2-cane and 4-cane treatments, respectively.

During harvest, selectively hand-picked fruit (clusters with > 5% botrytis diseased fruit were excluded) from each plot was transferred from picking bins into 68 L containers with

lids and 50 p.p.m. SO$_2$ added before shipping in a refrigerated truck (exact temperature unknown) overnight to the Lincoln University Winery where it was processed. Thus, on each harvest date (i.e. for each pruning treatment), 16 containers were dispatched. However, the fruit from replicate treatments within the same row were combined during vinification to produce 8 wines from each pruning treatment (2-cane, 4-cane). Thus, 16 wines were produced in total representing 4 replicate rows of each of the 4 treatments.

**Processing**

Fruit from the 2-cane harvest was processed on 8 April, 2005 and that from the 4-cane harvest on 14 April, 2005. On arrival at the winery, fruit was separated according to row number and treatment. Grapes from the same row and treatment (i.e. 50% and 100% exposure) were combined, crushed and de-stemmed.

Enzyme (Ultrazyme 3*L) was added  at the rate of 30 mL/1000 L. Grapes were left on skins at ambient temperature post-crushing for 3 hours. The fruit was then transferred to an 80 L water pressure press and pressed off using standard conditions (5 min at 0.5 bar, 5 min at 1 bar and 5 min at 2 bar) to obtain *c.* 500 L/t, then settled overnight at 10 °C.

Juice was racked off solids although a small proportion (5-10%) of light solids was included in the ferment. Samples of juice were frozen for later analysis. Duplicate 15 L aliquots of juice were racked into two separate 20 L glass fermentation vessels, one 23 L round bottom flask and one 23 L carboy. A duplicate of each ferment was made to insure against losses due to possible sample loss. Wines produced from the subsequent duplicate ferments were combined post-fermentation.

The juice was inoculated with EC1118 yeast (200-250 mg/L) by re-hydration and acclimatisation of yeast to +/- 5 °C of bulk juice temperature using standard procedures. Diammonium phospate (DAP) or other nutrients were not added. Fermentation occurred under controlled cold storage conditions in an effort to keep fermentation temperature between 12-15 °C. The progress of the fermentation was monitored by daily measurement of specific gravity (hydrometer) and temperature, and an assessment of aroma.

Wines were fermented to dryness (measured using Clinitest tablets, Bayer, USA, manufactured in UK), then racked off gross lees and checked for protein stability. Appropriate concentrations of bentonite required to achieve protein stability were determined using the method outlined by Rankine (2004). Effective bentonite addition rates for the wines were assessed to highlight any differences between pruning and exposure treatments in protein instability. Initial tests were carried out using combined samples for wines from both 2-cane and 4-cane treatments. Bentonite (at addition rates of 0, 400, 500, 600, and 700 p.p.m.) was added to 100 mL wine samples and settled overnight. The sample was filtered using GF/F filter in a small vacuum filter and the clear wine was then heated to 80 °C for 6 hours. The presence of visible haze indicated protein instability. Subsequently, fining trails using bentonite concentrations up to 800 mg/L were conducted using the same method on each replicate wine. Wine protein stability was assessed visually. On assessment wines were given a score from 1-5 on the severity of visible haze. The score was ascribed a descriptor to the level of haze present 1 bright, 2 clear, 3 light, 4 moderate and 5 severe.

**Figure 5**. Filtration and bottling of wines (courtesy of Mr. T. Walsh).

Wines were racked (1 June, 2005) with the addition of $SO_2$ at 50 mg/L (based on an estimated final volume of wine) to a $CO_2$-filled receiving vessel. Duplicate racked wines were combined. It was decided, however, that two series of wines would be produced, with and without bentonite treatment to assess the effect of bentonite fining on wine composition. Thus, the (now) combined wines were split into duplicate vessels and bentonite added at either 0 or 700 mg/L. The wines were then placed in a chiller and cold stabilised at 0-1 °C for ten days. Immediately prior to filtration and bottling, free $SO_2$ levels were adjusted to 20-25 mg/L. Filtration and bottling took place 15 June 2005. Each wine was filtered and bottled separately into dark green Bordeaux-style bottles (cleaned with dilute $SO_2$ (approximately one level dessert spoon to 20 L of water) and citric acid (approximately ½ cup to 20 L water) solutions and flushed with $CO_2$, the bentonite-treated replicates being filtered first. The first bottle of wine of each replicate was discarded to prevent contamination between replicates due to wine residue in the filter well. The filtration apparatus consisted of two cartridge filters, 1 μm and 0.45 μm pore size, in series with a nitrogen pressure system (Fig. 5). Bottles were then sealed with a Stelvin closure (screw-cap).

## Analyses

### Thirty-berry samples

Thirty berry samples were assessed for weight, °Brix, pH and titratable acidity (TA) using common methodology (Iland et al., 2000). Berry samples were weighed using bench top scales (BP3100P, Sartorius, Goettingen, Germany). Samples for analysis were prepared by breaking the intact berries in the sample bag using the palm of the hand and then homogenised using a stomacher (Seward 400 Stomacher) for 30 seconds on high speed. The juice was strained through two layers of muslin cloth into a 30 mL screw top sample tube. Soluble solids (°Brix) was estimated using a refractometer (Pocket PAL-1, Atago, Japan). The pH of sample was measured using a Metrohm 744 pH meter (Herisau, Switzerland). Titratable acidity was determined by titrating a 5 mL sample either manually using the Metrohm 744 pH meter or by using a Mettler Toledo DL50 auto-titrator with 0.1M NaOH to an end point of pH 8.2.

### Whole bunch samples

Whole bunch samples were prepared for further analysis as follows. The weight of each sample was recorded. Grapes from the sample bunches were pulled off while frozen. Care was taken to ensure that only healthy berries were taken; any diseased or brown berries were discarded. In addition, the pedicel was removed from the berries. Once removed, 100 g of sample berries were weighed (2 decimal places). The remaining sample berries were returned to the plastic sample bag in the freezer while still frozen. To the sample berries $SO_2$ was added at the rate of 100 mg/kg fruit, then covered with nitrogen and thawed overnight at 4 °C in beakers covered with Parafilm (Pechiney Plastic Packaging, Chicago, USA).

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Once thawed, the whole berries were processed while cool (< 10 °C) using a handheld Braun 300 Watt mixer, until a slush of homogenous consistency was achieved. This took around 2-3 minutes. The same blender was used for all samples. The berry sample was left to macerate at 4 °C overnight

The sample was split in the following way. A 10 g sub-sample was accurately weighed into a 20 mL centrifuge tube, coded, the weight recorded (2 decimal places) using a Mettler PE 1600 set of scales, and frozen (-20 °C) for subsequent analysis of quercetin concentration.

The remaining sample was weighed into a 250 mL centrifuge tube and centrifuged for 20 min using a JA20 rotor at 13800 rpm (23,000 $g$) at 4 °C (J2-MI, Beckman, USA). The recovered juice was decanted and weighed (2 decimal places). A 2 mL aliquot was pipetted into a syringe, passed through a 0.45 μm filter and transferred into a pre-weighed 2 mL tinted HPLC vial, weighed (4 decimal places), coded and frozen (-20 °C) for analysis of organic acids. A 20 mL aliquot of the recovered juice was pipetted into a brown glass, 40 mL storage vial, weighed (2 decimal places) and frozen at (-20 °C) for later extraction of thiols and thiol precursors. The remaining recovered juice was poured into a 40 mL tinted glass storage vial, weighed (2 decimal places) and frozen (-20 °C) for analysis of methoxypyrazines. Sample codes were written on glass vials using a metallic xylene-free permanent marker

### *Quercetin*

The method used for quercetin extraction and analysis (Anon., 2005a) was based on the method by Stricher (1993). The previously frozen 10 g sample was defrosted overnight at 4 °C and transferred into a 250 mL round-bottom flask using 50 mL ethanol and 20 mL

deionised water. After addition of 8 mL reagent grade concentrated HCl, the mixture was refluxed for 2.25 hours (using a six station Isopadisomantle model DEU/6/6, Borehamwood Herts, England). The mixture was then cooled to room temperature and filtered through a Whatman No. 1 filter. The filter and solids were washed with 20 mL ethanol. The filtrate was poured into a 100 mL volumetric flask and made up to volume with deionised water. A 2 mL sample was filtered through a PTFE membrane, non-sterile 0.45 μm syringe filter (Biolab, New Zealand) into a 2 mL, tinted HPLC vial and stored at a temperature of -20 °C.

The extracted samples were analysed using a Shimadzu HPLC, running LC-10 software. The column was a C18 Phenomenex 250 x 4.6 mm using Synergi 4u Hydro-RP 80A packing with 4 μm particle size and 80 Å pore diameter set at 35 °C; the detector was a SPD-M10A diode-array detector at 270 nm. A 10 μL aliquot was used with a mobile phase of methanol and 0.5% phosphoric acid (50:50). The column flow rate was set at 1.2-1.5 mL/min or 270 bar pressure.

**Organic acids**

A 2mL sample of juice was used for the analysis of malic and tartaric acids using a Shimadzu HPLC instrument. Samples were diluted 1:25 or 1:20 with purified (by reverse osmosis) water and a sample of 10 μL and run through a C18 Phenomenex 250 x 4.6 mm column with Synergi 4u Hydro-RP packing. The mobile phase used was a 20 mM potassium phosphate buffer pH 2.9 with a flow rate of 0.75 mL/min at a column temperature of 30 °C.

## Methoxypyrazines

Methoxypyrazine analysis was carried out by automated HS-SPME (Head Space Solid-Phase Micro-Extraction) GC-MS as described by Parr *et al.* (2007) except that NaOH was not added to the sample vial and juice was used instead of wine. In summary, 1.8 mL of juice (extracted as described above) was added to 5.12 mL of deionised water in a 12 mL SPME sample vial, followed by 80 µL of $D_3$-IBMP internal standard solution (*c.* 20 ng/L) and 3.0 g of crytalline NaCl. Samples were incubated (30 minutes, 50 °C) before the headspace was exposed to the SPME (1 cm, DVB/CAR/PDMS combination) fibre. The extracted volatiles were desorbed in a the heated (250 °C) injection port of a Shimadzu GCMS-QP2010 equipped with a 30 m × 0.25 mm Restek RTX5MS column at 90 °C with He carrier gas (28.3 cm/s).

## Musts

Two musts samples were collected in 100 mL bottles from the destemmer-crusher; one was frozen and the other was transported within 24 hours to the Pernod Ricard (NZ) Ltd., winery in Blenheim. There samples were analysed for pH, TA, and °Brix and YAN (yeast assimilable nitrogen), FAN (free available nitrogen), potassium and ammonium content using FTIR analysis.

## Wines

### Sulphur dioxide

Analysis of free- and bound-sulphur dioxide of wines was carried out prior to bottling using the aspiration method (Rankine, 2004). Analyses on 10 June, 2005, were used to calculate final $SO_2$ additions.  Free-sulphur dioxide was determined on 14 June using the

same method on replicate blends of each pruning treatment (2-cane, 4-cane) to check that the desired concentrations had been achieved.

**Glucose and fructose**

Wines were analysed for glucose and fructose after fermentation; 2-cane and 4-cane treatments were analysed on 30 May and 5 June, 2005, respectively. An enzymatic analysis method was used (RANDOX U.V. semi-micro method, procedure A). The spectrophotometer used was a Heλios α (Helios Alpha, England).

**Acidity (pH and titratable acidity)**

Wine pH was measured using a pH meter and titratable acidity was measured using a Metrohm 799 GPT Titrino (Herisau, Switzerland) autotitrator.

**Alcohol**

After bottling the alcohol content of the wines was determined using a Malligand ebulliometer using the method described by Rankine (2004).

**Methoxypyrazines**

Methoxypyrazine analyis was carried out by automated HS-SPME (Head Space Solid-Phase Micro-Extraction) GC-MS as described by Parr *et al.* (2007).

*Thiols (3-mercaptohexan-1-ol)*

The extraction and analysis of 3-mercaptohexan-1-ol was carried out following the method described by Tominaga *et al.* (1998b) except that 50 mL (rather than 500 mL) of wine was used and that the extracts in dichloromethane were reduced to 100 µL before direct injection onto the Shimadzu GCMS-QP2010 equipped with a 30 m × 0.25 mm Restek RTX5MS column at 90°C with He carrier gas (28.3 cm/s).

## Statistical analysis

Data were analysed using Genstat (version 8.2). Vine canopy, harvest, berry, whole bunch, and must data were statistically analysed using a split-plot design. Wine statistical analyses were undertaken using a split-split plot design.

# Results

## The vine canopy

A number of different methods were used to define differences in the vine canopy which might affect berry development and fruit exposure. These included Point Quadrat, pruning and leaf area analyses. There was no significant difference in canopy density shown by total vine leaf area (Table 2) or Point Quadrat (Table 3) analysis between 2-cane and 4-cane vines. There was an interaction effect between exposure treatments in 4-cane vine canopies with reduced canopy density in 100% exposed 4-cane vines (Table 3).

### The effect of pruning and exposure treatments on leaf area and canopy density

There was no significant difference ($P>0.05$) in leaf area per vine (calculated from weight) or leaf weight per vine between 2-cane and 4-cane vines (Table 2). Differences in shoot numbers between 2-cane and 4-cane vines (Table 4), resulted in significant differences ($P<0.01$) in the leaf area per shoot between pruning treatments. In 4-cane vines there were significant differences between exposure treatments with lower leaf layer numbers in 100% compare with 50% exposed treatments (Table 3). Lower leaf numbers was reflected in the lower on average external leaves and internal leaves in 100% treatments in addition to higher number of gaps and top gaps on average in 100% exposed treatments (Table 3). Lateral shoot removal was undertaken on vines on an ongoing basis and are not thought to interfere with the interpretation of the results.

**Table 2.** The effect of pruning treatment on leaf area per vine, leaf weight per vine and leaf area per shoot.

| Pruning treatment | Leaf area per vine (m$^2$) | Leaf weight per vine (kg) | Leaf area per shoot (cm$^2$) |
|---|---|---|---|
| 2-cane | 7.6 | 2.4 | 303 |
| 4-cane | 7.0 | 2.2 | 182 |
| LSD$_{0.05}$[#1] | 1.38 | 0.53 | 74.9** |

[#1] P<0.001 ***, P<0.01 **, P<0.05 *

Table 3. The effect of pruning and exposure treatments on canopy density using Point Quadrat assessment method as illustrated by Smart and Robinson (1991). Measured at two points 130cm and 190cm above the fruiting zone where 100% leaf removal was performed.

| | Pruning treatments | | | Exposure treatments | | | All treatments | | | | | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | 2-cane | 4-cane | $LSD_{0.05}$[#1] | 50% | 100% | $LSD_{0.05}$[#1] | 2-cane 50% | 2-cane 100% | 4-cane 50% | 4-cane 100% | $LSD_{0.05}$[#1] | $LSD_{0.05}$[#1,2] |
| % Gaps | 7.3 | 9.6 | 7.23 | 6.8 | 10.2 | 5.02 | 7.3 | 7.3 | 6.3 | 13.0 | 7.16 | 7.10 |
| % Internal leaves | 16.0 | 18.5 | 7.14 | 18.2 | 16.3 | 4.80 | 15.3 | 16.7 | 21.1 | 16.0 | 7.00 | 6.79 |
| Leaf layer number | 1.91 | 1.98 | 0.348 | 2.01 | 1.88 | 0.216 | 1.88 | 1.94 | 2.14 | 1.83 | 0.335 | 0.305 |
| Leaf layer number top | 2.01 | 2.14 | 0.298 | 2.11 | 2.04 | 0.183 | 1.98 | 2.04 | 2.24 | 2.04 | 0.286 | 0.258 |
| All leaves | 45.6 | 47.6 | 8.51 | 48.2 | 45.1 | 5.33 | 45.0 | 46.2 | 51.4 | 43.9 | 8.21 | 7.54 |
| External leaves | 38.1 | 37.9 | 5.63 | 39.0 | 37.0 | 3.58 | 38.0 | 38.1 | 40.0 | 35.9 | 5.45 | 5.07 |
| Gaps | 1.75 | 2.31 | 1.730 | 1.62 | 2.44 | 1.207 | 1.75 | 1.75 | 1.50 | 3.12 | 1.715 | 1.708 |
| Internal leaves | 7.56 | 9.69 | 4.42 | 9.19 | 8.06 | 3.19 | 7.00 | 8.12 | 11.38 | 8.00 | 4.43 | 4.51 |
| Top gaps | 1.31 | 1.75 | 1.05 | 1.25 | 1.81 | 1.06 | 1.38 | 1.25 | 1.12 | 2.38 | 1.23 | 1.49 |

[#1] P<0.001 ***, P<0.01 **, P<0.05 *
[#2] For means at the same level of pruning treatment

**The effect of pruning treatment on budburst, effective shooting, shoot size and pruning weights**

Pruning data analysis showed pruning treatment influenced most measures of canopy structure with the exception of new cane weight per vine. Exposure treatments showed no significant (P>0.05) influence on canopy architecture.

