Rootstock and Canopy Density Effects on Grape Berry Composition: Organic Acid Composition, Potassium Content and pH.

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The influence of rootstock and canopy density on grape berry composition was investigated over the summer of 2003-2004 on a commercial vineyard at Waipara, North Canterbury. This experiment was designed to investigate the influence of rootstock and canopy density on the acid composition, potassium (K) content and final pH of harvested fruit (Pinot Noir AM 10/5 Lincoln Selection). The trial block consisted of eight rootstocks laid out to an 8 x 8 latin square, each plot consisting of five vines of the same rootstock. Two canopy treatments were overlaid the block (down whole rows, assigned randomly, four rows to each treatment); one treatment allowed to grow naturally, in the other treatment the canopy was thinned removing double burst shoots and laterals. The bunch numbers were adjusted in the Unthinned canopy treatment (UCT) to match the Thinned canopy treatment (TCT).

Information was gathered to assess: the canopy size and density (Pinot Quadrat Leaf Layer and Percent Gaps and canopy porosity), the plant yield (and berry size, berries per cluster, cluster weight, clusters per plant), plant K levels at flowering and veraison (from petioles and leaf blades) and berry composition at harvest (soluble solids (as brix), K, titratable acidity (TA), tartaric acid concentration, malic acid concentration and pH). The trial area was non-irrigated on clay loam soils and viticultural management was to best commercial practice.

It was found that although rootstock influenced the levels of K in the plant and in the juice at harvest, the level of K in the juice did not influence pH in this experiment (range

of rootstock juice K: 808 ppm to 928 ppm, l.s.d. = 75 ppm). The level of tartaric acid concentration in the juice was found to be the dominant influence on the level of pH in this experiment (rootstock pH range: 3.21 to 3.39, l.s.d. = 0.05). The juice concentration of tartaric acid was influenced by both rootstock (rootstock range 4.0 to 4.7 g/L, l.s.d = 0.4) and canopy density (UCT = 4.1, TCT = 4.7, l.s.d. = 0.4), decreased shading positively increasing the level of tartaric acid. The malic acid concentration in the juice was positively influenced by increasing canopy density (UCT = 4.7 g/L, TCT = 4.1 g/L, l.s.d = 0.4) and this played a minor role in the determination of pH in this experiment; an influence of rootstock on the level of malic acid concentration was found. The malic acid concentration strongly influenced the determination of TA (UCT = 11.0 g/L, TCT = 10.2 g/L, l.s.d = 0.5); tartaric acid concentration had a minor influence on the recorded TA.

Attempts to characterise the influence of rootstock on malic acid, tartaric acid and pH were inconclusive. Rootstock was found to influence the canopy variables measured in this experiment and the recorded average plant yield. Crosses of *Vitis rupestris* were found to exhibit the most canopy vigour and those derived from *Vitis berlandieri* and *Vitis riparia* the least. The Canopy treatment did not show an influence over yield but the rootstock was found to influence plant yield, through the numbers of berries set in a cluster and the final harvest cluster weight. The influence of rootstock on pH may be described by the influence it exerts on canopy growth and yield but this was thought unlikely. Further research is required to describe the nature of the rootstock influence on K, malic acid, tartaric acid and pH.

Key Words: -rootstock, - grape vine, - canopy density, - leaf area, -yield, - fruit composition, - juice, -malic, - tartaric, - acid, -pH, -potassium,-Pinot Noir

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1. Chapter 1: Introduction

The interactions between wine grapevines and the environmental conditions in which they are grown are ultimately expressed in the composition of the harvested fruit (Jackson, 2001). The soil environment plays a critical role in deciding levels of plant health, canopy growth and yield bearing potential and the complex exchanges between grapevine roots and the soil provide all necessary nutrients and water for plant functions (Mullins, 1992). There are a number of external factors that can influence the success of the interaction between plant roots and the soil. The soil has a number of biological, physical and chemical elements that can influence a plant's ability to extract the requirements for uninhibited growth (McLaren and Cameron, 1990; Mullins, 1992). Viticultural management can influence the availability of water and nutrients to a grape vine (Mullins, 1992). The management of soil-based stress on grapevine roots is an important area of research. Most famously, the biological attack on roots by soil borne phylloxera forced a fundamental change to the husbandry of wine grapes (Pongrazc, 1983).

Following the destruction of European vineyards by the root louse *Phylloxera vastatrix* (phylloxera) in the later decades of the nineteenth century, vigorous investigation of its behaviour was instigated. As early as 1868 it was discovered infesting the roots of infected vines, and shortly afterwards it was established that the roots of American vines were tolerant of the pest. The investigation of the potential of wild American vines began with the first consignment of cuttings in 1878 and the first grafting experiments were the beginning of a new phase of viticultural management.

The haphazard success of the early grafting experiments (primarily field grafts), with a high percentage of failures from early vine deaths, prompted a systematic assessment of the different North American species and their suitability for grafting to *Vitis vinifera* scions and adaptability to the European soils and climate. Eighteen distinct American species were identified through a process of breeding and selection, and a group of rootstocks with desirable characteristics was identified. This selection of rootstocks,

made in the late eighteenth and early nineteenth centuries, is still the group of rootstocks that account for the majority of plantings around the world today (Delas, 1992; Pongracz, 1983).

It is recognised that these rootstocks can impart different influences on the grafted scion (usually *Vitis vinifera* species) and rootstocks are recognised as a management tool, to be assessed against the site and used when planning vineyard plantings (Pongrazc, 1983). A number of characteristics have been categorised in Europe and other countries around the world for rootstock selection. These include: adaptation to calcareous soils, nematode resistance, compatibility with *Vitis vinifera* scions, adaptation to soil chemical and physical properties, imparted vigour to the scion, influence on scion yield and quality of the harvested fruit (Pongracz, 1983). The last four areas were of particular importance to this experiment. They are intrinsically entwined in the management of wine grape varieties and interact to largely determine the structure and strength of the acidity found in the harvested juice.