Vines from the 2-cane pruning treatment had a greater proportion of effective shoots (having a diameter greater than 1.5 cm approximately) per vine; this is reflected by heavier individual new cane weights from 2-cane vines compared with 4-cane. In addition, 2-cane vines had fewer blind nodes (count shoots grown from count nodes that are one or more centimetres from the base of the parent cane (Bennett, 2005)). As expected the total number of shoots was significantly higher (P<0.001) and total cordon weight was significantly heavier (P<0.001) from vines with 4 canes compared with 2 canes laid down the previous year.

**Table 4.** Shoot data collected as part of the pruning weight determination carried out at the end of the trial (cont'd below).

| | Pruning treatments | | | Exposure treatments | | | All treatments | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2-cane | 4-cane | $LSD_{0.05}$[1] | 50% | 100% | $LSD_{0.05}$[1] | 2-cane 50% | 2-cane 100% | 4-cane 50% | 4-cane 100% | $LSD_{0.05}$[1] | $LSD_{0.05}$[1,2] |
| Count Nodes (A) (vine$^{-1}$) | 19.7 | 41.5 | 3.72*** | 30.2 | 31.0 | 1.47 | 19.1 | 20.3 | 41.3 | 41.8 | 3.84 | 2.07 |
| Blind Nodes (vine$^{-1}$) | 2.9 | 7.6 | 1.49*** | 4.8 | 5.7 | 0.93 | 2.5 | 3.3 | 7.2 | 8.0 | 1.64 | 1.31 |
| Thick (>2 cm diameter) shoots (B) (vine$^{-1}$) | 1.5 | 0.3 | 0.74** | 0.8 | 1.0 | 0.39 | 1.4 | 1.7 | 0.3 | 0.3 | 0.79 | 0.55 |
| Medium (1.5-2 cm diameter) shoots (C) (vine$^{-1}$) | 18.1 | 28.5 | 2.66*** | 23.9 | 22.8 | 1.52 | 18.7 | 17.4 | 29.0 | 28.1 | 2.88 | 2.14 |
| Thin (1-1.5 cm) shoots (D) (vine$^{-1}$) | 2.3 | 7.7 | 1.31*** | 4.8 | 5.2 | 0.98 | 2.2 | 2.4 | 7.3 | 8.0 | 1.52 | 1.38 |
| Very thin (<1 cm) shoots (E) (vine$^{-1}$) | 0.5 | 2.4 | 0.44*** | 1.5 | 1.3 | 0.76 | 0.4 | 0.6 | 2.6 | 2.1 | 0.83 | 1.07 |
| Total shoots (B+C+D+E) (vine$^{-1}$) | 22.4 | 38.8 | 3.47*** | 31.0 | 30.2 | 2.11 | 22.8 | 22.1 | 39.2 | 38.4 | 3.81 | 2.99 |
| Effective shoots (B+C)/(B+C+D+E) (%) | 88 | 74 | 3.3*** | 81 | 80 | 2.6 | 88 | 87 | 75 | 74 | 3.8 | 3.7 |
| Budburst (B+C+D+E)/A (%) | 115 | 94 | 13.1** | 108 | 101 | 6.0* | 120 | 111 | 95 | 92 | 13.8 | 8.4 |
| Count shoots (F) (vine$^{-1}$) | 16.8 | 34.0 | 3.83*** | 25.3 | 25.4 | 1.33 | 16.6 | 16.9 | 34.1 | 33.8 | 3.91 | 1.88 |
| Non-count shoots (B+C+D+E-F)/(B+C+D+E) (%) | 25.1 | 12.2 | 10.8* | 19.9 | 17.4 | 3.9 | 26.7 | 23.4 | 13.0 | 11.4 | 11.1 | 5.5 |

[1] P<0.001 ***, P<0.01 **, P<0.05 *
[2] For means at the same level of pruning treatment

**Table 4 (cont'd).** Shoot data collected as part of the pruning weight determination carried out at the end of the trial.

| | Pruning treatments | | | Exposure treatments | | | All treatments | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2-cane | 4-cane | LSD[#1] | 50% | 100% | LSD[#1] | 2-cane 50% | 2-cane 100% | 4-cane 50% | 4-cane 100% | LSD[#1] | LSD[#1,2] |
| Total fresh weight new canes (kg vine⁻¹) | 2.18 | 1.90 | 0.348 | 2.07 | 2.01 | 0.211 | 2.24 | 2.11 | 1.90 | 1.91 | 0.382 | 0.299 |
| Canes (vine⁻¹) | 1.92 | 3.64 | 0.300*** | 2.70 | 2.86 | 0.123* | 1.91 | 1.94 | 3.50 | 3.78 | 0.139* | 0.081* |
| New cane weight (g cane⁻¹) | 106.6 | 55.0 | 16.9*** | 80.8 | 80.8 | 9.87 | 107.7 | 105.5 | 53.8 | 56.2 | 18.4 | 14.0 |
| Total weight old canes (kg vine⁻¹) | 0.258 | 0.462 | 0.056*** | 0.344 | 0.376 | 0.068 | 0.244 | 0.272 | 0.444 | 0.480 | 0.083 | 0.097 |

[#1] P<0.001 ***, P<0.01 **, P<0.05 *
[#2] For means at the same level of pruning treatment

**Fruit zone light and temperature results**

Data for the following analysis was supplied courtesy by Mr. R. Titheridge (Visiting Fellow, Marlborough Wine Research Centre). The data was collected over an 8-hour period from 8:00 am to 4:00 pm on 22 March, 2005. The readings were taken from row 778 (4-cane vines) for both 50% and 100% exposure treatments. It was assumed that there would be no significant difference in berry exposure of 2-cane and 4-cane pruning treatments due to the 100% leaf removal imposed on the trial vines. During the day, weather was variable, generally overcast with breaks of sunny intervals. At 2:00pm the weather was noticeably cooler, at 2:30pm there was a cool westerly breeze and at 3:00pm there was northerly breeze. However, it was during the cooler weather and cool westerly breeze that the highest berry temperatures were recorded during the recording period (Table 5).

On two occasions exposure treatment had a significant effect on berry surface temperature (Table 5). The average temperature measured from berries were generally, but not always, higher under the 100% exposure treatment. However, when significant differences occurred (10:00 to 10:41 and 14:03 to 14:21), berries under the 100% exposure treatment were warmer than those under the 50% exposure treatment. Differences in temperature were greatest between 14:03 to 14:21 when the light intensity was greatest (Table 5).

There were highly significant differences between exposure treatments on all light reading occasions taken over the day (Table 5). Light intensity was approximately 2-3 times greater for 100% exposed berries in comparison with 50% exposed berries. Similarly, there were highly significant differences in the proportion of ambient light received by berries under each exposure treatment (Table 5). The proportion of ambient light received by 50% exposed berries was much lower. However, the proportion of light received in both

treatments increased during the day up to the 14.03 to 14.21 measurement period before

declining in the 16.00 to 16.25 measurement period.

**Table 5.** The effect of exposure on berry temperature, berry incident light intensity and the proportion of ambient light incident at the berry recorded on the 22nd March 2005 (Solar Noon at 13:33:07 +/- 15s (Internetworks, 2007))(cont'd below).

| Measurement Period | Berry surface temperature (°C) | | | Berry incident light intensity (klx) | | | Prop. ambient light at berry (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 50% | 100% | $LSD_{0.05}$[1] | 50% | 100% | $LSD_{0.05}$[1] | 50% | 100% | $LSD_{0.05}$[1] |
| 8:00 – 8:53 Overcast and light cloud | 15.7 | 14.8 | 1.99 | 2.5 | 6.7 | 2.02** | 8.7 | 26.4 | 4.3*** |
| 10:00 – 10:41 Sun piercing cloud 10:00-10:30, cloud over sun | 18.4 | 19.1 | 0.69* | 4.6 | 11.8 | 1.81*** | 9.2 | 23.7 | 6.2*** |
| 12:00 – 12:22 Sun behind cloud | 19.7 | 20.3 | 0.69 | 5.4 | 11.8 | 1.13*** | 10.3 | 24.9 | 2.3*** |
| 14:03 – 14:21 Overcast, light cloud sun not visible | 24.2 | 26.5 | 1.15** | 9.8 | 28.0 | 8.23*** | 14.8 | 35.5 | 3.1*** |

[1] $P<0.001$ ***, $P<0.01$ **, $P<0.05$ *

**Table 5 (cont'd).** The effect of exposure on berry temperature, berry incident light intensity and the proportion of ambient light incident at the berry recorded on the 22nd March 2005 (Solar Noon at 13:33:07 +/- 15s (Internetworks, 2007)).

| Measurement Period | Berry surface temperature (°C) | | | Berry incident light intensity (klx) | | | Prop. ambient light at berry (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 50% | 100% | $LSD_{0.05}$[#1] | 50% | 100% | $LSD_{0.05}$[#1] | 50% | 100% | $LSD_{0.05}$[#1] |
| 16:00 – 16:25 | 19.1 | 18.9 | 0.32 | 4.7 | 13.2 | 1.22*** | 13.3 | 36.6 | 2.9*** |
| Light cloud, sun visible | | | | | | | | | |

[#1] $P<0.001$ ***, $P<0.01$ **, $P<0.05$ *

## Harvest results

Two harvest dates were chosen, one for each pruning treatment. Two separate harvest dates were chosen to achieve similar fruit sugar concentration of 21.5 °Brix. Harvest for 2-cane vines was 7 April, 2005 and for 4-cane vines almost a week later, 13 April, 2005.

Statistical analysis of the harvest data showed that there were significant differences (P<0.01) in yield between the pruning treatments (Table 6), as expected. Thus, yields per vine (healthy, diseased and total) from 4-cane vines were almost double those from 2-cane vines. The proportion of diseased fruit per vine was higher from 4-cane vines but this was not statistically significant (P>0.05). Exposure level had a significant influence on 4-cane disease incidence (Table 6). Crop loss to disease was significantly higher in 4-cane 50% exposure treatments than 100% exposure treatment of the same pruning level (Table 6). There was no effect (P>0.05) of pruning treatment on average bunch weight (115 g).

In contrast, the exposure treatment was found to have some effect on yield (Table 6). Average bunch weight was somewhat greater for the 50% compared to the 100% exposure treatment (119 g and 112 g, respectively) although the difference was not statistically significant. Although yields per vine (healthy, diseased and total) from the 50% exposure treatment were to some extent greater those from the 100% exposure treatment, this was only statistically significant for the weight of diseased fruit (P<0.05).

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**Table 6.** Data collected at fruit harvest: on 7 and 13 April, 2005 for 2-cane and 4-cane vines, respectively.

| | Pruning treatments | | | Exposure treatments | | | All treatments | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2-cane | 4-cane | $LSD_{0.05}$[1] | 50% | 100% | $LSD_{0.05}$[1] | 2-cane 50% | 2-cane 100% | 4-cane 50% | 4-cane 100% | $LSD_{0.05}$[1] | $LSD_{0.05}$[1,2] |
| Bunches (vine$^{-1}$) | 33.8 | 58.6 | 4.27*** | 46.0 | 46.4 | 2.52 | 33.0 | 34.7 | 59.0 | 58.1 | 4.65 | 3.57 |
| Healthy bunches (vine$^{-1}$) | 30.5 | 49.4 | 3.79*** | 38.7 | 41.2 | 3.08 | 30.0 | 31.0 | 47.4 | 51.3 | 4.54 | 4.35 |
| Diseased bunches (vine$^{-1}$) | 3.4 | 9.2 | 1.86*** | 7.3 | 5.2 | 1.96* | 3.0 | 3.7 | 11.6 | 6.7 | 2.50** | 2.76** |
| Healthy fruit weight (kg vine$^{-1}$) | 3.38 | 5.55 | 0.390*** | 4.49 | 4.44 | 0.444 | 3.37 | 3.39 | 5.62 | 5.48 | 0.551 | 0.628 |
| Diseased fruit weight (kg vine$^{-1}$) | 0.51 | 1.15 | 0.310** | 0.97 | 0.69 | 0.272* | 0.47 | 0.55 | 1.47 | 0.83 | 0.383* | 0.385* |
| Total fruit weight (kg vine$^{-1}$) | 3.89 | 6.70 | 0.438*** | 5.46 | 5.13 | 0.386 | 3.84 | 3.94 | 7.09 | 6.31 | 0.541* | 0.545* |
| Healthy fruit (% w/w) | 86.8 | 83.3 | 3.86 | 83.4 | 86.7 | 5.10 | 87.2 | 86.4 | 79.6 | 87.0 | 6.00 | 7.22 |
| Diseased fruit (% w/w) | 13.2 | 16.7 | 3.86 | 16.6 | 13.3 | 5.10 | 12.8 | 13.6 | 20.4 | 13.0 | 6.00 | 7.22 |
| Bunch weight (g bunch$^{-1}$) | 115 | 115 | 8.6 | 119 | 112 | 7.1 | 117 | 114 | 120 | 109 | 10.3 | 10.0 |

[1] P<0.001 ***, P<0.01 **, P<0.05 *
[2] For means at the same level of pruning treatment

## Berry development

Although quantitative data were not collected, it was apparent by visual inspection that berry colour was affected by exposure treatments. Berries that had 50% exposure were greener and had less brown, patchy discolouration at harvest in comparison with 100% exposed berries (Fig. 6). Furthermore, berries on the outside of the bunch from the 100% exposure treatment were more discoloured than the internal berries. Frozen berries retained these colour differences, with 100% exposure berries being more yellow and 50% exposure berries being greener. The juice from the berries reflected these colour differences. During sampling it was noted that juice from the exposed berries had a yellow hue, whereas juice from the shaded berries was bright lime, green.



**Figure 6**. Grapes from 50% and 100% exposure treatments, left and right, respectively (courtesy of Dr. Jeff Bennett).

Anecdotal observations during sampling suggested that the flavour of berries from 50% and 100% exposure treatments differed. Berries from 100% exposed treatment seemed to

have riper flavour characteristics with stronger passionfruit and cat's pee integration in flavour profile. In addition, exposed berries had a less harsh acidic flavour. The berry skins from the 100% exposure treatment were thought to be sweeter and more aromatic in flavour the influence of the skin was thought to be important for the overall berry flavour. Also, greater discolouration e.g. brown and patchy the skin, appeared to be associated with riper flavours. Therefore, flavour variation of individual berries within bunches was evident with level of exposure. Fruit from 2-cane treatment tasted sweeter and riper than 4-cane fruit leading up to harvest. Flavour was assessed on a purely informal manner over the ripening period.

**Berry developmental stages**

Flowering occurred from 6 December, 2004 to mid-January, 2005. In general most grape growing areas in New Zealand had a poor fruit-set in the 2005 season due to cold weather (Dr. M. Trought, *pers. comm.*). However, due to high cropping ability, Marlborough Sauvignon blanc attained reasonable crop levels regardless of the cool flowering period. Veraison was predicted to occur around 20 February (Dr. M. Trought, *pers. comm.*) and in this study was found to occur around the 22 February 2005 (Tables 7 & 8). The onset of veraison was approximately four days later in 4-cane than 2-cane fruit (Tables 7 & 8).

**Berry weight**

Throughout the monitored period (pre-bunch closure to harvest), 2-cane berries were consistently heavier than 4-cane berries (Table 7). Statistical analysis revealed that there was a significant difference ($P<0.05$) in berry weight between 2-cane and 4-cane berries on four occasions during the ripening period. On 4 April 2005, 3 days before harvest of the 2-cane vines, berries from the 2-cane treatments were approximately 5% heavier than those from

the 4-cane treatments. In contrast, berry weight was not influenced by exposure treatment

(Table 7).

**Table 7.** Mean berry weight (g) on dates up to harvest.

| Sampling date | Pruning treatments | | | Exposure treatments | | | All treatments | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2-cane | 4-cane | $LSD_{0.05}$[1] | 50% | 100% | $LSD_{0.05}$[1] | 2-cane 50% | 2-cane 100% | 4-cane 50% | 4-cane 100% | $LSD_{0.05}$[1] | $LSD_{0.05}$[1,2] |
| 16 Feb 2005 | 0.86 | 0.82 | 0.061 | 0.83 | 0.85 | 0.034 | 0.82 | 0.89 | 0.83 | 0.81 | 0.064* | 0.048* |
| 22 Feb 2005 | 0.92 | 0.84 | 0.089 | 0.87 | 0.88 | 0.049 | 0.90 | 0.93 | 0.85 | 0.83 | 0.096 | 0.070 |
| 24 Feb 2005 | 1.02 | 0.93 | 0.065* | 0.97 | 0.99 | 0.044 | 1.00 | 1.05 | 0.95 | 0.92 | 0.073 | 0.062 |
| 02 Mar 2005 | 1.23 | 1.17 | 0.056 | 1.17 | 1.23 | 0.040** | 1.20 | 1.26 | 1.14 | 1.21 | 0.064 | 0.056 |
| 04 Mar 2005 | 1.21 | 1.12 | 0.064* | 1.15 | 1.18 | 0.044 | 1.18 | 1.24 | 1.13 | 1.11 | 0.072 | 0.062 |
| 08 Mar 2005 | 1.38 | 1.29 | 0.051** | 1.34 | 1.33 | 0.040 | 1.37 | 1.38 | 1.30 | 1.28 | 0.060 | 0.056 |
| 16 Mar 2005 | 1.51 | 1.49 | 0.061 | 1.50 | 1.50 | 0.054 | 1.51 | 1.50 | 1.48 | 1.51 | 0.076 | 0.076 |
| 22 Mar 2005 | 1.60 | 1.57 | 0.057 | 1.62 | 1.55 | 0.052* | 1.63 | 1.57 | 1.61 | 1.53 | 0.072 | 0.074 |
| 31 Mar 2005 | 1.81 | 1.80 | 0.070 | 1.79 | 1.82 | 0.067 | 1.81 | 1.81 | 1.77 | 1.83 | 0.090 | 0.094 |
| 04 Apr 2005 | 1.88 | 1.79 | 0.037*** | 1.83 | 1.84 | 0.056 | 1.85 | 1.90 | 1.81 | 1.77 | 0.064 | 0.080 |
| 12 Apr 2005[3] | | | | 1.70 | 1.72 | 0.058 | | | | | | |

[1] P<0.001 ***, P<0.01 **, P<0.05 *
[2] For means at the same level of pruning treatment
[3] Data for 4-cane treatments only; sampling data was after 2-cane vine harvest

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**Berry composition**

*Soluble solids*

Soluble solids accumulation was measured from pre-bunch closure to harvest in the 30-berry samples. The concentration of soluble solids in berries at pre-bunch closure was around 4-6 °Brix and increased steadily until verasion when soluble solids reached approximately 11 °Brix. After veraison, soluble solids accumulation was more rapid. The onset of rapid soluble solids accumulation at veraison was approximately four days later in 4-cane than 2-cane berries. In addition, the accumulation rate was higher in 2-cane than in 4-cane pruned vines berries post veraison.

On 12 April 2005, the day before harvest, 4-cane vine fruit had not reached the designated target maturity level of 21.5 °Brix. On a per berry basis, 4-cane berries were over two weeks behind 2-cane berries in soluble solids accumulation. Furthermore, soluble solids accumulation in 4-cane berries slowed toward the end of the ripening period whereas this was not true for 2-cane fruit.

Statistical analysis indicated that there were significant differences ($P<0.05$) between 2-cane and 4-cane soluble solids accumulation from veraison on a per berry basis (Table 9). On average, the mass of soluble solids per berry in the period immediately post-veraison was approximately 17 percent greater in 2-cane compared to 4-cane fruit.

Closer to harvest, results continued to show significant differences in soluble solids per berry and the concentration of soluble solids between 2-cane and 4-cane berries. Berries from 2-cane vines had approximately 18 percent more soluble solids per berry than 4-cane vine berries. Soluble solids concentration within berries was also higher in 2-cane vine fruit.

**Table 8.** Soluble solids concentration (°Brix) in 30-berry samples on dates up to harvest.

| Sampling date | Pruning treatments | | | Exposure treatments | | | All treatments | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2-cane | 4-cane | LSD$_{0.05}$[1] | 50% | 100% | LSD$_{0.05}$[1] | 2-cane 50% | 2-cane 100% | 4-cane 50% | 4-cane 100% | LSD$_{0.05}$[1] | LSD$_{0.05}$[1,2] |
| 16 Feb 2005 | 4.43 | 4.50 | 0.365 | 4.46 | 4.47 | 0.150 | 4.49 | 4.36 | 4.43 | 4.58 | 0.378 | 0.212 |
| 22 Feb 2005 | 5.62 | 5.35 | 0.309 | 5.36 | 5.61 | 0.292 | 5.54 | 5.70 | 5.18 | 5.53 | 0.395 | 0.413 |
| 24 Feb 2005 | 7.04 | 6.70 | 0.465 | 6.71 | 7.03 | 0.334 | 6.96 | 7.11 | 6.45 | 6.95 | 0.534 | 0.473 |
| 02 Mar 2005 | 9.49 | 8.78 | 0.401** | 9.08 | 9.19 | 0.389 | 9.34 | 9.65 | 8.81 | 8.74 | 0.519 | 0.550 |
| 04 Mar 2005 | 11.22 | 9.97 | 0.797** | 10.58 | 10.61 | 0.604 | 11.21 | 11.22 | 9.95 | 10.00 | 0.930 | 0.855 |
| 08 Mar 2005 | 12.93 | 11.70 | 0.348*** | 12.34 | 12.29 | 0.647 | 13.07 | 12.79 | 11.60 | 11.80 | 0.702 | 0.915 |
| 16 Mar 2005 | 15.89 | 14.28 | 0.817** | 14.97 | 15.19 | 0.789 | 15.77 | 16.00 | 14.17 | 14.39 | 1.055 | 1.116 |
| 22 Mar 2005 | 18.93 | 16.67 | 0.461*** | 17.97 | 17.63 | 0.750 | 19.07 | 18.79 | 16.86 | 16.48 | 0.835 | 1.061 |
| 31 Mar 2005 | 20.41 | 18.28 | 0.966** | 19.52 | 19.16 | 0.666 | 20.75 | 20.06 | 18.30 | 18.26 | 1.094 | 0.942 |
| 04 Apr 2005 | 21.33 | 18.84 | 0.922*** | 20.23 | 19.93 | 0.345 | 21.43 | 21.23 | 19.04 | 18.64 | 0.947 | 0.487 |
| 12 Apr 2005[3] | | | | 20.59 | 20.77 | 0.640 | | | | | | |

[1] P<0.001 ***, P<0.01 **, P<0.05 *
[2] For means at the same level of pruning treatment
[3] Data for 4-cane treatments only; sampling data was after 2-cane vine harvest

**Table 9.** Soluble solids content (mg/berry) in 30-berry samples on dates up to harvest.

| Sampling date | Pruning treatments | | | Exposure treatments | | | All treatments | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2-cane | 4-cane | LSD$_{0.05}$[1] | 50% | 100% | LSD$_{0.05}$[1] | 2-cane 50% | 2-cane 100% | 4-cane 50% | 4-cane 100% | LSD$_{0.05}$[1] | LSD$_{0.05}$[1,2] |
| 16 Feb 2005 | 37.8 | 36.7 | 4.30 | 36.9 | 37.6 | 2.17 | 37.0 | 38.6 | 36.7 | 36.7 | 4.43 | 3.07 |
| 22 Feb 2005 | 51.5 | 44.8 | 4.42** | 46.8 | 49.5 | 4.02 | 49.7 | 53.2 | 43.9 | 45.8 | 5.54 | 5.68 |
| 24 Feb 2005 | 72.1 | 62.6 | 7.55* | 65.4 | 69.3 | 4.69 | 69.7 | 74.4 | 61.0 | 64.2 | 8.32 | 6.63 |
| 02 Mar 2005 | 117 | 103 | 8.1** | 107 | 113 | 7.1 | 112 | 121 | 101 | 105 | 10.0 | 10.1 |
| 04 Mar 2005 | 136 | 112 | 13.3** | 122 | 125 | 9.8 | 132 | 139 | 112 | 112 | 15.4 | 13.9 |
| 08 Mar 2005 | 178 | 152 | 9.0*** | 166 | 164 | 10.1 | 180 | 177 | 152 | 152 | 12.6 | 14.3 |
| 16 Mar 2005 | 239 | 213 | 17.7* | 224 | 228 | 16.3 | 239 | 240 | 210 | 217 | 22.3 | 23.0 |
| 22 Mar 2005 | 303 | 262 | 12.9*** | 292 | 274 | 19.0 | 311 | 295 | 272 | 253 | 21.7 | 26.9 |
| 31 Mar 2005 | 370 | 329 | 25.8** | 350 | 349 | 20.1 | 376 | 363 | 324 | 335 | 30.4 | 28.4 |
| 04 Apr 2005 | 400 | 338 | 19.9*** | 371 | 367 | 12.8 | 396 | 404 | 345 | 330 | 22.1 | 18.0 |
| 12 Apr 2005[3] | | | | 349 | 357 | 8.3 | | | | | | |

[1] P<0.001 ***, P<0.01 **, P<0.05 *
[2] For means at the same level of pruning treatment
[3] Data for 4-cane treatments only; sampling data was after 2-cane vine harvest

*Acidity (pH and titratable acidity)*

In general, both pruning and exposure treatments had little effect on the pH and the titratable acidity of 30-berry samples (Tables 10 & 11). However, there was a significant difference ($P<0.01$) in the pH of 2-cane and 4-cane berry juice in the latter stages of ripening; the juice of 2-cane berries had a slightly higher pH than juice of 4-cane berries, although juice titratable acidity was not significantly affected by pruning treatment ($P>0.05$). There was no significant effect ($P>0.05$) of exposure treatment on the pH or titratable acidity of berry juice.

**Table 10.** The pH of juice from 30-berry samples on dates up to harvest.

| Sampling date | Pruning treatments | | | Exposure treatments | | | All treatments | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2-cane | 4-cane | LSD$_{0.05}$[1] | 50% | 100% | LSD$_{0.05}$[1] | 2-cane 50% | 2-cane 100% | 4-cane 50% | 4-cane 100% | LSD$_{0.05}$[1] | LSD$_{0.05}$[1,2] |
| 16 Feb 2005 | 2.52 | 2.55 | 0.045 | 2.54 | 2.53 | 0.020 | 2.53 | 2.52 | 2.56 | 2.54 | 0.046 | 0.018 |
| 22 Feb 2005 | 2.47 | 2.46 | 0.016 | 2.46 | 2.47 | 0.014 | 2.46 | 2.47 | 2.45 | 2.46 | 0.020 | 0.019 |
| 24 Feb 2005 | 2.51 | 2.51 | 0.015 | 2.51 | 2.51 | 0.013 | 2.50 | 2.52 | 2.51 | 2.51 | 0.019 | 0.019 |
| 02 Mar 2005 | 2.55 | 2.55 | 0.022 | 2.55 | 2.56 | 0.022 | 2.55 | 2.56 | 2.55 | 2.55 | 0.029 | 0.031 |
| 04 Mar 2005 | 2.58 | 2.54 | 0.037* | 2.57 | 2.56 | 0.034 | 2.59 | 2.58 | 2.55 | 2.53 | 0.047 | 0.048 |
| 08 Mar 2005 | 2.71 | 2.70 | 0.018 | 2.71 | 2.70 | 0.025 | 2.70 | 2.72 | 2.71 | 2.69 | 0.029 | 0.036 |
| 16 Mar 2005 | 2.77 | 2.76 | 0.047 | 2.77 | 2.76 | 0.029 | 2.78 | 2.77 | 2.76 | 2.75 | 0.051 | 0.041 |
| 22 Mar 2005 | 2.88 | 2.85 | 0.021** | 2.88 | 2.85 | 0.015** | 2.89 | 2.87 | 2.86 | 2.83 | 0.024 | 0.022 |
| 31 Mar 2005 | 2.96 | 2.92 | 0.026** | 2.94 | 2.95 | 0.028 | 2.95 | 2.97 | 2.92 | 2.93 | 0.036 | 0.040 |
| 04 Apr 2005 | 2.91 | 2.86 | 0.033** | 2.89 | 2.88 | 0.038 | 2.92 | 2.90 | 2.87 | 2.85 | 0.047 | 0.053 |
| 12 Apr 2005[3] | | | | 3.00 | 3.03 | 0.029 | | | | | | |

[1] $P < 0.001$ ***, $P < 0.01$ **, $P < 0.05$ *
[2] For means at the same level of pruning treatment
[3] Data for 4-cane treatments only; sampling data was after 2-cane vine harvest

**Table 11.** Titratable acidity (g/L as tartaric acid) in 30-berry samples on dates up to harvest.

| Sampling date | Pruning treatments | | | Exposure treatments | | | All treatments | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2-cane | 4-cane | LSD$_{0.05}$[1] | 50% | 100% | LSD$_{0.05}$[1] | 2-cane 50% | 2-cane 100% | 4-cane 50% | 4-cane 100% | LSD$_{0.05}$[1] | LSD$_{0.05}$[1,2] |
| 16 Feb 2005 | 36.0 | 36.6 | 1.94 | 35.9 | 36.7 | 1.00 | 35.6 | 36.4 | 36.2 | 37.0 | 2.07 | 1.42 |
| 22 Feb 2005 | 37.5 | 37.8 | 1.68 | 37.6 | 37.7 | 0.60 | 37.7 | 37.3 | 37.5 | 38.1 | 1.72 | 0.84 |
| 24 Feb 2005 | 34.7 | 34.4 | 1.69 | 34.9 | 34.1 | 0.61* | 35.0 | 34.3 | 34.8 | 33.9 | 1.73 | 0.86 |
| 02 Mar 2005 | 28.4 | 29.2 | 1.55 | 29.6 | 28.1 | 1.17* | 28.9 | 28.0 | 30.3 | 28.2 | 1.81 | 1.66 |
| 04 Mar 2005 | 25.9 | 26.7 | 1.95 | 26.1 | 26.5 | 1.67 | 25.4 | 26.4 | 26.8 | 26.6 | 2.38 | 2.36 |
| 08 Mar 2005 | 18.8 | 19.1 | 1.16 | 19.4 | 18.5 | 1.46 | 19.2 | 18.4 | 19.6 | 18.6 | 1.75 | 2.07 |
| 16 Mar 2005 | 14.9 | 14.7 | 1.68 | 14.9 | 14.6 | 0.74 | 15.1 | 14.6 | 14.8 | 14.6 | 1.76 | 1.05 |
| 22 Mar 2005 | 11.8 | 12.1 | 0.55 | 11.9 | 12.0 | 0.74 | 11.7 | 12.0 | 12.1 | 12.0 | 0.87 | 1.05 |
| 31 Mar 2005 | 10.4 | 10.2 | 0.65 | 10.3 | 10.3 | 0.53 | 10.4 | 10.4 | 10.2 | 10.2 | 0.78 | 0.75 |
| 04 Apr 2005 | 10.3 | 10.4 | 0.82 | 10.5 | 10.2 | 0.53 | 10.3 | 10.2 | 10.7 | 10.1 | 0.91 | 0.74 |
| 12 Apr 2005[3] | | | | 9.7 | 8.8 | 0.59* | | | | | | |

[1] P<0.001 ***, P<0.01 **, P<0.05 *
[2] For means at the same level of pruning treatment
[3] Data for 4-cane treatments only; sampling data was after 2-cane vine harvest

***Quercetin***

Quercetin concentrations were determined on whole-bunch samples taken on a number of selected dates representing pre-veraison, close to veraison, post-veraison and harvest (both 2-cane and 4-cane harvest dates). Quercetin concentrations in berries decreased from the first sampling occasion until the third sampling occasion after which quercetin concentrations showed an increased (i.e. at harvest).

There were differences in quercetin concentrations as a result of both pruning and exposure treatments (Table 12). For all the sampling occasions analysed, exposure treatments showed highly significant differences ($P<0.001$) in quercetin concentrations with berries from 100% exposed treatments having more than double the concentration of 50% exposed treatments.