The composition of organic acids in harvested fruit is considered to be one of the most important elements in deciding the final wine quality and is an essential structural component of the final wine (Jackson, 1994). The significance of acidity to wine structure goes beyond organoleptic assessments, it is the skeleton on which a wine is able to live and develop (Jackson, 1994). Organic acid content of wine grapes largely determines the final pH of a wine (Margalit, 1997; Ribereau-Gayon *et al.*, 2000). The level of this important oenological measurement has been shown to strongly influence a number of important quality parameters. Low acid levels (or low acid disassociation) in grape juice and the resulting high wine pH levels have been found to be detrimental to wine quality (Jackson, 1994; Mpelasoka *et al.*, 2003); the colour intensity of red pigments (anthocyanins), microbiological stability, oxidative stability and aging potential are all affected by wine pH (Jackson, 1994).

This study concentrated on the common measures used in viticulture and oenology to define the composition of juice, must or wine acidity; titratable acidity (TA) and pH. The

levels of the two main grape acids, tartaric and malic acid, and their salts largely determine these measures (Margalit, 1997; Ruffner, 1982 a and b). The levels of metal cations in the grape berry determine the levels of the salt forms of these acids. Potassium (K) is the most significant mineral cation found in grape berries, constituting more than 90% of the total mineral cations (Iland, 1987; Mpelasoka *et al.*, 2003). Increased berry potassium levels have been implicated in a reduction in Titratable Acidity (TA) and increased pH levels of pressed juice at harvest and in the resulting wine (Boulton, 1980 a, b, c; May, 1994).

Grafting on to different rootstocks may result in altered scion nutritional status and this can affect the vigour of the growth of vegetative parts of the vine (Mullins, 1992). Rootstock and scion combinations can both devigorate or increase the growth of the vegetative parts of the vine (Mullins, 1992; Jackson, 1994). Excessive vine vigour and the resulting increase in canopy density have been implicated in altering levels of berry acids and the accumulation of berry potassium (Jackson & Lombard, 1993; Kliewer & Lider, 1968; Smart *et al.*, 1985).

The influence of rootstock and canopy density on grape berry composition was investigated over the summer of 2003-2004 on a commercial vineyard at Waipara, North Canterbury. This experiment was designed to investigate the influence of rootstock and canopy density on the acid composition, potassium (K) content and final pH of harvested fruit (Pinot Noir AM 10/5 Lincoln Selection). The trial block consisted of 8 rootstocks laid out to an 8 x 8 latin square, each plot consisting of 5 vines of the same rootstock. Two canopy treatments were overlaid the block (down whole rows, assigned randomly, 4 rows to each treatment); one treatment allowed to grow naturally, in the other treatment the canopy was thinned removing double burst shoots and laterals. The bunch numbers were adjusted in the Unthinned canopy treatment (UCT) to match the Thinned canopy treatment (TCT) (for details see Section 3.2).

Information was gathered to assess: the canopy size and density (Pinot Quadrat Leaf Layer and Percent Gaps, and a canopy porosity measurement was taken using digital

video against a magenta backdrop), the plant yield (and components that make up yield: berry size, berries per cluster, cluster weight, clusters per plant), plant K levels at flowering and veraison (from petioles and leaf blades) and berry composition at harvest (soluble solids (as brix), K, titratable acidity (TA), tartaric acid concentration, malic acid concentration and pH). The trial area was non-irrigated on clay loam soils and viticultural management was to best commercial practice.

The nature of rootstock potassium supply to the scion and the resulting fruit potassium level has not been fully investigated (Mpelasoka *et al.*, 2003) and this research seeks to describe the rootstocks in relation to their effect on potassium supply to a scion (Pinot Noir). This study investigated how much of the resulting potassium in the harvested fruit is a result of indirect canopy density effects and how much attributable to seasonal grapevine potassium levels, potentially influenced by the rootstock. Similarly, the influence of rootstock and canopy treatment on the level of malic acid, tartaric acid and potassium in the harvested fruit was investigated with respect to the influence of canopy and rootstock. Finally, the influence of acid and potassium levels and the influence of canopy and rootstock on juice pH were described.

2. Chapter 2: Literature Review: Rootstock and Canopy Density Effects on Grape Berry Composition (Organic acid composition, Potassium content and pH).

2.1. Introduction

The measurement of TA and pH has allowed the easy and inexpensive characterisation of juice, must and wine acidity. The measurement of both requires inexpensive equipment and a small investment in chemicals and time. The monitoring of wine TA and pH throughout the ripening process of grapes and fermentation of the must is important as it helps to manage the final acid composition and pH of the completed wine (Margalit, 1997). Timing of grape harvest can have implications on the composition of grape berry acidity. Warm climate grape growing requires considerable attention to level of organic acids prior to harvest, the degradation of acids (primarily malic acid) is advanced by warm temperatures leading up to harvest (Ruffner, 1992). Cooler growing regions may require longer ripening periods to meet the desired harvest concentrations of organic acids, with implications for and trade-offs to be made between acid levels and other compositional parameters (e.g. elevated brix levels or higher disease incidence may result) (Mullins, 1992).

There are oenological interventions that can be used to alter the level of must and wine acidity. For example, a malolactic fermentation of all or part of the must will reduce the level of malic acid in the finished wine, de-acidification of must or wine may also be employed to reduce the acid content and the addition of tartaric acid is commonly used to increase the acidity in a high pH must (Ribéreau-Gayon *et al*, 2000). The timing and intensity of the winemaking intervention will be largely determined by the measurements of TA and pH obtained.

More sophisticated tools are available for the characterisation of the acid composition of grape juice and wine the most common (and least expensive) is the use of the enzymatic determination of malic acid, a process now available in mass produced commercial kits. There is no enzymatic means of determining tartaric acid; however the determination of

TA and the subtraction of the determined malic acid will describe the approximate level of this acid (Margalit, 1997). There are other more sophisticated means of determining the levels of these acids (e.g. High Performance Liquid Chromatography – HPLC) that all require considerable outlay for equipment and technical knowledge and are not in widespread use in the industry. While the determination of the relative levels of the acids is desirable, frequently this is limited to the determination of TA and pH, with the additional input from the experience of the winemaker providing the judgement required for the management during the winemaking process.

Even if the levels of malic and tartaric acids have been determined by reliable means the formation of salts with mineral cations reduces the predictive accuracy of these measures (Margalit, 1997). Tartaric acid and the formation of its salts provide particular problems for the winemaker (Iland, 1987, Ribéreau-Gayon *et al*, 2000).