**Table 12.** Quercetin concentration (µg/g) in whole-bunch samples on selected dates up to harvest.

| Sampling date | Pruning treatments | | | Exposure treatments | | | All treatments | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2-cane | 4-cane | $\text{LSD}_{0.05}$[1] | 50% | 100% | $\text{LSD}_{0.05}$[1] | 2-cane 50% | 2-cane 100% | 4-cane 50% | 4-cane 100% | $\text{LSD}_{0.05}$[1] | $\text{LSD}_{0.05}$[1,2] |
| 15 Feb 2005 | 3.25 | 2.53 | 0.79 | 1.53 | 4.25 | 0.74*** | 1.61 | 4.88 | 1.44 | 3.62 | 1.00 | 1.04 |
| 08 Mar 2005 | 2.85 | 2.38 | 0.42* | 1.54 | 3.69 | 0.42*** | 1.65 | 4.06 | 1.43 | 3.33 | 0.55 | 0.59 |
| 22 Mar 2005 | 2.24 | 1.65 | 0.34** | 1.10 | 2.79 | 0.36*** | 1.29 | 3.18 | 0.91 | 2.40 | 0.46 | 0.52 |
| 04 April 2005 | 2.17 | 2.74 | 0.45* | 1.80 | 3.11 | 0.28*** | 1.48 | 2.86 | 2.12 | 3.35 | 0.49 | 0.39 |
| 12 April 2005[3] | | | | 1.87 | 4.84 | 0.59*** | | | | | | |

[1] P<0.001 ***, P<0.01 **, P<0.05 *
[2] For means at the same level of pruning treatment
[3] Data for 4-cane treatments only; sampling data was after 2-cane vine harvest

*Organic acids*

The concentrations of malic and tartaric acids were determined on whole-bunch samples taken on a number of selected dates (Tables 13 & 14). Some values are missing due to accidental sample loss.

Malic acid concentrations in berries decreased during ripening. Pruning treatment had no significant effect ($P > 0.05$) on malic acid concentration in berries. However, on two dates pruning treatment had a significant effect ($P < 0.05$) on tartaric acid concentrations: namely, around veraison and at mid-ripening (8 and 22 March, 2005, respectively). On these two sampling occasions 4-cane fruit had greater concentrations of tartaric acid.

In contrast, there was no effect ($P > 0.05$) of exposure treatments on tartaric acid concentration but significant differences ($P < 0.01$) in malic acid concentration. Fruit from the 100% exposure treatments had lower concentrations of malic acid than that from the 50% exposure treatments.

**Table 13.** Malic acid concentration (g/L) in whole-bunch samples on selected dates up to harvest.

| Sampling date | Pruning treatments | | | Exposure treatments | | | All treatments | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2-cane | 4-cane | LSD$_{0.05}$[1] | 50% | 100% | LSD$_{0.05}$[1] | 2-cane 50% | 2-cane 100% | 4-cane 50% | 4-cane 100% | LSD$_{0.05}$[1] | LSD$_{0.05}$[1,2] |
| 15 Feb 2005 | 16.1 | 15.7 | 1.45 | 15.7 | 16.1 | 1.29 | 16.0 | 16.3 | 15.4 | 15.9 | 1.80 | 1.83 |
| 08 Mar 2005 | 9.1 | 10.8 | 2.34 | 10.9 | 9.0 | 0.86 | Not calculated because missing data resulted in non-orthogonal components. | | | | | |
| 22 Mar 2005 | 5.08 | 5.65 | 0.666 | 5.65 | 5.08 | 0.390** | 5.26 | 4.91 | 6.05 | 5.25 | 0.725 | 0.551 |
| 04 Apr 2005 | 4.27 | 4.26 | 0.792 | 4.98 | 3.55 | 0.517*** | 4.85 | 3.70 | 5.12 | 3.41 | 0.884 | 0.731 |
| 12 Apr 2005[3] | | | | 4.94 | 3.64 | 0.607** | | | | | | |

[1] P<0.001 ***, P<0.01 **, P<0.05 *
[2] For means at the same level of pruning treatment
[3] Data for 4-cane treatments only; sampling data was after 2-cane vine harvest

**Table 14.** Tartaric acid concentration (g/L) in whole-bunch samples on selected dates up to harvest.

| Sampling date | Pruning treatments | | | Exposure treatments | | | All treatments | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2-cane | 4-cane | $LSD_{0.05}$[1] | 50% | 100% | $LSD_{0.05}$[1] | 2-cane 50% | 2-cane 100% | 4-cane 50% | 4-cane 100% | $LSD_{0.05}$[1] | $LSD_{0.05}$[1,2] |
| 15 Feb 2005 | 12.5 | 13.1 | 1.00 | 12.6 | 13.0 | 0.69 | 12.2 | 12.8 | 13.0 | 13.1 | 1.13 | 0.98 |
| 08 Mar 2005 | 6.23 | 6.85 | 0.458* | 6.51 | 6.53 | 0.617 | Not calculated because missing data resulted in non-orthogonal components. | | | | | |
| 22 Mar 2005 | 4.86 | 5.14 | 0.257* | 4.98 | 5.03 | 0.277 | 5.00 | 4.73 | 4.95 | 5.33 | 0.352* | 0.392* |
| 04 Apr 2005 | 4.82 | 5.15 | 0.576 | 4.89 | 5.08 | 0.601 | 4.73 | 4.91 | 5.05 | 5.26 | 0.774 | 0.850 |
| 12 Apr 2005[3] | | | | 4.41 | 4.67 | 0.347 | | | | | | |

[1] $P<0.001$ ***, $P<0.01$ **, $P<0.05$ *
[2] For means at the same level of pruning treatment
[3] Data for 4-cane treatments only; sampling data was after 2-cane vine harvest

***Methoxypyrazines***

The concentrations of iso-butyl and iso-propyl methoxypyrazines (IBMP and IPMP, respectively) were determined on whole-bunch samples taken on a number of selected dates (Tables 15 & 16). Methoxypyrazine concentrations decreased during sampling. The rate of decrease was sharp between pre-veraison sampling and veraison then slowed from veraison onward.

Pruning treatment had no consistent effect on either IBMP or IPMP concentration in berries. IBMP was significantly higher ($P < 0.05$) at harvest in the 2-cane treatments than 4-cane treatments, with the greatest concentration in the 2-cane 50% exposed berries. There was a statistically significant effect ($P < 0.01$) of exposure on IPMP concentration on one sampling occasion (22 March 2005, post-veraison) with higher concentration in the less exposed berries of each pruning treatment.

**Table 15.** Iso-butyl methoxypyrazine concentration (ng/L) in whole-bunch samples on selected dates up to harvest.

| Sampling date | Pruning treatments | | | Exposure treatments | | | All treatments | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | 2-cane | 2-cane | 4-cane | 4-cane | | |
| | 2-cane | 4-cane | LSD$_{0.05}$[1] | 50% | 100% | LSD$_{0.05}$[1] | 50% | 100% | 50% | 100% | LSD$_{0.05}$[1] | LSD$_{0.05}$[1,2] |
| 15 Feb 2005 | 47.9 | 47.2 | 8.24 | 47.8 | 47.2 | 8.91 | 49.0 | 46.7 | 46.6 | 47.8 | 11.29 | 12.60 |
| 08 Mar 2005 | 9.1 | 10.4 | 5.15 | 10.4 | 9.0 | 4.05 | 8.2 | 9.9 | 12.7 | 8.1 | 6.09 | 5.73 |
| 22 Mar 2005 | 3.84 | 4.07 | 2.029 | 4.91 | 3.00 | 2.044 | 4.95 | 2.73 | 4.87 | 3.26 | 2.673 | 2.891 |
| Harvest (7 and 13 Apr 2005 for 2-cane and 4-cane, respectively) | 3.26 | 1.51 | 1.731* | 3.04 | 1.73 | 1.510 | 4.57 | 1.94 | 1.51 | 1.52 | 2.125 | 2.135 |

[1] P<0.001 ***, P<0.01 **, P<0.05 *
[2] For means at the same level of pruning treatment

**Table 16.** Iso-propyl methoxypyrazine concentration (ng/L) in whole-bunch samples on selected dates up to harvest.

| Sampling date | Pruning treatments | | | Exposure treatments | | | All treatments | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2-cane | 4-cane | $\text{LSD}_{0.05}$[1] | 50% | 100% | $\text{LSD}_{0.05}$[1] | 2-cane 50% | 2-cane 100% | 4-cane 50% | 4-cane 100% | $\text{LSD}_{0.05}$[1] | $\text{LSD}_{0.05}$[1,2] |
| 15 Feb 2005 | 17.5 | 15.8 | 3.82 | 16.0 | 17.3 | 3.76 | 17.2 | 17.7 | 14.7 | 16.9 | 4.98 | 5.32 |
| 08 Mar 2005 | 2.36 | 2.47 | 1.018 | 2.52 | 2.30 | 0.877 | 2.20 | 2.51 | 2.85 | 2.08 | 1.248 | 1.240 |
| 22 Mar 2005 | 0.63 | 0.77 | 0.348 | 0.91 | 0.48 | 0.308** | 0.86 | 0.40 | 0.97 | 0.56 | 0.431 | 0.436 |
| Harvest (7 and 13 Apr 2005 for 2-cane and 4-cane, respectively) | 0.72 | 0.43 | 0.295 | 0.62 | 0.52 | 0.221 | 0.86 | 0.57 | 0.37 | 0.48 | 0.342 | 0.313 |

[1] $P<0.001$ ***, $P<0.01$ **, $P<0.05$ *
[2] For means at the same level of pruning treatment

## Fermentation results

### Must analyses

It should be noted that different harvest dates were implemented for 2-cane and 4-cane treatments. The must analysis samples were analysed at harvest for each treatment.

The later (one week) harvest date for 4-cane compared to 2-cane fruit did not have significant effect on °Brix. In contrast titratable acidity was significantly lower for the 4-cane must (at equivalent °Brix to 2-cane must) although must samples from 2-cane treatments exhibited higher pH than 4-cane must. However, this could not be related to differences in must potassium content between pruning treatments (Table 17). Potassium concentrations were significantly lower in 4-cane compared with 2-cane must (Table 17).

The concentrations of nitrogenous species in must were influenced by fruit exposure within 4-cane treatments (Table 17). Both FAN (free assimilable nitrogen) and YAN (yeast available nitrogen) were higher in must from the higher exposure treatment within 4-cane treatments. Neither pruning nor exposure treatments influenced ammonia or potassium concentrations in fruit.

**Table 17.** Analyses of must samples.

| Analysis | Pruning treatments | | | Exposure treatments | | | All treatments | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2-cane | 4-cane | LSD$_{0.05}$[1] | 50% | 100% | LSD$_{0.05}$[1] | 2-cane 50% | 2-cane 100% | 4-cane 50% | 4-cane 100% | LSD$_{0.05}$[1] | LSD$_{0.05}$[1,2] |
| Soluble solids (°Brix) | 21.3 | 21.0 | 0.79 | 21.1 | 21.2 | 0.40 | 21.2 | 21.3 | 20.9 | 21.1 | 0.74 | 0.57 |
| pH | 3.08 | 2.94 | 0.03*** | 3.01 | 3.01 | 0.02 | 3.09 | 3.08 | 2.94 | 2.94 | 0.03 | 0.02 |
| Titratable acidity (g/L as tartaric acid) | 9.49 | 8.54 | 0.39** | 9.24 | 8.78 | 0.29** | 9.61 | 9.36 | 8.87 | 8.21 | 0.40 | 0.42 |
| NH$_4$ (mg/L) | 108 | 115 | 24.6 | 113 | 110 | 16.2 | 105 | 112 | 121 | 108 | 24.0 | 22.9 |
| FAN (mg/L) | 160.9 | 160.1 | 27.2 | 172 | 149 | 29.9 | 163 | 159 | 182 | 138 | 33.7 | 42.2 |
| YAN (mg/L) | 269 | 275 | 46.7 | 286 | 258 | 44.2 | 268 | 271 | 303 | 246 | 52.7 | 62.4 |
| K (mg/L) | 1133 | 889 | 123** | 1018 | 1004 | 85 | 1119 | 1147 | 918 | 860 | 122 | 120 |

[1] P<0.001 ***, P<0.01 **, P<0.05 *
[2] For means at the same level of pruning treatment

**Progress of fermentation**

There was little influence of crop load or exposure treatment on the rate of fermentation (Figs. 7 & 8). Temperature results indicate that 2-cane fermentation was warmer after the start of fermentation (Fig. 9). However, 4-cane wines began to decline in soluble solids earlier than 2-cane wines after inoculation (Fig. 7). Level of exposure had no significant influence on fermentation progress (Fig. 8). There were a couple of cold weather events that influenced the temperature of must during fermentation. The fermentation room had the facility to reduce temperature but not warm the room. The weather events occurred on days 2 and 17 after inoculation for 4-cane wines and days 14 and 29 for 2-cane wines (Fig. 9).

Daily aroma checks were done on each wine for signs of stuck fermentation. Some characteristic burnt onion aromas were noted in a large proportion of wines to signify struggling ferment. However, the wines fermented to completion with no stuck ferments.
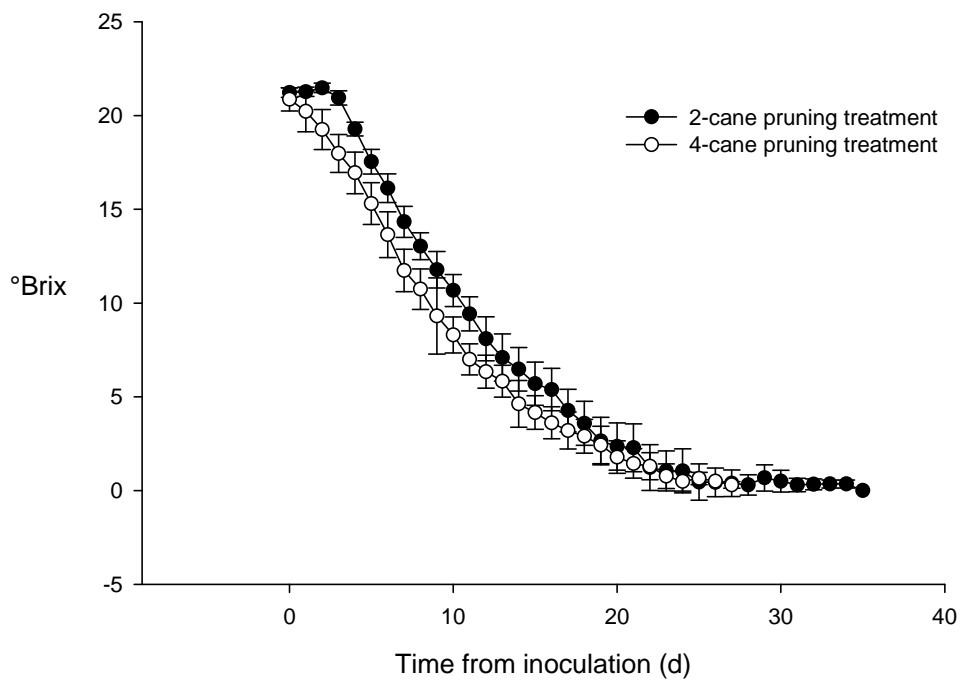
**Figure 7.** The influence of pruning treatments on the course of fermentation.
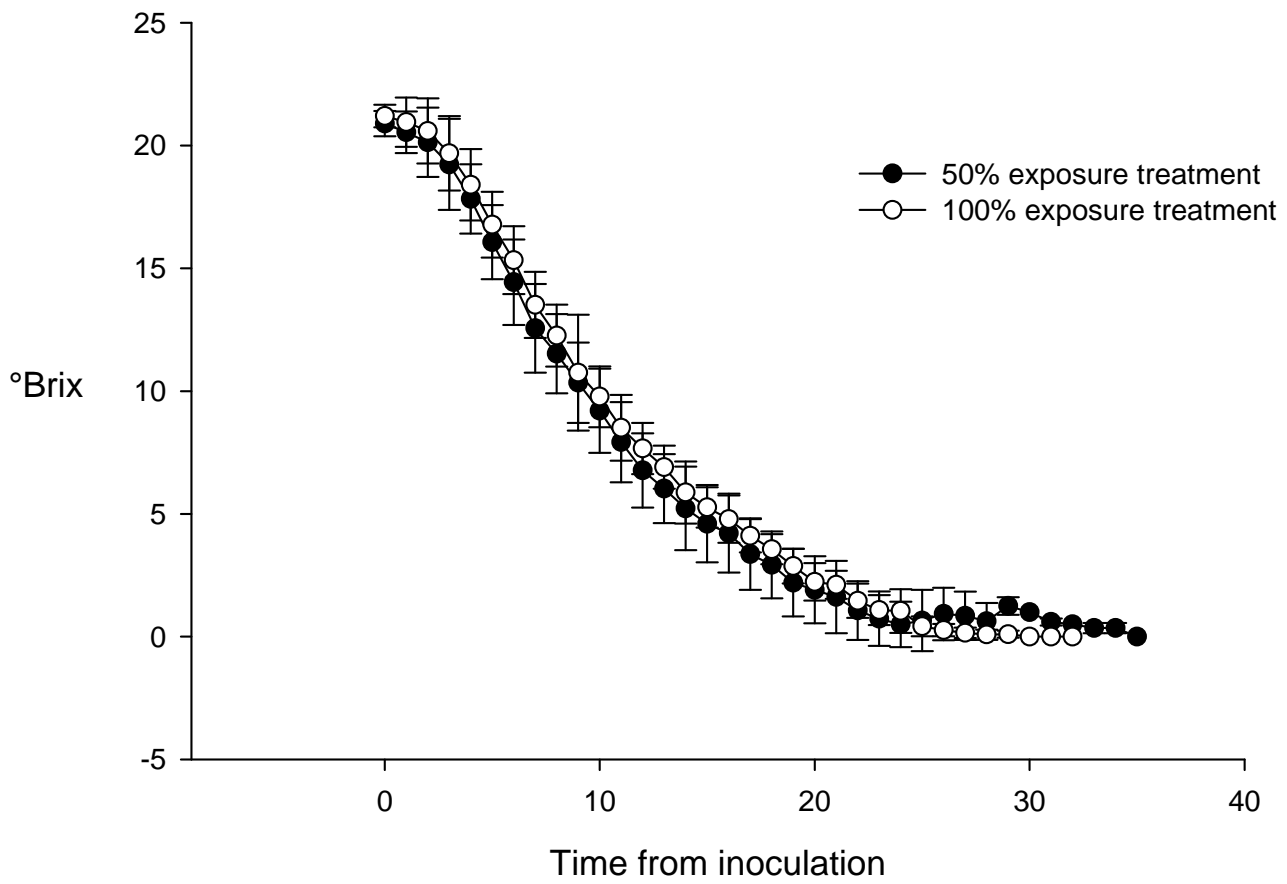
**Figure 8.** The influence of exposure treatments on the course of fermentation.
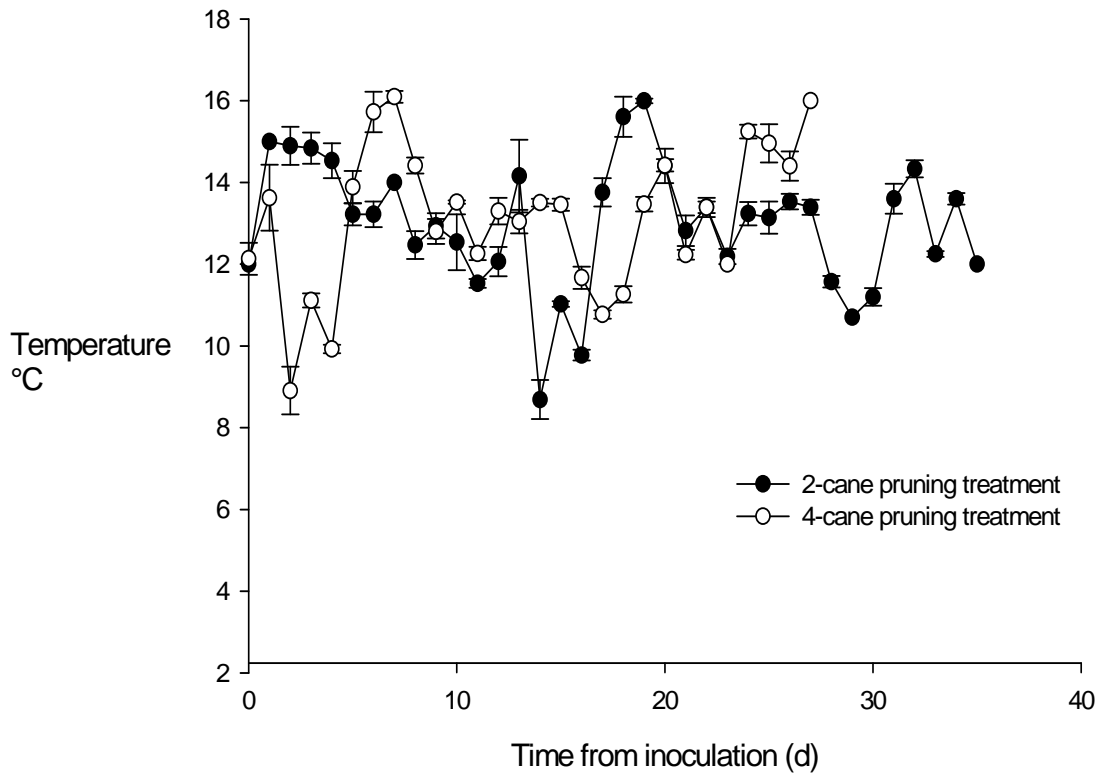
**Figure 9.** Average temperatures during the course of fermentation of 2-cane and 4-cane treatments.

## Wine analyses

### Sulphur dioxide

Although the addition of bentonite had no significant (P>0.05) effect on free $SO_2$ concentration, there was a significant interaction between pruning treatment and bentonite addition; free $SO_2$ concentration was lowest for bentonite-fined wines from the 4-cane treatment and highest for bentonite-fined wines from the 2-cane treatment (Table 18).

It should be noted when interpreting these results that higher addition rates of $SO_2$ were made to 4-cane wines at bottling. Higher rates of $SO_2$ were needed to achieve free sulphur levels 20-25 mg/L in 4-cane wines. This is made evident by the significant differences (P <0.01) in bound and total $SO_2$ concentrations between pruning treatments. Wines from 2-cane vine fruit had about thirty percent less bound $SO_2$ than 4-cane wines (Table 19). Once 20-25 mg/L was achieved at bottling, there were no significant differences in free sulphur reduction between pruning treatments after bottling (Table 18). Bentonite addition significantly (P <0.05) lowered levels of bound sulphur in wines.

**Table 18.** Free $SO_2$ (mg/L) concentration in finished wines. An additional 30% $SO_2$ was added to 4-cane wines.

| Treatment | | Pruning treatments | | Exposure treatments | | Bentonite treatments | | Pruning $LSD_{0.05}$[1] | Exposure $LSD_{0.05}$[1] | Bentonite $LSD_{0.05}$[1] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2-cane | 4-cane | 50% | 100% | (+) | (-) | | | |
| Main | | 12.2 | 10.2 | 12.6 | 9.8 | 11.5 | 10.9 | 5.88 | 3.77 | 1.70 |
| Split plot | 2-cane | | | 12.2 | 12.2 | 13.5 | 10.9 | | 5.69, 5.32[2] | 5.49, 2.40*[2] |
| | 4-cane | | | 13.0 | 7.3 | 9.5 | 10.8 | | | |

| | | Exposure.Bentonite treatments | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 50% (+) | 100% (+) | 50% (-) | 100% (-) | | | | | |
| Split-split plot | 2-cane | 13.2 | 13.8 | 11.2 | 10.6 | | | 5.90, 5.51[2] | 5.90, 3.40[3] | 5.90, 5.51[4] |
| | 4-cane | 12.8 | 6.2 | 13.2 | 8.4 | | | | | |

[1] P<0.001 ***, P<0.01 **, P<0.05 *
[2] For means at the same level of pruning treatment
[3] For means at the same level of pruning and exposure treatment
[4] For means at the same level of pruning and bentonite

**Table 19.** Bound $SO_2$ (mg/L) concentration in finished wines. An additional 30% $SO_2$ was added to 4-cane wines.

| Treatment | | Pruning treatments | | Exposure treatments | | Bentonite treatments | | Pruning | Exposure | Bentonite |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2-cane | 4-cane | 50% | 100% | (+) | (-) | LSD$_{0.05}$[1] | LSD$_{0.05}$[1] | LSD$_{0.05}$[1] |
| Main | | 51.6 | 87.8 | 68.1 | 71.3 | 68.6 | 70.8 | 12.05** | 10.21 | 1.80* |
| Split plot | 2-cane | | | 51.9 | 51.2 | 51.3 | 51.8 | | 12.87, 14.44[2] | 11.74, 2.54[2] |
| | 4-cane | | | 84.3 | 91.3 | 85.8 | 89.8 | | | |

| | | Exposure.Bentonite treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 50% (+) | 100% (+) | 50% (-) | 100% (-) | | | | |
| Split-split plot | 2-cane | 50.8 | 51.8 | 53.0 | 50.6 | | 12.98, 14.46[2] | 12.98, 3.60[3] | 12.98, 14.46[4] |
| | 4-cane | 81.8 | 89.8 | 86.8 | 92.8 | | | | |

[1] $P<0.001$ ***, $P<0.01$ **, $P<0.05$ *
[2] For means at the same level of pruning treatment
[3] For means at the same level of pruning and exposure treatment
[4] For means at the same level of pruning and bentonite

**Residual sugar**

Wines were analysed for residual sugars at the end of fermentation (Table 20). In general, residual sugars were similar for all treatments. Although there was a statistically significant (P<0.05) effect of exposure treatments on D-fructose concentration, (wine produced from 100% exposure fruit ending fermentation with approximately 6 g/L D-fructose compared to 4 g/L for wine from 2-cane fruit) this difference was relatively minor and would not be expected to markedly influence wine sensory characteristics. The results suggest that the effect of the two separate harvest dates, and hence different periods during which the fermentations took place, did not have a major influence on the outcome of the fermentation at least as far as the metabolising of grape sugars was concerned.

**Table 20.** Residual sugars concentrations (g/L) in wines at the end of fermentation.

| | Pruning treatments | | | Exposure treatments | | | All treatments | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2-cane | 4-cane | LSD$_{0.05}$[1] | 50% | 100% | LSD$_{0.05}$[1] | 2-cane 50% | 2-cane 100% | 4-cane 50% | 4-cane 100% | LSD$_{0.05}$[1] | LSD$_{0.05}$[1,2] |
| D-fructose | 4.1 | 5.7 | 2.91 | 3.8 | 6.0 | 1.32* | 3.6 | 4.6 | 4.0 | 7.4 | 2.70 | 1.86 |
| D-glucose | 0.2 | 0.7 | 0.78 | 0.4 | 0.5 | 0.22 | 0.1 | 0.2 | 0.7 | 0.8 | 0.73 | 0.32 |

[1] $P<0.001$ ***, $P<0.01$ **, $P<0.05$ *
[2] For means at the same level of pruning treatment

**Acidity (pH and titratable acidity)**

Pruning and exposure treatments had no significant effect on (P>0.05) on wine pH (Table 21). This is in contrast to berry and must analysis results which found pH higher in 2-cane pruned fruit and must. Wine processing may have lowered pH in wines. Prior to processing 2-cane mean pH of must was 3.09, post processing wine pH from the same treatment was 3.01. Significant differences between pruning treatments in pH and titratable acidity in must were not reflected in wines post processing.

There were significant effects of both exposure (P<0.05) and bentonite (P<0.001) treatments on titratable acidity. Wine from 100% exposure treatments were significantly lower in titratable acidity compared with 50% exposure treatment wines whereas ripening analysis found no influence of exposure on titratable acidity in fruit or must.

Bentonite addition reduced titratable acidity by 0.3 g/L as tartaric acid but also decreased pH by 0.01 units. Although these effects were statistically significant (P<0.01), the numerical differences were very small and can be considered to have no meaningful consequence for wine chemistry and sensory characteristics.

**Table 21.** pH of finished wines.

| Treatment | | Pruning treatments | | Exposure treatments | | Bentonite treatments | | Pruning | Exposure | Bentonite |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2-cane | 4-cane | 50% | 100% | (+) | (-) | $LSD_{0.05}$[#1] | $LSD_{0.05}$[#1] | $LSD_{0.05}$[#1] |
| Main | | 3.01 | 3.00 | 3.01 | 3.00 | 3.00 | 3.01 | 0.06 | 0.02 | 0.01** |
| Split plot | 2-cane | | | 3.03 | 3.01 | 3.00 | 3.03 | | | |
| | 4-cane | | | 2.99 | 3.00 | 2.99 | 3.00 | | 0.05, 0.02[#2] | 0.05, 0.01*[#2] |

| | | Exposure.Bentonite treatments | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 50% (+) | 100% (+) | 50% (-) | 100% (-) | | | |
| Split-split plot | 2-cane | 3.01 | 3.00 | 3.04 | 3.02 | | | |
| | 4-cane | 2.99 | 3.00 | 2.99 | 3.00 | 0.05, 0.03[#2] | 0.05, 0.02[#3] | 0.05, 0.03[#4] |

[#1] P<0.001 ***, P<0.01 **, P<0.05 *
[#2] For means at the same level of pruning treatment
[#3] For means at the same level of pruning and exposure treatment
[#4] For means at the same level of pruning and bentonite

**Table 22.** Titratable acidity (g/L as tartaric acid) of finished wines.

| Treatment | | Pruning treatments | | Exposure treatments | | Bentonite treatments | | Pruning $LSD_{0.05}$[1] | Exposure $LSD_{0.05}$[1] | Bentonite $LSD_{0.05}$[1] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2-cane | 4-cane | 50% | 100% | (+) | (-) | | | |
| Main | | 8.6 | 8.5 | 8.7 | 8.4 | 8.4 | 8.7 | 0.39 | 0.20* | 0.10*** |
| Split plot | 2-cane | | | 8.7 | 8.5 | 8.4 | 8.8 | | 0.37, 0.28[2] | 0.37, 0.15[2] |
| | 4-cane | | | 8.7 | 8.3 | 8.3 | 8.6 | | | |

| | | Exposure.Bentonite treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 50% (+) | 100% (+) | 50% (-) | 100% (-) | | | | |
| Split-split plot | 2-cane | 8.5 | 8.3 | 8.9 | 8.7 | 0.37, 0.30[2] | 0.37, 0.21[3] | 0.37, 0.30[4] | |
| | 4-cane | 8.5 | 8.2 | 8.9 | 8.4 | | | | |

[1] P<0.001 ***, P<0.01 **, P<0.05 *
[2] For means at the same level of pruning treatment
[3] For means at the same level of pruning and exposure treatment
[4] For means at the same level of pruning and bentonite

**Alcohol**

Regardless of differences in berry sugar concentrations (Table 9) trial wines showed no influence of pruning treatments on wine alcohol levels (Table 23). Exposure showed no significant influence on alcohol concentrations, as expected. Unexpectedly, bentonite addition was the only treatment to influence wine alcohol (P<0.001). Although statistically significant, reductions in alcohol of approximately 0.2 % w/v are thought to have little impact the sensory attributes of the wines.

**Table 23.** Alcohol content (% w/v) of finished wines.

| Treatment | | Pruning treatments | | Exposure treatments | | Bentonite treatments | | Pruning | Exposure | Bentonite |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2-cane | 4-cane | 50% | 100% | (+) | (-) | $LSD_{0.05}$[1] | $LSD_{0.05}$[1] | $LSD_{0.05}$[1] |
| Main | | 13.3 | 12.9 | 13.0 | 13.2 | 13.0 | 13.2 | 0.58 | 0.26 | 0.06*** |
| Split plot | 2-cane | | | 13.1 | 13.4 | 13.2 | 13.3 | | 0.54, 0.36[2] | 0.57, 0.09[2] |
| | 4-cane | | | 12.8 | 13.0 | 12.8 | 13.0 | | | |

| | | Exposure.Bentonite treatments | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 50% (+) | 100% (+) | 50% (-) | 100% (-) | | | | |
| Split-split plot | 2-cane | 13.1 | 13.3 | 13.2 | 13.5 | 0.53, 0.36[2] | 0.53, 0.12[3] | 0.53, 0.36*[4] | |
| | 4-cane | 12.7 | 13.0 | 13.0 | 13.1 | | | | |

[1] P<0.001 ***, P<0.01 **, P<0.05 *
[2] For means at the same level of pruning treatment
[3] For means at the same level of pruning and exposure treatment
[4] For means at the same level of pruning and bentonite

**Methoxypyrazines**

Concentrations of methoxypyrazines in finished wines were very low (Tables 24 & 25). The average concentrations of iso-butyl and iso-propyl methoxypyrazine (IBMP and IPMP) were 1.32 and 0.07 ng/L, respectively. These concentrations were close to the limit of quantitation. Nevertheless, the low concentrations and the lack of any significant effects ($P > 0.05$) of pruning and exposure treatments were contrary to expectations. The concentration of IBMP in commercial Sauvignon blanc wines from New Zealand are commonly in the range 10 to 35 ng/L, although concentrations of IPMP are normally lower (4 to 5 ng/L) (Lacey et al., 1991).