2.2. Relationship of Berry K levels to observed TA & pH in Juice, Must or Wine.

To fully evaluate and understand the measurement of acidity it is necessary to consider the relationship between the actual concentration of organic acids (total acidity), the measured titratable acidity, monovalent metal cations and the pH.

2.2.1. Juice and Wine and the Measurement of pH.

The concept of pH is a mathematical description of the concentration of hydronium ions (H_3O^+, abbreviated to H^+ or hydrogen ions in some contexts) in an electrically conductive solution (e.g. must or wine) expressed as:

$$pH = -\log^{10}[H_3O^+]$$

It is an abstract measure with no units, measured using a pH meter with a glass electrode after calibration with two buffer solutions. It is a measure that is based upon the dissociation equilibrium of the various acids found in the solution. This can be expressed by the equation:

$$AH + H_2O \Leftrightarrow A^- + H_3^+O$$

The emission of the H_3O^+ ions defines the acidity of the acid (AH) molecule.
The level of the dissociation depends upon the value of the equilibrium constant, Ka, of the acid:

$$K_a = \frac{[A^-]\,[H_3O^+]}{[AH]}$$

Grape juice and wines are mixtures of weak acids that combine to form salts to a greater or lesser extent according to their pKa (-log Ka). The pKa of tartaric acid (3.01) indicates that it is a 'stronger' acid that malic (3.46), lactic (3.81) or citric (5.74) acids (Margalit, 1997; Ribéreau-Gayon *et al.*, 2000). The proportion of the salts formed depends upon genetic, environmental and management variables, but theory suggests that tartaric acid will take priority in forming salts (Ribéreau-Gayon *et al.*, 2000). The contribution of each acid to TA and pH is determined by its dissociation, as well as the degree to which it has combined with cations to form salts.

Wines possess an acidobasic buffering capacity, in that a modification of their chemical composition produces only a limited variation in pH. This explains the relatively small shifts in pH as a response to alcoholic and malolactic fermentations (Ribéreau-Gayon *et al.*, 2000). Wine generally has a lower buffering capacity than the juice from which it is made, which is why it is more efficient to add acid to wine rather than must. However, must is a dilute aqueous medium and wine a dilute alcohol medium and acid salts become less soluble as a result of an increase in alcohol content (Ribéreau-Gayon *et al.*, 2000). This is the case for the most abundant wine salt potassium bi-tartrate (KHT), which causes a decrease in TA on crystallisation (Ribéreau-Gayon *et al*, 2000). A decrease in pH may occur during the cold stabilisation of tartrates, despite the reduction in TA, due to the removal of the mono-K salt from the wine (Ribéreau-Gayon *et al.*, 2000).

2.2.2. The Relationship between Titratable Acidity and Total Acidity

Titratable acidity is defined as the number of protons (hydrogen ions, H^+) recovered during a titration with a strong base to an end point (in New Zealand commonly pH 8.2) (Margalit, 1997). Total acidity is the proton equivalence of the organic acid anions found in the juice. This is therefore the number of protons that would be expected in the wine or juice given the number of acid anions (Boulton, 1980a; Margalit, 1997). The key concept in the relationship between the titratable acidity and actual concentration of organic acids, in grape juice and wine, is that not all the protons expected are found when determining titratable acidity and pH (Boulton, 1980a). This deficit is thought to occur due to the relationship between hydrogen ions and monovalent metal cations in the grape berry.

2.2.3. The Relationship between Monovalent Metal Cations and pH

Previous attempts to describe the relationship between titratable acidity and pH concentrated on the cause and effect model, altering the environment in which the vine was grown and measuring the changes elicited in the berry acid composition. The descriptions of the synthesis and metabolism mechanisms of organic acids in wine grapes developed by these studies failed to adequately explain the relationship between titratable acidity and pH. In a series of papers Boulton (1980 a, b, c, d) described the relationship between monovalent metal cations and hydrogen ions (H^+) in grape berries, grape juice and wine. He has developed a hypothesis to explain the variation found in measures of TA and pH.

The level of organic acid anions found in juice or wine cannot explain the TA and pH measures. By predicting the level of protons expected from equilibrium ionisation of the acid anions, Boulton (1980a) tested the hypothesis that monovalent metal cations were exchanged for protons in berry cells. He was able to show that the deficit in expected protons (from the disassociation of known concentrations of acid anions) could be explained in by the inclusion of the concentration of K^+ and sodium (Na^+) ions. Relating this to the development of the grape berry Boulton (1980b) was able to explain the deficit occurring in phase 1 and phase 3 in terms of this relationship. The juice pH is dependent

not only on the level of cation to acid exchange, but also on the net level of acid synthesis and degradation.

In phase 1 the synthesis of acids outstrips their degradation and the net effect is a steady rise in berry titratable acidity. During this early phase of development (and over the whole period of berry maturation) there is a net rise in berry K (Possner & Kliewer, 1985); the effect of this is offset by the rise in total number of acid anions found in the juice. A drop in pH can only occur when acid synthesis is not accompanied by a - corresponding increase in mineral uptake (Boulton, 1980b). Post-veraison, a shift toward malic acid degradation by either gluconeogenesis (at lower temperatures) or respiration occurs and this leads ultimately to a reduction in total acidity (Ruffner, 1982). The impact of the reduction in malic acid can be for pH to remain constant, as the tartaric to malic acid ratio increases, increasing the dissociation of tartaric acid (with a higher disassociation constant); cation uptake at this stage may result in a rise in pH due to salts being formed with tartaric acid (Boulton, 1980b).

The relationship outlined above goes some way to describe the variances found in titratable acidity measures and pH found between vineyards, seasons and scions. The relative proportion of the major organic acids found in berries does influence the impact of cation uptake on pH. Grapes with the same K^+ contents and TA measures can vary in pH according to the relative amounts of tartaric and malic acids in the juice (Margalit, 1997). In general, however, the pH value is more sensitive to changes in K^+ and Na^+ concentrations than the ratio of the two acids. In typical juice samples the free proton pool is generally between 0.001 and 0.0001 mol/l, the concentration of K and Na between 0.02 to 0.05 mol/l and the total acid concentration between 0.05 to 0.10 mol/l (Boulton, 1980c). A 10% increase in cation concentration (at typical juice pH) will elicit an increase in pH of approximately 0.1 pH units (Boulton, 1980c).

Implicit in the effect of an increase in cation content of the berry and the corresponding decrease in the proton pool is a mechanism for exchange. The exchange is thought to occur across the membranes of berry cells and relates to a specific uptake system that has

a strong preference for K^+ ions over Na^+ and other monovalent metal cations (Boulton, 1980a and c). There are only four mechanisms identified for the transfer of mineral elements across cellular membranes: bulk flow, diffusion, active transport and enzymatic exchange (Boulton, 1980 c and d). Boulton (1980c) points out that if minerals enter the cell by the first three mechanisms the protons from the acids would be retained and fully recovered in the measurement of TA. The enzyme system proposed at the time as the most likely candidate for the transfer involved adenosine triphosphatase (ATPase) (Boulton, 1980d). This enzyme activity has been demonstrated in the ion transfer into cells in the roots of plants, exhibiting strong preference for K over other monovalent metal cations (Boulton, 1980d). Each molecule of ATPase hydrolysed exchanges three protons for three metal cations and in general the movement of the cations is against a concentration gradient (Boulton, 1980d). The rapid uptake of K^+ by berry cells relative to other plant tissues is hypothesized to be the result of higher ATPase availability (Boulton, 1980d).

Whatever the mechanism, Boulton has described a statistically proven relationship between the actual protons found in grape juice and wine, the mineral cations and the number that is expected by the level of acid anions. This has implications for the accurate description of wine acidity, the inclusion of monovalent cation concentrations may be required explain the measured TA and pH.

2.3. Impact of High pH from Increased Juice Potassium Concentration on Wine Quality

2.3.1. The Effect pH on Sulphur Dioxide

The importance of juice pH to winemaking is central to the quality, integrity and stability of the final wine produced. Low acid levels or low acid dissociation causing elevated pH have a negative impact on wine stability and ability to store in the bottle (Margalit, 1997). The impact of elevated pH on the dissolved protectant sulphur dioxide has ramifications on the storage potential of all wine, without the action of this agent the storage life of

bottled wine is only a few weeks (Jackson, 1994). The pH level affects the relative proportions of inter-convertible states that sulphur dioxide forms in solution:

Figure 2.1 Forms of Sulphur Dioxide Found when Added to Juice, Must and Wine (Jackson, 1994).

$$SO_2 + H_2O \Leftrightarrow H_2SO_3 \Leftrightarrow HSO_3^- + H^+ \Leftrightarrow SO_3^{2-} + 2H^+$$

Active Molecular SO_2	Sulphurous Acid	Bisulphite Ion	Sulphite Ion

The sulphur dioxide is found in free and bound forms. The amount of bound sulphur dioxide is determined by the concentration of various binding compounds found in the wine (e.g. acetaldehyde formed during fermentation, anthocyannins, and tannins) (Jackson, 1994). The remaining free form of sulphur dioxide is divided up as the undissociated or molecular form, bi-sulphite or sulphite anions. At typical wine pH the bi-sulphite form dominates (Rankine, 1998):

$$pH = 3,\ HSO_3^- = 94\%,\ SO_2 = 6\%$$

$$pH = 4,\ HSO_3^- = 99.4\%,\ SO_2 = 0.6\%.$$

The amount of free sulphur dioxide is important for determining the relative quantity of these forms at the existing wine pH. There are legal restraints placed on the total amount of sulphur dioxide that can be added to wine so the wine pH becomes of great importance when determining absolute concentrations of these forms (Rankin, 1998). Red wines also require that the quantity of free sulphur dioxide is kept to a minimum, as excess levels can have a detrimental effect on wine colour (see section 2.3.2).

Of the free forms of sulphur dioxide molecular SO_2 is the most toxic anti-microbial form. Bound molecular SO_2 is very weakly anti-microbial and the bisulphate form is probably

only effective against some (non-wine) yeast (Jackson, 1994). Therefore, the wide-spectrum molecular SO_2 is of most interest to winemakers and levels of about 1.5 mg/l is considered sufficient to inhibit most spoilage yeasts and bacteria (Jackson, 1994). Obtaining sufficient protection at low total sulphur dioxide levels is dependent upon low rather than higher wine pH. The level of free molecular SO_2 is also important in determining protection from wine oxidation; it suppresses the activity of several oxidases (Jackson, 1994; Rankin 1998). The sulphites (HSO_3^-, SO_3^{2-}) are capable as acting reductively converting oxidation products back to their reduced forms. Therefore, achieving sufficient microbiological and oxidative protection in all wines, without impacting on other quality parameters, is largely dependent upon maintaining as low a pH as possible.

2.3.2. The Effect of pH on Wine Colour

Colour compounds (anthocyanins) in red grape varieties predominantly exist in grapes in a form in conjunction with a glucose molecule and are called an anthocyanidin. The sugar component improves the chemical stability and solubility of anthocyanins. In young red wine anthocyanins occur as a dynamic equilibrium of five states (see Figure 2.2).

Most of these forms are colourless at wine pH; red colour comes primarily from the flavylium form. The proportion of this form is dependent upon the wine pH and the free sulphur dioxide level. As pH rises the concentration of this form rapidly decreases. At typical red wine pH of 3.4 to 3.6, 20 to 25% are in the flavylium state; at pH 4 only 10% (see Figure 2.3). The blue-mauve colour of high pH wines comes from the slight increase in the quinoidal form.