**Thiols (3-mercaptohexan-1-ol)**

Results of the thiol extraction and analysis did not give concentrations of 3-mercaptohexan-1-ol in the range expected from other workers (Tominaga et al., 2000). On investigation, this was traced to the way in which the internal standard was added to the wine sample prior to extraction. It is thought that the approach adopted would have resulted in a low, although probably consistent, transfer of internal standard to the wine. The method for thiol extraction and analysis is laborious and there was insufficient time to repeat the procedure. Rather than discard the results completely, the peak height data were analysed. There were no statistically significant ($P > 0.05$) effects of pruning, exposure or bentonite addition treatments.

**Table 24.** Iso-butyl methoxypyrazine concentration (ng/L) of finished wines.

| Treatment | | Pruning treatments | | Exposure treatments | | Bentonite treatments | | Pruning | Exposure | Bentonite |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2-cane | 4-cane | 50% | 100% | (+) | (-) | $LSD_{0.05}$[1] | $LSD_{0.05}$[1] | $LSD_{0.05}$[1] |
| Main | | 1.63 | 1.01 | 1.39 | 1.26 | 1.38 | 1.26 | 0.627 | 0.295 | 0.320 |
| Split plot | 2-cane | | | 1.68 | 1.59 | 1.60 | 1.66 | | 0.584, 0.418[2] | 0.585, 0.452[2] |
| | 4-cane | | | 1.09 | 0.92 | 0.92 | 1.09 | | | |

| | | Exposure.Bentonite treatments | | | | | |
|---|---|---|---|---|---|---|---|
| | | 50% (+) | 100% (+) | 50% (-) | 100% (-) | | |
| Split-split plot | 2-cane | 1.86 | 1.35 | 1.49 | 1.83 | | 0.669, 0.566*[2] 0.669, 0.639*[3] 0.669, 0.566*[4] |
| | 4-cane | 1.12 | 0.73 | 1.07 | 1.12 | | |

[1] P<0.001 ***, P<0.01 **, P<0.05 *
[2] For means at the same level of pruning treatment
[3] For means at the same level of pruning and exposure treatment
[4] For means at the same level of pruning and bentonite

**Table 25.** Iso-propyl methoxypyrazine concentration (ng/L) of finished wines.

| Treatment | | Pruning treatments | | Exposure treatments | | Bentonite treatments | | Pruning | Exposure | Bentonite |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 2-cane | 4-cane | 50% | 100% | (+) | (-) | $LSD_{0.05}$[1] | $LSD_{0.05}$[1] | $LSD_{0.05}$[1] |
| Main | | 0.13 | 0.00 | 0.08 | 0.06 | 0.06 | 0.08 | 0.261 | 0.142 | 0.148 |
| Split plot | 2-cane | | | 0.15 | 0.11 | 0.11 | 0.15 | | 0.246, 0.201[2] | 0.248, 0.201[2] |
| | 4-cane | | | 0.00 | 0.00 | 0.00 | 0.00 | | | |

| | | Exposure.Bentonite treatments | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 50% (+) | 100% (+) | 50% (-) | 100% (-) | | | |
| Split-split plot | 2-cane | 0.30 | 0.00 | 0.00 | 0.22 | 0.296, 0.266*[2] | 0.296, 0.295*[3] | 0.296, 0.266*[4] |
| | 4-cane | 0.00 | 0.00 | 0.00 | 0.00 | | | |

[1] P<0.001 ***, P<0.01 **, P<0.05 *
[2] For means at the same level of pruning treatment
[3] For means at the same level of pruning and exposure treatment
[4] For means at the same level of pruning and bentonite

# Discussion

The objective of this research was to study the effect of crop load and fruit exposure on the composition of Sauvignon blanc berries and of the resulting wines, particularly in relation to the important flavour and aroma compounds that are required to give wines made from this variety their distinctive characteristics.

The important flavour and aroma characteristics of Sauvignon blanc wines include a dry to off-dry flavour with a fresh, lively acidity, moderate alcohol levels, the aromas of sweet passionfruit, box wood or cat's pee (Peyrot des Gachons, 2002), and green herbaceous capsicum (Marais, 1994). Important constituents include tartaric and malic acids, ethanol, methoxypyrazines (especially IBMP) and volatile thiols (including 3MH, 4MMPOH and 4MMP). A number of these compounds were analysed at stages during berry development and again in finished wines.

It was important to determine how the treatments influenced the characteristic Marlborough wine style from berry to finished wine. Therefore, assessments including of vine balance, berry development and composition, wine fermentation and wine finishing (namely bentonite fining) were undertaken to assess the influence of the treatments on fruit and wine quality.

## Carbohydrate allocation and vine balance

The concept of the "carbon budget" is related to the use and allocation of limited carbon within the vine. At different stages of development during the year, carbon reserves and

photosynthates are manufactured, mobilised, transported, utilised and stored within the vine (Zapata et al., 2004).

The amount of carbon within the vine at anytime is limited. Carbon is allocated to various sinks and organs within the vine in response to a complex array of genetic and environmental signals. The related concept of "vine balance" (Howell, 2001) implies that there is a distribution of carbon within the grape vine which is optimal (under given conditions) to produce vines that achieve root, vegetative and reproductive harmony.

All vine structures represent an investment by the vine. Root and leaf structures actively return nutrients to the vine system. Shoots, roots and trunks serve as carbon storehouses for the vine (Bates et al., 2002; Zapata et al., 2004). Grape flowers and berries function as the reproductive system, returning very little back to the vine, and contributing almost no metabolites to their own development. Fruit is removed from the vine; the resources expended are lost permanently.

Factors that control total potential photosynthesis in the vine are leaf area (Petrie et al., 2003), canopy density (Smart and Robinson, 1991) and availability of stored carbohydrates (Zapata et al., 2004). Photosynthesis is vital for plant function and of the products of photosynthesis fructose and glucose are the most abundant in grape berries. At maturity there is slightly more fructose than glucose in berries grapes (Kliewer, 1967). The majority of photosynthesis occurs in the palisade cells directly below the epidermal layer of the leaf (Jackson, 2000). Leaf area available for carbon fixation is crucial to vine and fruit development. Because canopy shading quickly reduces the strength of sunlight below the compensation point, an open canopy is important. This ensures that the leaf surface

available for light interception is efficiently utilised for the light-initiated process of water splitting, a procedure in photosynthesis which is energy intensive (Jackson, 2000).

In this study, the effect of increased crop load via pruning treatment was explored. The trial involved a comparison between 2-cane and 4-cane pruned vines. Pruning treatment results in a manipulation of carbon allocation within the vine; both in terms of how much carbon is allocated to the different parts of the vine e.g. root, shoot, leaf, and in how these structures are spatially configured. Whole-vine architecture is limited by factors such as canopy training (Archer et al., 1988) and manipulation practice, in addition to vine spacing (Archer et al., 1988), irrigation and environmental conditions.

A further carbon manipulation carried out as part of this trial was 100% leaf removal in the fruit zone. Leaf removal was undertaken to remove the confounding effect of leaf area on exposure treatment. The influence leaf area loss on the trial vines was not investigated *per se*.

Pruning results revealed significant differences between 2- and 4-cane vines. Old cane weight in 4-cane vines was effectively double, as was the number of old canes compared with that of 2-cane vines (Table 4). Shoots produced by 2-cane vines were significantly heavier (approximately double) those of 4-cane vines (Table 4). Not only were the individual shoots heavier for 2-cane vines but the new cane weight per vine was also greater (Table 4). This is reflected in the proportion of effective shoots (i.e. > 1.5 cm in diameter) which was significantly higher in 2-cane vines (Table 4). The number of individual shoots was almost double for 4-cane vines compared with 2-cane (Table 4). As a consequence, 2-cane vines generated thicker shoots as the number of shoots produced was fewer. Such findings are supported by others who have found carbohydrate partitioning to

shoots is reduced in vines carrying higher crop loads. Vines with a high percentage of carbohydrates accumulating as fruit do so at the expense of vegetative structures (Howell, 2001). Howell (2001) found that carbon allocation to leaves, shoots and roots was significantly reduced by increased crop load.

Yields in 2005 were slightly lower in Marlborough than average which was attributed to cool flowering and fruit set in December (Anon., 2005c). In this study, total fruit yield per vine was 3.89 and 6.70/vine for 2-cane and 4-cane respectively (Table 6). This is higher than yields obtained in Oyster Bay vineyards of 4.47 kg/vine (Anon., 2005b). There were highly significant differences in yield between 2-cane and 4-cane vines (Table 6). Using the criterion developed by Smart (1991), both 2-cane and 4-cane vines would be classified as excessively vigorous. The yield-to-pruning weight ratios for the two pruning treatments were 1.8 and 3.5 for 2-cane and 4-cane respectively, where a value of 5-10 is considered optimal (Smart and Robinson, 1991). These data suggest that vines for both pruning treatments are out of balance with too much carbon allocated to the production of vegetative structures rather than fruit.

The results suggested that the effect of exposure treatments influenced the two pruning treatments differently. Increased exposure significantly ($P<0.05$) reduced total yield in 4-cane vines by approximately 11% (Table 6). Berry weights (Table 7) were similar but bunches were heavier for 50% exposed treatments compared with those from 100% exposure (Table 6) suggesting a larger number of berries per bunch in 4-cane 50% exposure compared to 100% exposure. Yields from the 4-cane 100% exposed treatment had a higher proportion of healthy fruit (Table 6). Differences in diseased fruit between shaded and exposed treatments may have been a result of reduced air flow and sunlight exposure in the berry microclimate due to the shade cloth in shaded treatments.

Total above-ground carbon accumulation (the sum of total yield, new cane weight and fresh leaf weight) for the 2005 season was 8.5 kg for 2-cane vines and 10.8 kg for 4-cane vines. The difference between the two was mainly a result of increased yields from 4-cane vines (Table 6). A similar result was obtained by Miller (1998) who found that increasing crop load up to a vines capacity increased the allocation of carbohydrates to fruit.

Point Quadrat analysis revealed that there was little difference between pruning treatment and canopy density (Table 3). However, among 4-cane vines, those from the higher exposure treatment had reduced leaf layer numbers (Table 3). The reasons for this are unknown.

There were no significant differences in leaf area per vine and leaf weight per vine between 2-cane and 4-cane treatments (Table 2). This is of interest, as one might assume that if the number of canes doubled (Table 4) and fruit yield is doubled (Table 6) there would be a similar increase in leaf area and leaf weight. In fact the results indicate otherwise such that there is a smaller allocation of carbon for leaf production per shoot in 4-cane vines in comparison with 2-cane vines (Table 2). These findings are supported by work by Miller (1998) who showed that an increase in fruit yield resulted in a decrease in carbon allocation to vegetative structures. This study found that leaf area per shoot was highest in vines with the lowest number of shoots. Although leaf number and leaf size data were not gathered during this trial, a reduction in leaf size has been found with high crop loads (Bravdo et al., 1984). The impact of lateral shoot leaves was not assessed; lateral shoots were removed when visible during the growing season. However, with the number and vigour of the vines used in this trial is possible that some lateral shoot leaves were missed during the growing season and present in some vines.

According to Howell (2001) the leaf area needed to ripen one gram of fruit is 7-14 cm$^2$ depending on the growing region's climate. In this trial leaf area to fruit ratio (g/cm$^2$) was 19.53 for 2-cane and 10.45 for 4-cane vines, respectively, calculated from leaf area per vine and total yield per vine data in Tables 2 & 6 respectively. Such leaf area to fruit ratios indicate that 4-cane vines were well within the optimal range described by Howell (2001) above but 2-cane leaf area was excessive.

It is not known how the leaf removal imposed on the vines affected sugar accumulation. According to other workers (Howell, 2001; Jackson, 1986; Smart and Robinson, 1991) both pruning treatments resulted in leaf area to fruit ratios with the acceptable range. However, it has been shown that lower section of the canopy contributes more to whole vine photosynthesis (Petrie et al., 2003). Petrie (2003) established that berry sugar content was lower in vines with basal leaves removed compared with those that had been topped, even though both canopy treatments resulted in approximately the same leaf area loss. Petrie (2003) and Bennett (2004) (the latter using the vines in this trial) also found that other carbon stores were affected by basal leaf removal; namely lower cane weight and smaller cane diameters. Results indicate that 2-cane vines compensated more effectively for fruit zone leaf removal than 4-cane vines as evidenced by sugar accumulation rates (Table 8). This is attributed to a higher leaf/fruit ratio in 2-cane vines.

Literature indicates that leaves are more costly (meaning high concentrations of glucose are involved in their synthesis) to produce in comparison with other vine tissue (Vivin et al., 2003). This view is based on leaves having higher concentrations of carbon and nitrogen based compounds than fruit and shoots (Vivin et al., 2003). Costly compounds include proteins, lipids and phenolics; and less costly ones include soluble sugars, mineral and

organic acids, cellulose and hemicellulose (Vivin et al., 2003). Of the yield parameters measured, vegetative and fruit, leaf area per vine was the only one not significantly increased by pruning treatment (Tables 2, 4 & 6).

Point Quadrat canopy assessments showed that there were no significant differences in leaf layer number, internal/external leaf numbers or percentages or total leaf numbers (Table 3). From this, it is assumed that there was little difference in level of canopy shading of interior leaves between pruning treatments. Therefore, whole vine photosynthesis was assumed to be similar and differences in berry composition were ascribed to leaf area/fruit ratio rather the level of shading within the canopy.

## Berry development

The pattern of berry development follows a double sigmoid curve pattern. The three stages of berry development are: Stage I, the initial or rapid growth phase; Stage II, the lag phase; and Stage III, resumed growth and maturation (Mullins et al., 1992). Veraison occurs at the end of the lag phase and initiates rapid sugar accumulation, the beginning of the maturation phase (Mullins et al., 1992).

Pruning treatment affected the timing of berry development. Berries on 4-cane vines took longer to reach key stages of berry development, namely veraison and maturity. Flowering and fruit set occurred after the imposition of pruning treatments and data were not collected during this part of the season. Exposure treatments were imposed after flowering and fruit-set, so it is not known if exposure may have influenced the timing of flowering and fruit-set, therefore veraison and maturity.

**Veraison**

Veraison occurs during Stage II of berry development. It is characterised by the onset of rapid sugar accumulation, decrease in berry acid concentration, berry softening and colour change in red varieties and a transparent appearance in white varieties. The timing of veraison can vary significantly from one year to another. Differences in the timing of veraison can be seen between vineyards, individual vines in the same vineyard and even between individual berries in a cluster. Many suggestions have been put forward on what influences the timing of veraison including water stress, air temperature after flowering and date of anthesis (Coombe, 1992).

On the basis of the data, the end of the lag phase and the onset of veraison was thought to occur in the last week of February (Tables 8 & 11). The onset of veraison was interpreted in this study as the onset of rapid sugar accumulation and increase in berry weight. Other indicators of veraison during the same week were also noted from the data such as a drop, then an increase in pH (Table 10) and a decrease in titratable acidity (Table 11).

Veraison occurred later in 4-cane vines, lagging behind 2-cane vines by approximately four days. The effect of pruning treatment on the timing of veraison has been found by other workers (Bennett and Trought, 2004). The delay in veraison in 4-cane berries may be related to a lower ratio of leaf area to fruit influencing the amount of photosynthates available for berry development. Increase in berry temperature or radiant light exposure appear to have little influence on the timing of veraison in this study.

**Maturation**

Each grape berry is thought to develop independently within a cluster and unlike some other fruit cannot continue to ripen independently of the vine (Coombe, 1992). The ratio between sugars and acids within the berry changes throughout the developmental period; sugars increase and acids decrease in concentration from veraison. Ripeness can be measured using a number of parameters or by the more subjective taste evaluation (Simoni, 2007). The timing of berry maturation is influenced by a number of factors including veraison date. Variation in weather and crop load can influence the balance of sugars and other flavour compounds appreciably (Howell, 2001; Marais et al., 1999).