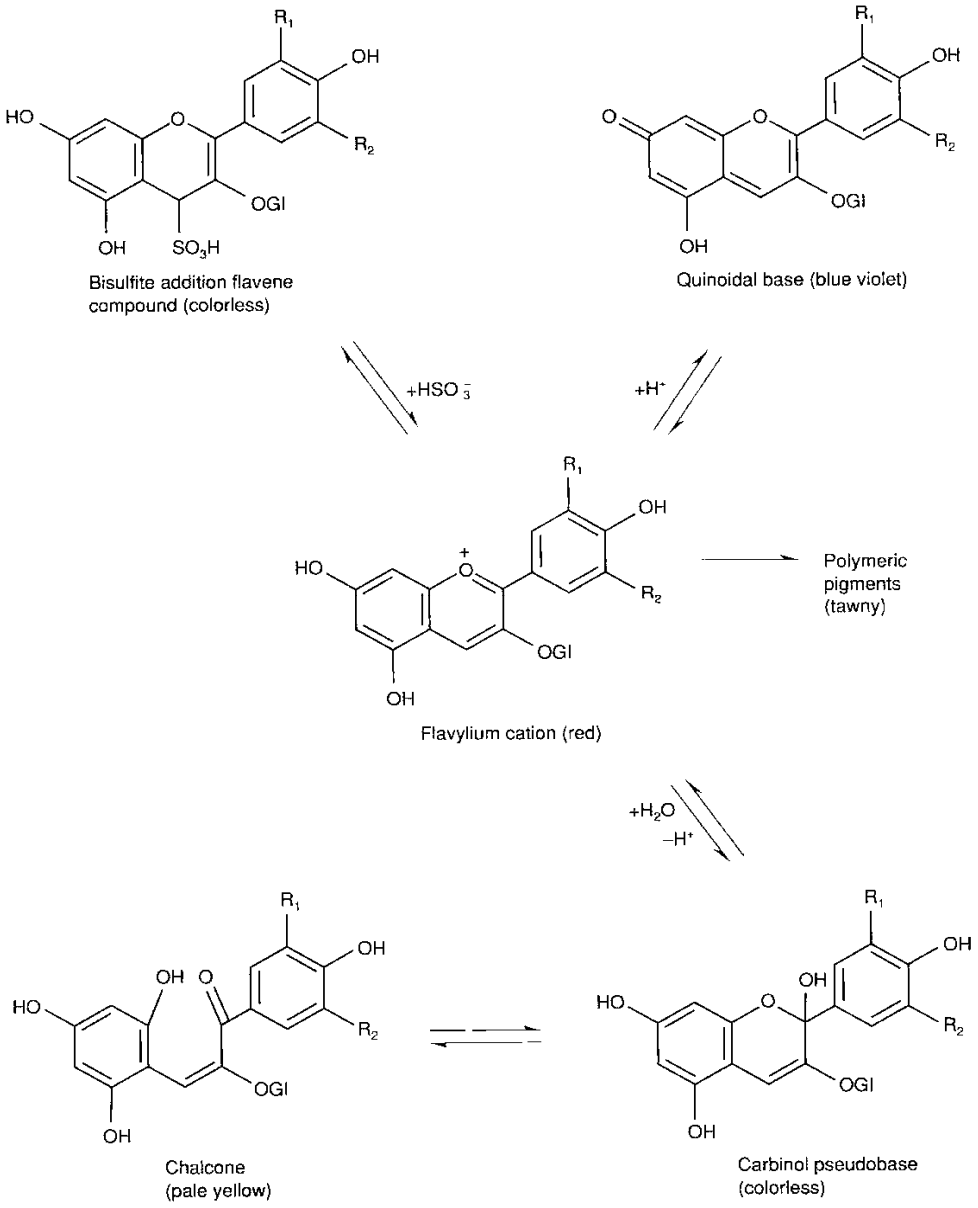
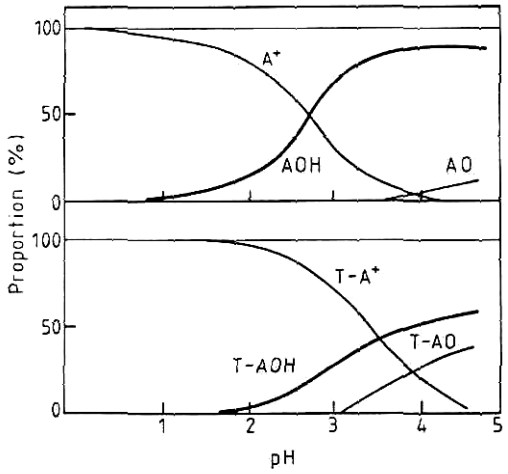


Figure 2.2 Equilibrium between the Forms of Anthocyanins in Red Wine (Jackson, 2000)

These compounds form complexes with other compounds (mainly other phenolic compounds) and these complexes increase colour density and light absorption. For example, approximately 60% of the polymerized anthocyanins are coloured at pH 3.4 where only 20% of the free anthocyanins are coloured (Figure 2.3, T-A combined anthocyanins). The combination of low pigment and phenolic compounds in some

varieties (e.g. Pinot Noir; Jackson, 1994) will show greater colour loss during the heating that occurs during fermentation (thermovinification) due to the breakdown of the anthocyanin complexes (Jackson, 1994). The polymerization of these compounds (copigmentation) also is important in stabilising wine colour by protecting the anthocyanidin molecule from oxidation and other chemical modification. Polymerization also increases solubility and reduces precipitation of tannins (Boulton, 2001). The formation of coloured copigments is also favoured when anthocyanins are predominantly in the flavylium state, at low pH (Boulton, 2001; Jackson, 1994). Therefore, low pH is important to the creation of full, bright colour in red wines.

Figure 2.3 The Effect of Wine pH on the Different Forms of Free Anthocyanins (A) and Combined Anthocyanins (T-A) (Ribéreau-Gayon and Glories, 1987).



\+ = red flavylium cation

OH = colourless carbinol pseudobase

O = blue-violet quinoidal base

The K^+ content of berries has been shown to affect the final pH of a wine. All of the K^+ found in grapevines is sourced from the soil. The soil availability and uptake of K^+ in to the vine has a profound effect on the quantity of K^+ that is in the fruit at harvest and is therefore an important determinant of the final pH of the wine made.

2.4. Factors Affecting Plant Uptake of Monovalent Metal Cations: Rootstocks, Root Structure and the Soil Interface

2.4.1. The Relationship between Soil, Plant Roots and Plant Nutrition

Plant growth is dependent upon the ability of roots to absorb nutrients and water from the soil. This is affected by the biological, chemical and physical conditions in the soil (McLaren and Cameron, 1990). The root system of a plant has a variety of characteristics including the physical structure, nutrient uptake mechanisms and root growth patterns that may alter their expression in different soil conditions (McLaren and Cameron, 1990).