Other research (Bravdo et al., 1984) has found reduced rates of sugar accumulation in berries from vines with higher crop loads. In this study, a later date of veraison and slower rate of sugar accumulation resulted in 4-cane vines not reaching the target harvest ripeness of 21.5 °Brix (Table 8). At harvest, 2-cane berries had a soluble solids content of approximately 400 mg/berry whereas 4-cane fruit only reached about 340 mg/berry. After 31 March, the rate of sugar accumulation in 4-cane berries reduced significantly so that final soluble solids per berry was equivalent to that of 2-cane vines two weeks prior. This drop in rate of sugar accumulation in 4-cane vines also coincided with a slight reduction in average berry weight (Table 7). It was noted that sugar concentration slightly increased on the last sampling occasion before harvest in 4-cane berries but the amount of sugar in the berries dropped (Tables 8 & 9); sugar accumulation had halted in 4-cane berries. The trend for 2-cane fruit was different; soluble solids continued to accumulate and berry weight increased at the same rate right up until harvest. At harvest 4-cane berries were smaller and had less sugar than 2-cane berries.

Exposure treatment appeared to have no significant effect on berry sugar accumulation (Table 9). This is in contrast with other researchers who have found that fruit grown at higher berry temperatures (Buttrose et al., 1971) and greater light exposure (Morrison and Noble, 1990) have higher concentrations of soluble solids. Morrison (1990) asserts that there is an incomplete metabolic shift at veraison from metabolising to accumulating sugars in shaded fruit that results in lower sugar concentrations in berries. In addition, variation within exposed fruit populations is higher than within shaded fruit in regard to sugar concentrations; in exposed bunches it was found that the external sun-facing berries were always lower in soluble solids than the less exposed berries within the same cluster (Kliewer and Lider, 1968). It may be that the degree of shading is important in influencing the metabolic shift described by Morrison (1990). The degree of shading imposed on the 50% exposed fruit may not have been sufficient to retard the metabolic shift to the same extent found by Morrison (1990).

## Berry composition

One measure of grape quality is the sugar to acid ratio within the berry. The ability to achieve an appropriate sugar to acid ratio is one advantage of growing many grape cultivars in a cool climate. Bravdo (1984) maintains that low acid relative to sugar concentration is an indicator of over cropping. Berry acidity is measured as pH and as titratable acidity (TA) expressed as g/L of tartaric acid. The two most important acids in grape berries are malic and tartaric acids (Ruffner, 1982). The concentration of malic acid can be considered a key indicator of harvest date (Jackson, 2000). The concentrations of both acids follow known trends during berry ripening and the influences of climate, crop load and exposure are well documented (Kliewer, 1977a; Ruffner, 1982).

Titratable acidity of berries dropped during ripening from 35 to 10 g/L at harvest (Table 11). There was no consistent effect of exposure treatment on berry TA. On two occasions around veraison TA was significantly greater (P<0.05) in the 50% compared to the 100% exposure treatments. The expected influence of exposure on berry TA is not clear from the literature; some workers (Bergqvist et al., 2001; Crippen and Morrison, 1986) have found that exposure does not affect berry TA, while others (Bergqvist et al., 2001) have found that berry acidity is reduced with berry exposure. This study, however, is supported by findings by researchers such as Morrison (1990) who found that shading fruit did not affect the total acid concentrations.

Nevertheless, berry pH showed different trends to those of TA. Berry pH was significantly lower in 4-cane berries during ripening. In addition, pH in 4-cane berries was lower at the same °Brix of 2-cane berries. These findings were supported by must analysis at harvest (Table 17). However, at the same °Brix, 2-cane fruit retained higher TA than 4-cane fruit (Figure 10). These results were supported also by must analysis results at harvest (Table 17). Lower TA in 4-cane fruit was a result of extended berry hang time taken for 4-cane fruit to achieve given concentrations of berry sugar; differences were most evident between 10 and 17 °Brix (Figure 10). The reasons for the opposing results of berry pH and titratable acidity are not known. Must analysis showed that potassium content was not significantly different between pruning treatments; however, on average 2-cane must had higher potassium content than 4-cane must (Table 17).
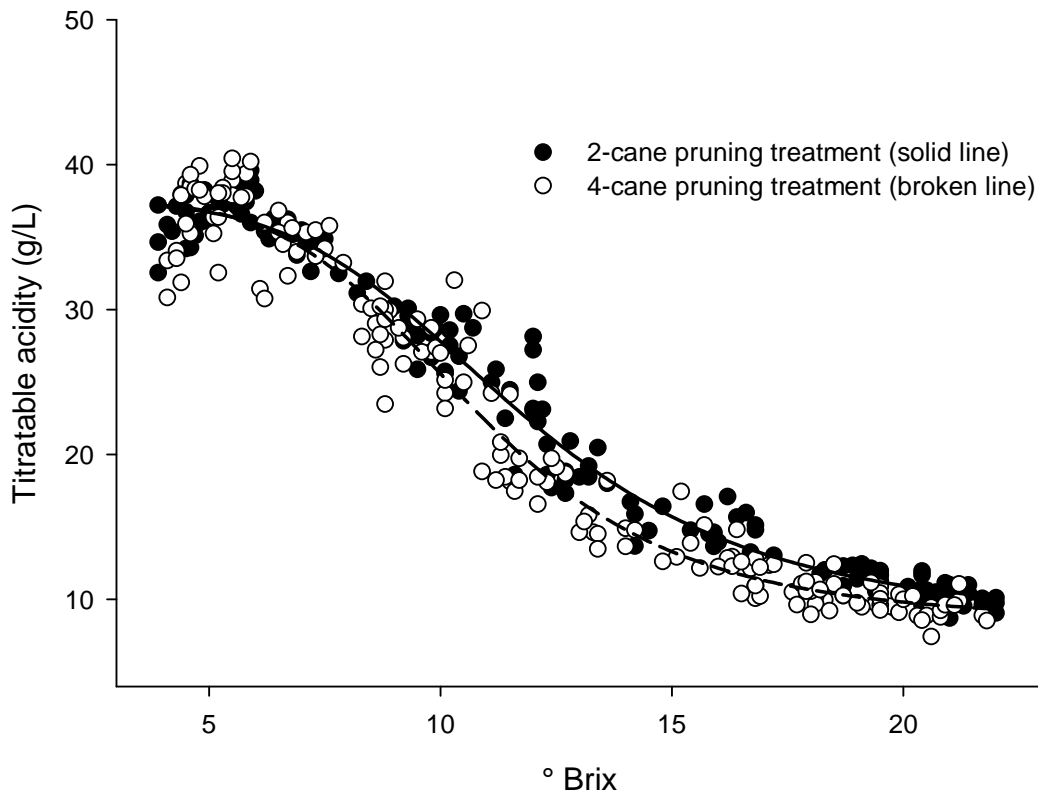
**Figure 10**. The relationship between °Brix and titratable acidity for berries from 2-cane and 4-cane pruning treatments.

**Malic acid**

The initial concentration of malic acid in berries was approximately 16 g/L which decreased to around 4 g/L at harvest (Table 13). Concentrations of malic acid in berries in this study were higher on average than those found during other studies in berries from the same region (Naylor et al., 2000). It is not known whether these differences could be a result of seasonal variability or leaf removal imposed on the trial vines. As in other studies (Morrison and Noble, 1990), concentrations dropped sharply after veraison until harvest. Malate accumulation is rapid prior to veraison reaching concentrations of approximately

18-24 mg/g fresh weight, then rapidly decreasing to approximately 2-8 mg/g at harvest (Morrison and Noble, 1990).

The final concentration of malic acid in berries is thought to be a function of four factors: initial concentration, timing of veraison during berry development, berry exposure and berry shrinkage. Results show that 4-cane fruit had slightly higher concentrations of malic acid after veraison but prior to harvest (Table 13). During veraison there is a metabolic shift within the grape berry (Ruffner, 1982). Metabolism of sugars ceases and the berry begins to metabolise malic acid so that sugars accumulate and malic acid concentrations begin to decline. The date of veraison occurred later in 4-cane berries compared with 2-cane berries and although not statistically significant, on average 4-cane berries were higher in malic acid veraison to harvest. On the last sampling occasion there was an increase in malic acid in 4-cane 100% berries that coincided with a slight reduction in berry weight from both exposure treatments. Interestingly, the same increase in malic acid was not seen berries from the lower exposure treatment.

The most significant factor influencing malic acid concentrations at harvest was exposure treatment (Table 13). Lower levels of malic acid were evident in fruit from the 100% exposure treatments. This supports the assertion that temperature is the dominant factor in determining malate concentrations at harvest (Ruffner, 1982). The temperature of fully exposed berries is known to exceed the ambient temperature (+7°C) and those of shaded berry temperatures (+10°C) significantly (Bergqvist et al., 2001). Readings on temperature differences were taken during only one day and the weather during recording was overcast for the most part which may have diminished possible differences between exposure treatments. However, significant differences in berry temperature were recorded on two occasions (Table 5). On the first occasion the sun was piercing the clouds and on the

second occasion it was approximately 30 mins after solar noon when the sun is at its most intense. However, it is thought that the intensity of berry sun exposure when directly overhead is diminished due to canopy shading (Dr. M. Trought, *pers. comm.*). On the 22nd of March 2005 differences in berry temperatures were not consistent (Table 5). Under clear weather conditions higher u.v. exposure and higher berry temperatures are thought to be likely. Higher berry temperatures result in increased rates of berry respiration and malic enzyme activity (Ruffner, 1982).

Interestingly, differences in malic acid concentrations between exposure treatments were not apparent in berry titratable acidity or consistently in pH results (Tables 10 & 11). This is supported by others (Morrison and Noble, 1990) who have found that malate content of fruit is not closely related to titratable acidity.

**Tartaric acid**

During ripening tartaric acid combines with cations to form tartrate salts (Jackson, 2000). The predominant cation for tartrate formation in berries is potassium (Jackson, 2000). In this study there was no significant difference in potassium levels between pruning treatments. Trends found in this study indicate that tartaric acid concentrations within berries at harvest are a function of concentrations at veraison and the increase in berry size post-veraison.

Tartaric acid concentrations dropped during berry development up until the 22 March and then remained relatively constant (Table 14). Such findings are supported by others (Coombe, 1992; Jackson, 2000) who attribute the drop in berry acid concentration to different factors including berry volume and changes in free versus salt ratios (Jackson,

2000). In stage III of berry development significant berry expansion occurs which in effect dilutes the acid. Once maximum berry size is achieved tartaric acids levels stabilise (Jackson, 2000).

The influence of pruning treatment on tartaric acid concentrations (Table 14) can be explained in part by larger berry size of 2-cane compared with 4-cane grapes. Berry weight results show that 2-cane berries continued to increase in size up until harvest; on the other hand, 4-cane berries began to shrink as harvest approached. Berries from 4-cane treatments continued to show reductions in tartaric acid concentrations (Table 14) even though berry volume was decreasing in the final two sampling occasions. Both tartaric and malic acid results are in contrast to the findings of Bravdo (1984) who found that highly cropped vines had high levels of tartaric acid and relatively low levels of malic acid compared to vines with lower yields: at equivalent °Brix, concentrations of these acids were lower but not on specific sampling occasions.

Bledsoe et al. (1988) explored the effects of leaf removal on Sauvignon blanc grape berry composition namely: potassium, malate, tartrate content and titratable acidity. It was found that leaf removal reduced organic acid content, titratable acidity and potassium content but increased berry soluble solids. Bledsoe et al. (1988) attributed higher soluble solids content to higher rates of berry exposure after leaf removal whereas results in this study show exposure treatment did not influence °Brix  (Table 8). In this study, differences in malate, tartrate, potassium and titratable acidity show similar trends to those found in the Bledsoe et al. (1988) trial at equivalent °Brix (Tables 11, 13 & 17).

**Methoxypyrazines**

Due to losses during wine processing (Roujou de Boubee, 2003), Sauvignon blanc grapes at harvest need to achieve levels of approximately 50 ng/L of iso-butylmethoxypyrazine (IBMP) to contain commonly found levels of 26 ng/L (Lacey et al., 1991) in New Zealand finished wine. In Bordeaux (France) and Wagga Wagga (Australia) grapes at veraison have around 40 times the concentration of IBMP in grapes at harvest (Lacey et al., 1991; Roujou de Boubee, 2003). If the same proportions of residual IBMP are true for New Zealand Sauvignon blanc grapes then concentrations of IBMP could be expected to reach around 2200 ng/L at or prior to veraison. The results from this study show that a week prior to veraison concentrations in the berries were a great deal less at 10-48 ng/L (Table 15).

Results indicate that the accumulation and subsequent decrease of methoxypyrazines and malic acid during berry development are attributed to different factors. Malic acid is synthesised from sucrose within the berry (Ruffner, 1982) whereas results support research by Roujou de Boubee (2003) that methoxypyrazine synthesis occurs within the leaf and then transported to the berry and IBMP movement within the vine.

Toward the end of veraison berries had a concentration of approximately 9.5 ng/L (Table 15). This is similar to levels found in Australian studies (Lacey et al., 1991) from Sauvignon blanc grapes grown in Wagga Wagga and the Adelaide Hills. It is significantly lower however, than concentrations found in Cabernet Sauvignon during a season in Bordeaux (Roujou de Boubee, 2003). In Bordeaux mid-veraison levels have been recorded at approximately 90 ng/L; a week after veraison, concentrations dropped to around 30 ng/L. Thirty days after anthesis Sauvignon blanc grapes have been found the have concentrations of approximately 100 ng/L whereas Semillon was found to have around 300 ng/L (Hashizume and Samuta, 1999). Grapes grown in both Wagga Wagga and Bordeaux show a

sharp decrease in IBMP concentrations from veraison until 10-14 days prior to harvest when the rate of decrease slows. A similar trend is noted in this study. The sharp decrease after veraison, common to all these studies indicates that the concentration of IBMP in the grapes prior to or at veraison is very important in determining the concentration at harvest.

At harvest the concentration of IBMP was significantly higher in 2-cane berries at (3.26 ng/L) than 4-cane berries (1.51 ng/L) (Table 15). Due to delayed ripening, berries from 4-cane vines were left a week longer on the vine in an effort to achieve the target 21.5 °Brix. As methoxypyrazines levels were similar pre-veraison, the extra hang time leading to harvest further degraded methoxypyrazines within berries resulting in significantly lower concentrations compared with 2-cane fruit at harvest.

Concentrations of methoxypyrazines were much lower than anticipated. In fact, the concentrations in fruit from all treatments were considerably lower than the average concentration for New Zealand wines of 25.9 ng/L (Lacey et al., 1991). Only once during the four sampling occasions (prior to veraison) did the concentration come close to this level (47 ng/L). The very low concentrations of IBMP found in grape berries may be related to the removal of shoot basal leaves, estimated to be around a third of the vine canopy. In addition, the higher leaf area to fruit ratio may have had some bearing on the higher concentrations of IBMP in 2-cane compared with 4-cane grapes at harvest.

Recent studies have found that basal leaves contain a much higher concentration of IBMP than other leaves or clusters (Roujou de Boubee, 2003). In addition, IBMP was found to be transported from the leaves to berries. Roujou de Boubee (2003) discovered that rainfall resulting in vegetative vine growth during ripening also affects the concentration of IBMP

in fruit. The consequences of this were high concentrations of IBMP in fruit at harvest after a rainfall event that caused resumed vegetative growth during the ripening period.

During the early stages (I and II) of fruit development high rates of vegetative growth are also occurring. Methoxypyrazine synthesis is related to the vegetative growth cycle of the grapevine (Roujou de Boubee, 2003). At the beginning of the growth season high levels of IBMP are synthesised in the leaves and transported to berries where it is broken down during ripening (Roujou de Boubee, 2003). Once the canopy reaches its maximum size, less IBMP is synthesised and transported to berries so rate of photo-degradation outstrips the rate of synthesis (Roujou de Boubee, 2003). A slowed rate of IBMP photo-degradation (Hashizume and Samuta, 1999) and a high initial IBMP concentration in the berries due to canopy vigour (Roujou de Boubee, 2003) may result in berries at harvest retaining high IBMP concentrations. Irrigation practices (Sala et al., 2005) and vine rootstock may also take a role in Marlborough Sauvignon blanc's high IBMP concentrations. SO4, which is a commonly used rootstock in Marlborough, is regarded as having high vigour. In addition, on-going irrigation, as is common practice in Marlborough, allows the vegetative growth of vines to continue throughout the season. These factors could help explain why grape IBMP levels are high in Marlborough.