Root growth and physiology are affected by a number of factors. Physical restraint of root penetration is affected by the soil strength (determined largely by sand, silt and clay content and by level of compaction). As soil strength increases the rate of root elongation decreases and root diameter increases (Marschner, 1995; McLaren and Cameron, 1990). Seasonal soil temperatures affect the growth and activity of roots, generally cooler soil temperatures promote slower root growth. Soil water and nutrient availability have a great effect on the ability of roots to extract soil resources required for plant growth (McLaren and Cameron, 1990). Soil aeration is important for gas exchange during respiration to provide the energy required for growth and nutrient uptake (Marschner, 1995). The presence of root inhibiting toxins (high levels of aluminium, manganese, hydrogen and herbicides) and mineral deficiencies (especially calcium in acidic soils) will adversely affect roots' expansion and performance (McLaren and Cameron, 1990).

In addition to the structure and chemical composition of the soil is the importance of bacterial and fungal activity around the roots of a plant. The health of microbiological activity in the root rhizosphere can have a profound effect on the behaviour and success

of root systems. Central to the effective exploitation of the soil is the association between the root and mycorrhizae fungi (McLaren and Cameron, 1990). These organisms have a symbiotic relationship with the plant and effectively increase the root surface area supplying nutrients to the host plant. The relationship between the root and soil micro-organisms varies between species and this can have a profound impact on ability of a plant to fully exploit the soil.

Soil properties such as soil moisture, aeration, soil temperature and pH will affect uptake of potassium (K^+) by plant roots (McLaren and Cameron, 1990). A gradient of depleted soil in the rhizosphere is created by the uptake of K^+ by plant roots and this must be recharged. Increasing the volume of water in the soil will increase the diffusion of K^+ to plant roots increasing plant available supply (Bogoni *et al.*, 1995). Soil aeration is reported to have a greater effect on K^+ uptake than of other ions (Barber, 1995). When oxygen levels in the soil drop below 10%, K uptake by the roots will decrease. This may be related to the energy requirements of the assimilation of K^+ into plant roots via active transport mechanisms (Barber, 1995).

The soil temperature can have a similarly inhibitory effect on the active absorption of mineral ions. Low soil temperatures inhibit respiration and the subsequent energy release required by these mechanisms (only passive uptake occurs below 2^oC as this is too low for respiration). Soil temperature affects the plant growth rate and nutrient influx. In general soil temperatures above 5^o C increase influx until a maximum is reached, determined by the plant species and the type of nutrient, after which a decline in uptake rate occurs (Barber, 1995). The soil temperature is related to the air temperature, which also affects the plant transpiration rate. This will affect the water influx through the roots; however the decrease in ion influx associated with high soil temperatures is generally not mirrored in a corresponding decease in water influx (Barber, 1995).

Soil pH has an affect on the availability of mineral cations. Low pH (acidic) soils reduce the availability of macronutrient cations (Ca^{2+}, Mg^{2+}, Na^+ and K^+) as these are replaced on soil colloids by acidic cations (H^+ and Al^{3+}) and are leached from the soil (McLaren

and Cameron, 1990). At higher pH (pH 6 to 7) the availability of calcium and magnesium increases potentially affecting the availability and uptake of K ions (Janick, 1969; McLaren and Cameron, 1990). The addition of fertiliser to the soil alters the balance of cations held on soil colloids and in the soil solution the net result is the increase in soil available cations especially of the added species.

The interactive nature of the relationship between the soil and the plant root results in management interventions having subtly different results depending upon the configuration of climate, rootstock, soil type and water management on vineyards. Soil variances across grape growing regions and individual vineyards create viticultural challenges in the management of wine grapes. Similarly, the management of field research trials investigating anything affected by vine nutrition must be prepared to accommodate the inherent variability of the soil medium when planning for and assessing results.

2.4.2. Grapevine Rootstocks: Selection Criteria and their Influence on Plant Nutrition

2.4.2.1. Rootstock Selection Criteria

While phylloxera resistance is, by necessity, the primary criterion by which a rootstock is evaluated it is evident that other desirable characteristics can be selected as well (Pongracz, 1983). Over time the available rootstocks have been assessed for nematode resistance, grafting affinity (with *Vitis vinifera* varieties), adaptation to calcareous (high limestone content) soil, affinity for soils of different physical and chemical properties, drought tolerance, the influence on the vigour of the scion, and the influence on the size and quality of the crop. Out of the available North American species only *Vitis riparia*, *V. rupestris*, *V. berlandieri* and *V. cordifolia* adapted well to the conditions in Europe, and most rootstocks used today are varieties or hybrids of these species (Pongracz, 1983).

Most of the initial work on rootstock identification and classification was completed in Europe. Rootstocks were selected and bred to ensure suitability for soil and climatic

conditions. This allowed a robust selection of about 20 rootstocks to provide sufficient variation to cover most European conditions (Delas, 1992). For example, warm regions (also found in South Africa and Australia) that have a dry climate suit themselves to rootstocks that are drought resistant (*V. berlandieri* and *V. cordifolia* hybrids) and that impart vigour to the scion in difficult growing conditions. Generally American *Vitis* species do not grow well in calcareous soils, the notable exception for rootstock selection are those with *V. berlandieri* as a parent species. Conversely, regions with acidic soils will do best to target species adapted to these conditions, *V. riparia*, *V. cordifolia* and *V. labrusca* (Pongraz, 1983).

There are a number of physiological differences between rootstock species that alter their performance in different soil conditions. The adaptation of the species to their native environment has imparted different growth patterns to the roots and aerial vine parts that have implications when grafted to *V. vinifera* varieties. A good example of this is *V. riparia*, an important rootstock parent species and also available in 'pure' varietal forms for grafting. The natural habitat of this species, while widespread throughout America and Canada, is usually found on deep moist, fertile soils (e.g. on river banks). It is naturally suited to cool climate areas with deep, fertile soils that are well supplied with water, consequently warm climate conditions or dry, sandy, excessively free draining soils devigorate this species and scions grafted on to it (Pongracz, 1983).

The transfer of phylloxera to other wine producing countries has forced the grafting of vines in conditions different from that found in European vineyards. The early selections made by the Europeans have proven to be adequate for the needs of other countries providing the rootstock varieties have been assessed for their suitability and specific application in local conditions. For example, it has been noted that in European conditions the grafting of different rootstocks on the same scion can frequently bring about pronounced differences in the quantity of the resulting crop. This effect has been moderated by favourable climatic conditions in South African vineyards during initiation, flowering and fruit set, reducing the expression of this characteristic (Pongracz, 1983).

2.4.2.2. The Relationship Between Rootstocks, Plant Nutrition and Potassium

The interaction of the rootstock and the environment also expresses itself through the ability of its root system to develop and to extract adequate nutrients and water from the soil. This can be considered the source of the vigour of a rootstock and vigour differences conferred to the scion, are often more apparent in poor soils. Soils low in soil moisture or soil available nutrients are often better exploited by rootstocks known to impart vigorous growth to scions. This ability to extract nutrients from the soil has been studied in great detail in relationship to one cation: K^+.

The importance of potassium to plant growth is recognised and while it has no role in the structure of organic molecules it is involved in many important plant functions (Mullins 1992). The list of physiological processes involving this cation emphasise its central role in regulating plant metabolic function. It is used by the plant: to neutralise organic acids, for enzyme activation (of energy metabolism, starch synthesis, photosynthesis, sugar degradation), for membrane transport processes, osmotic water pressure regulation in cells and the regulation of stomatal opening (Iland, 1988). The uptake and accumulation of K^+ in grapes has implications for the resulting acidity and pH in wine (see Section 2.2). This relationship has been studied, particularly where high K^+ availability in the soil results in high K^+ content of juice for wine making (Mpelasoka *et al.*, 2003).

A number of relationships have been established between the uptake of K^+ by vine roots and the content of grape juice at harvest. Firstly, high levels of K^+ uptake from the soil generally result in high berry K^+ content at harvest (May, 1994). There is agreement that rootstocks do influence the composition of the scion grapes (May, 1994). The degree by which rootstock K^+ uptake varies is according to the ability to exploit soil reserves and this is at least partially dependent upon the uptake mechanisms present in the roots.

2.4.2.3. Rootstock Influence on Plant Potassium Uptake

It is believed that all rootstocks possess an active transport mechanism (i.e. movement of metabolites against the concentration gradient by expending energy), but high-absorbing rootstocks possess a passive transport mechanism, tied to the transpiration stream (Ruhl, 1992). The active transport system is believed to operate in soil available K^+ levels up to 0.5 mmol/L and the passive system at higher soil available concentrations of about 1 mmol/L and above (Ruhl, 1992). In addition rootstocks may differ in the mechanism for the transfer of K^+ from the roots to the scion. There is evidence of some rootstocks retaining higher concentrations of K^+ in the vacuoles of root cells thus providing relatively smaller concentrations to the grafted scions (e.g. 140 Ruggeri, 1103 Paulsen) (Ruhl, 1989).

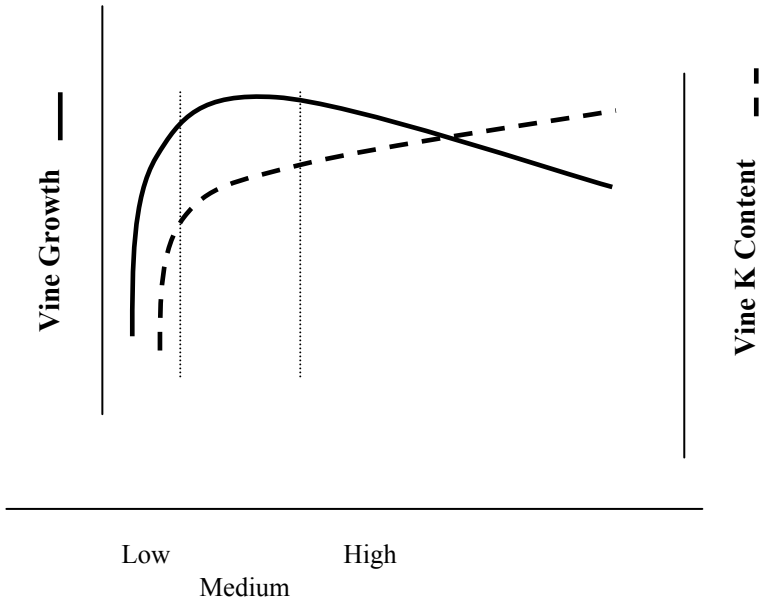
In addition to these mechanisms, other factors influence the rootstocks ability to exploit fully soil K^+ reserves. We can include soil structural elements such as layers in the profile impervious to root penetration, soil water availability especially where the vine is dependent upon irrigation, internal root structure, root architecture and mycorrhizal fungal associations. The external and internal influences on a root system's ability to extract K^+ from the soil environment are many and varied. Close study of rootstocks has indicated those most likely to inhibit uptake and those able to more efficiently extract this cation from the soil (Delas 1992, Pongraz, 1983).

Study of rootstock behaviour in warm climate regions of Australia established that rootstocks from crosses of *V. berlandieri* and *V. rupestris* (e.g. 110 Richter, 140 Ruggeri, 1103 Paulsen) exhibited lower foliar K^+ and lower transfer to fruit. Conversely, rootstocks descended from *V. champinii* (e.g. Dog Ridge and Freedom) showed high petiole K^+ and higher transfer to fruit (Ruhl, 1989). Similarly studies in France found that rootstocks SO4 and Fercal absorb and transfer more K^+ to scion leaves and fruit than those of *V. riparia* (Delas, 1992).

While the exact mechanism for the restriction of K^+ uptake and transfer is unknown studies in Australia and France have implicated mineral antagonism between magnesium

(Mg^+) and K^+ (Delas, 1992; Ruhl, 1991; Ruhl *et al.*, 1992). The implication is that rootstocks that are most susceptible to Mg^+ deficiency are the most resistant to K^+ deficiency (e.g. SO4, 110 Richter and Fercal). Conversely those most susceptible to K^+ deficiency are most resistant to Mg^+ deficiency (e.g. 1103 Paulsen, 140 Ruggeri, 41 B Millard et de Grasset) (Delas, 1992). The similarity between the K^+ susceptible and the low uptake rootstocks and the converse Mg^+ susceptible and high K^+ uptake rootstocks implies that mineral antagonism may be a factor in the regulation of K^+ uptake by vines. It has been shown that the response of some rootstocks to fertiliser application makes the mediation of K^+ uptake by the application of Mg theoretically possible in some situations (Ruhl *et al.*, 1992).