Most studies conclude that the exposure of berries to sunlight prior to and after veraison is paramount in determining methoxypyrazine concentration in grapes at harvest. Although not statistically significant, 100% exposed berries were on average higher in methoxypyrazine concentrations than 50% exposed berries prior to veraison. Such findings are supported by Hashizume and Samuta (1999) who found immature berries had increased IBMP concentrations with light exposure. The increased u.v. radiation and higher

berry temperatures experienced by the berries in the 100% exposed treatment resulted in lower concentrations of methoxypyrazines on average post veraison (Tables 15 & 16).

Climatic, environmental and viticultural factors affect the concentration of IBMP in grapes and other grapevine organs. It is accepted that temperature has a strong influence on the concentration of IBMP within grapes (Lacey et al., 1991; Marais, 1994; Marais et al., 1999). Grapes grown in cooler climates have higher concentrations of IBMP at harvest than grapes grown in warm climates (Lacey et al., 1991; Marais, 1994). An interesting point made by Lacey *et al* (1991) is that temperature has a greater effect on the IBMP level than the accumulation of sugars. This means that in a cooler year one could expect to have higher concentrations of IBMP at an equivalent °Brix in Sauvignon blanc grapes. However, results from this study showed no statistically significant difference in IBMP concentration and level of exposure, but increased berry exposure appeared to reduce IBMP concentrations in 2-cane fruit at harvest on average (Table 15).

**Quercetin**

The synthesis of quercetin begins early during berry development (Jackson, 2000). In this study, quercetin concentrations in berries followed trends mentioned in other research (Downey et al., 2004). Concentrations declined up to 22 March and then increased at harvest (Table 12). Maximum quercetin concentrations were measured prior to veraison in 2-cane berries, probably as a result of small berry size and the consequent high skin to pulp ratio. On most sampling occasions quercetin concentrations were higher in 2-cane compared to 4-cane grapes. However, quercetin concentrations showed a sharp increase in 4-cane grapes from 22 March to harvest but this was not evident in 2-cane berries (Table 12). In part, this could be a result of a reduction in berry size on the part of 4-cane berries.

Similar differences have been found at harvest with quercetin 83% higher from fully exposed berries compared to moderately exposed Pinot noir berries (Price et al., 1995). Once the initiation of phenolic accumulation occurs light is needed to keep synthetic rate at maximum levels (Dokoozlian and Kleiwer, 1996). It is believed that shading results in a reduction of flavonol synthesis (Downey et al., 2004). Researchers have found that light exposure has little effect on anthocyanins and tannins, but significantly influences flavonols measured as quercetin glycosides. In addition, it is asserted that all flavonoid accumulation in Shiraz berries is light induced due to the light dependent expression of the FLS (flavonol synthase) present in leaves, flowers and fruit (Downey et al., 2004).

Exposure had a significant influence on berry appearance also. Berries from the higher exposure treatment were yellow in colour and had patchy brown almost scarred appearance in some cases. It appears that exposure may influence berry skin health and integrity also (Fig. 7). A change in berry colour and skin appearance may indicate a change in berry skin composition, particularly quercetin. Quercetin is known to have a yellow colour (Beninger and Hosfield, 1999). The increase in concentration of berry quercetin in the skin could be responsible for the yellow appearance of berries from the 100% exposure treatment. Colour differences were apparent in juice from whole cluster samples prepared for analysis. Juice from berries from the lower exposure treatments was bright lime green whereas juice from exposed treatments looked more yellow.

In this study, the different exposure treatments were imposed during the later part of stage I of berry development. The quercetin concentration in 100% exposed berries was at least double that of those from the 50% exposure treatment throughout the measurement period including the first sampling. This was not long after the exposure treatments were

imposed on the vines prior to veraison (Table 12). Therefore, changes in berry quercetin levels occured quickly in response to changes in u.v. exposure.

## Must analysis

Must analysis results were consistent with berry maturation in °Brix and titratable acidity. However there were differences in must pH and berry maturation results (Tables 10, 11 & 17). Must analysis revealed significant differences in must pH and titratable acidity between pruning treatments (Table 17). Differences in juice pH were likely related to differences in potassium content. Differences in titratable acidity reflect maturation results in that 4-cane fruit had lower titratable acidity content at equivalent °Brix. Differences in titratable acidity in must between exposure treatments reflected that of titratable acidity differences in berry ripeness data on 12th April (Table 11). Differences in titratable acidity between exposure treatments reflected lower malic acid concentrations in fruit from 100% exposure treatments (Table 13).

## Wine fermentation

Temperature is known to be one of the most important factors influencing fermentation. A desirable temperature for white wine fermentation is between 10-15 °C (Jackson, 2000). This is due to cooler fermentation temperatures favouring the development of fresh fruit driven wines (Jackson, 2000).

Although initial temperatures were acceptable, a cold weather event during the initiation of 4-cane fermentation occurred and this resulted in lower temperatures in the lag phase. Day 1 to day 5 after inoculation 2-cane wines had higher fermentation temperatures (Fig. 10). It

is thought that higher temperatures of 2-cane wines would be conducive to initiating fermentation. It is known that cooler temperatures prolong the lag phase (i.e. the initial stage after inoculation occurs and during which yeasts acclimatise to the conditions of the grape must) of fermentation considerably (Jackson, 2000). However, this did not appear to be the case. Wines made from 2-cane fruit appeared to have a longer lag phase and took about two days longer than 4-cane to begin fermentation (Fig. 8). However, the rate at which sugars declined was similar for all treatments. Completion of fermentation took around twenty eight days (Fig. 9).

## Wine composition

Wine composition was determined by measuring pH, titratable acidity, and concentrations of residual sugars, alcohol, sulphur dioxide, organic acids and methoxypyrazines. The influence of bentonite fining at a single rate of addition (700 ppm) on wine composition was also assessed. Comparing results of fruit and wine composition allows one to evaluate the usefulness of fruit ripeness parameters as indicators of wine quality.

### Alcohol

Ethanol is the most important by product of fermentation (Jackson, 2000) and influences wine quality appreciably. The effect of ethanol on the metabolic rates of yeasts influences the types and amounts of aromatic compounds produced. In addition, its action as a solvent acts to reduce the release of aromatic compounds from wine with carbon dioxide while fermentation is taking place (Jackson, 2000). It is not known how the solvent action of ethanol may influence the concentration of methoxypyrazines retained in a wine after fermentation.

The alcohol concentrations of the wines were approximately 13-13.5% w/v (Table 23) within the normal range for New Zealand Sauvignon blanc wine. However this is thought to be slightly higher than what was expected. According to Watson (Watson, 2003) multiplying the °Brix by 0.6 for white wine should give an estimate of what the resulting alcohol concentration would be. With an average of 21.2 °Brix for both 2-cane and 4-cane the estimated alcohol would be 12.7% w/v.

No significant differences in alcohol concentrations between pruning treatments were evident (Table 23) and did not relate to the higher sugar concentrations achieved by 2-cane versus 4-cane vine fruit (Table 23). In addition, alcohol concentrations in wines showed no differences between exposure treatments.

Bentonite fining appeared to have a significant influence on wine alcohol concentrations, the reasons for which are not known. However, in practical terms, bentonite addition made only about 0.2-0.3% w/v differences in alcohol concentration (Table 23). Such small differences in alcohol concentration would not be expected to impact the sensory characteristics of the wine.

**Organic acids**

As a group, acids are almost as important to wines as alcohols (Jackson, 2000). Acids are responsible for giving wine its structure, longevity and flavour. The titratable acidity (TA) of the wines was at the high end of the normal range found in most table wines (Jackson, 2000). Similarly, wines were within the normal range for pH (Rankine, 2004).

There was little influence of pruning treatment on TA in wines. This parallels grape ripening results. However, there was an apparent (although not statistically significant) difference between exposure treatments on wine TA. Wines made from 50% exposed treatments fruit were higher in TA than those made from 100% exposed fruit. Keeping in mind there was no significant differences in TA in berries between treatments, the difference in wine TA between treatments is thought to be a result of two factors: the higher malic acid concentrations and tartrate removal during cold stabilisation. The wines produced during this trial only underwent alcoholic fermentation. Therefore, malic acid content of the wines should be an accurate reflection of the malic acid profile of the fruit from which they were made. Differences in titratable acidity between fruit and wines (Tables 11 & 22) are a result of losses of tartaric acid during cold stabilisation. The higher malic acid in the 50% exposed fruit became apparent through wine titratable acidity once tartaric acid was removed during wine processing. Wines from 50% exposed treatments had significantly higher titratable acidity than 100% exposed treatments. Unfortunately, malic and tartaric acids of the wines were not measured therefore the influence of wine processing on the ratio between these two acids could not be assessed.

The addition of bentonite to wine also showed a significant influence on wine titratable acidity (Table 22). This trial's results support findings by Zoecklein (Zoecklein, 1988) which found that bentonite addition during cold stabilisation results in a greater degree of tartrate stability. Greater stability is bought about by the removal of proteins and polyphenolics that complex with tartrate inhibiting the formation of tartrate crystal formation (Zoecklein, 1988). Once complex forming compounds are removed more tartrate is lost from the wine resulting in lower titratable acidity.

**Sulphur dioxide**

Total sulphur dioxide ($SO_2$) in wine is the sum of the free and bound forms, the concentration of which needs to be within legal limits (250-400 p.p.m.) for human consumption in New Zealand (Rankine, 2004). An ideal free $SO_2$ concentration in a wine depends on the wine style, residual sugar and pH. However for a dry white wine with a low pH (*c.* 3) a free $SO_2$ of 10-20 p.p.m. would be thought adequate (Rankine, 2004). The $SO_2$ concentrations of the wines in this trial were within the ideal range stated above at around 11 p.p.m. (Table 18).

Wines made from 100% exposed fruit were significantly lower in free $SO_2$ than 50% exposed fruit (Table 19). This was not related to any difference in pH (Table 21). Sulphur dioxide is known to bond with tannins in wine (Rankine, 2004). The fruit from 100% exposed treatments was found to have significantly higher concentrations of quercetin (Table 12). Wines are known to reflect this aspect of fruit composition (Price et al., 1995). The higher quercetin concentration of 100% exposed treatment wines may have reduced free $SO_2$ concentrations due to the binding of the sulphur dioxide to quercetin.

Results show significant differences between pruning treatments on bound $SO_2$ concentration (Table 19). At bottling 4-cane wines were found to require significantly more $SO_2$ to achieve the target free $SO_2$ level of 23 p.p.m. to protect wines from oxidation while bottle aging. This significantly increased bound sulphur concentrations in the wine (Table 19) and total sulphur (sum of bound plus free). Therefore 4-cane wines had considerably greater sulphur binding capacity compared with 2-cane wines. Higher addition rates for 4-cane wines make comparisons between 2-cane and 4-cane wines difficult as addition rates were different. After bottling, regardless of higher addition rates, 4-cane wines had on average less free $SO_2$ than 2-cane wines (Table 18). A greater haze density was observed

from 4-cane wines during testing for protein stability using the hot/cold method (Rankine, 2004), a likely indicator of higher concentrations of proteins.


**Methoxypyrazines**

The concentration of iso-butylmethoxypyrazine (IBMP) and iso-propylmethoxypyrazine (IPMP) in wines were very low (Tables 24 & 25). About half the IBMP and IPMP in grapes was lost during wine processing as found in other research (Roujou de Boubee, 2003). IBMP is easily extracted during grape processing with free run juice concentrations very similar to those from pressed juice fractions (Roujou de Boubee, 2003). The addition of bentonite to wine appeared to have little influence on methoxypyrazine concentrations (Tables 24 & 25) and is interesting as it is thought that settling, an important part of clarification, causes the loss of approximately half methoxypyrazines in wine (Roujou de Boubee, 2003). Therefore, IBMP concentrations may be determined after cold settling and prior to bentonite addition. It is not known what proportion of methoxypyrazines are lost due to volatilisation during winemaking; with $CO_2$ gas released 25% of aromatic compounds are lost during fermentation at around 15 °C (Miller et al., 1987).

As in the fruit, pruning treatment appeared to have a slight influence on the IBMP concentrations in the wines, which were on average higher in wines from the 2-cane compared with the 4-cane treatment (Table 24). However, both pruning treatment and exposure treatment had no statistically significant influence on IBMP or IPMP in wines.


**Thiols (3-mercaptohexan-1-ol)**

As described earlier, due to low recovery of the internal standard, it was not possible to use the data obtained from the thiol analysis to accurately quantify the concentrations of 3-

mercaptohexan-1-ol in the wines. Bearing in mind the limitations of these data, statistical analysis revealed no significant (P>0.05) differences due to pruning treatment, exposure or bentonite addition. The data were quite variable and no obvious trends due to the various treatments were apparent. This may have been a consequence of the limitations imposed by the analytical method and further analysis with an improved method is required. Nevertheless, current understanding suggests that there may be a link between glutathione and cysteine (the compound which the free volatile, 3MH thiol is cleaved to) concentrations which increase during ripening (Adams and Liyanage, 1993). It is believed that the 3MH precursor is involved in the transport of quercetin to berry skin cell vacuoles (Peyrot des Gachons et al., 2002a). Whether or not a relationship between leaf area to fruit ratio and/or exposure (perhaps quantified via quercetin) and 3MH precursor exists is yet to be established. From informal tasting of berries during the season it was believed that exposed fruit exhibited more of the characteristic thiol flavour profile of passionfruit and boxwood.

# Conclusions

Results from this study indicate that it is difficult to assess wine quality based only on commonly used berry ripeness indicators of °Brix, TA and pH. Berry concentrations of sugars, quercetin and methoxypyrazines can be translated to concentrations in finished wine easily. However, a wine's protein stability and acid profile are often unknown until processing is complete and can be very different in finished wine from that of the berry.

Leaf area to fruit ratio was thought to be the limiting factor for fruit quality in this study. Pruning treatment influenced many parameters of wine quality significantly. Vines from the higher pruning treatments produced fruit of inferior quality compared with 2-cane fruit. This is based on acid concentrations at equivalent °Brix, increased quercetin, and lower methoxypyrazine concentrations. In addition, protein instability was visibly evident in wines made from fruit from 4-cane vines. Greater potassium concentrations in 2-cane must resulted in increased pH regardless of higher titratable acidity compared with 4-cane. These differences were not seen in wines after cold stabilisation.

Exposure treatment was clearly dominant in influencing quercetin and malic acid concentrations within the berry. The effect of exposure on berry skin and juice colour indicated differences in composition which were supported by quercetin analysis. Berry malic acid concentrations support much previous research which has found berry temperature to be the decisive factor determining levels at harvest. This trial found that differences in both berry quercetin and malic acid levels were evident prior to veraison between exposure treatments.

Wine acidity profile was very different from that of the berry. Wine titratable acidity was lower and pH was higher than that of the berries from which it was made. Methoxypyrazine concentrations in wine were half that of berries. Bentonite addition changed wine profiles on a statistically significant level for many parameters including titratable acidity, pH and alcohol.

Leaf removal as part of the exposure treatments imposed appeared to impact berry composition greatly. Methoxypyrazine concentrations, particularly IBMP in berries was found to be a fraction of what is generally found in New Zealand Sauvignon blanc grapes and later the trial wines. Such low berry and wine concentrations were not expected. There is circumstantial evidence that Sauvignon blanc vine vigour and the role of leaves in the development of characteristic flavour and aroma compounds are important to Marlborough Sauvignon blanc wine style.

## Further work

Further study is needed to determine the true nature of methoxypyrazine synthesis and translocation to the berry in Marlborough. Is the concentration of IBMP a function of basal leaf area to fruit ratio?

Topics of interesting study on the influence of the following on methoxypyrazines content in berries and wine style include:

- Degree of basal leaf removal, and timing of removal on IBMP concentrations in berries

- Common Marlborough rootstock effects on basal leaf development

- Irrigation practice on fruit composition

- Canopy management practices – e.g. the initial impact of mechanical leaf removal and subsequent vine leaf growth and/or leaf repositioning in the fruit zone

Time constraints during this study prevented completion of the thiol analysis of berry and wine samples collected. Once completed the results may help build our understanding of the influence of crop load and exposure on thiol profiles of Marlborough Sauvignon blanc grapes and wines. Other areas of work on impacting thiol concentrations in Marlborough Sauvignon blanc could include:

- Exploring the relationship between berry quercetin levels and thiol precursors particularly 3MH
- Irrigation practice
- Exploring relationships between leaf area and thiol precursor concentrations

Areas of winemaking research that may prove worthy of study include the influence of crop load and physiological ripeness on protein stability and sulphur dioxide usage in Marlborough Sauvignon blanc wines and methods for reducing volatile thiol loss during winemaking.

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