Figure 2.4 Generalised Vine growth and berry K^+ accumulation relative to the K^+ levels in the root medium revealed in the potted vines in glasshouse using sand culture system (Li, 2003).



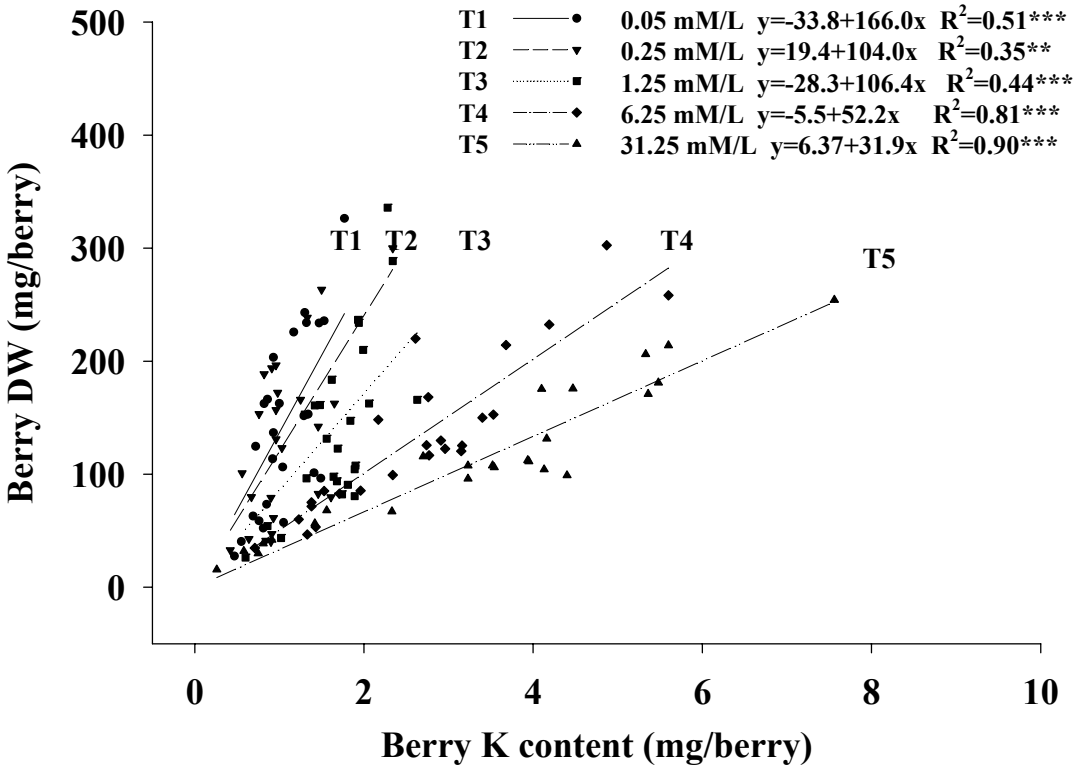


*Figure 2.5 Correlations of berry potassium (K) content and DW during berry growth based on each K treatment (Li, 2003) (** and *** represent the significance at p < 0.01 and 0.001 levels, respectively)*

In addition to the direct uptake and transfer of K^+ to the scion, other characteristics of rootstocks have potential to influence the transfer of this cation in to the fruit prior to harvest. The nutrient uptake and transfer to scions, particularly K^+ and nitrogen, has been implicated in the accumulation of dry matter (May, 1994; Ruhl *et al*., 1992). A study (conducted in a sand culture) established that increases in low (< 1.0 mM/l) to medium (1 to 6 mM/l) levels of plant available K^+ will stimulate vine growth and increase whole vine content of K^+. However, additions of K^+, where soil availability is already at high (>6mM/l) levels, potentially reduces vine growth (vigour declines at a modest rate, see Figure 2.4). More importantly increasing levels of plant available K^+ appears to

influence the plant content, concentration of K^+ and resulting berry K^+ concentration (Figures 2.4 and 2.5).

The stimulation of vegetative vigour also has implications for the increased transfer of K^+ to the maturing fruit (see Section 2.5.2). The vigour of rootstock/scion combinations can also be influenced by other factors, particularly rootstock compatibility to the vineyard environment, seasonal soil moisture levels, soil fertility, irrigation and fertiliser application. A rootstock that confers high vigour to a scion may prolong the growth period of the shoots into the maturation period of fruit potentially delaying sugar accumulation due to the additional competition for assimilates. There is a tendency for berry acidity levels to remain high due to bunch shading and therefore potential for additional K^+ transfer to berries throughout this elongated ripening period (see following sections).

2.5. The Influence of Climate, Plant Nutrition and Viticultural Management on Vine Growth (Canopy Density).

2.5.1. The Influence of Climate

To a great degree it is the influence of the seasonal climate that decides the individual compositional characteristics of a juice harvested from a wine grape cultivar grown in a particular region in a given season. The seasonal viticultural management of the vine can to some degree control the impact of weather patterns on the composition of the harvested fruit. As will be seen the mechanisms that elicit compositional changes are complex, subtle and little understood; in contrast vine management often appears coarse and clumsy. Factors such as seasonal temperature patterns, rainfall events, wind run and light intensity at key periods in the growing season can have a dramatic effect on vine growth patterns and plant physiology (Jackson & Lombard, 1993). Through the methods described below, the vine structure can be altered through direct management of vine nutrition and the structure of the canopy; however, these methods all add cost to the production of wine grapes.

2.5.2. Vine Nutrition

The soil/root interface is the other defining interaction the grapevine has with the environment. Availability of soil water and key nutrients (nitrogen (N), potassium (K) and phosphorus (P)) can greatly affect the vigour and eventual size of the vine canopy. To a degree we can influence the availability of these two growth stimulating factors, more so in situations where the soil and climatic environment offer the vine amounts less than that required for uninhibited growth. Through informed planning a vineyard can be designed aimed at limiting soil water and nutrients available to the vines. Examples of this are the selection of a rootstock with specific root growth characteristics to alter the manner of exploitation of the soil (e.g. shallow rooting in a site with a high water table) and the close planting of vines or sowing of an inter-row sward to deplete soil moisture and increase competition for nutrients.

In situations where there exists a deficit of either soil water or key nutrients we have the potential to manage the growth characteristics of vines. Techniques that may be used include; use of fertiliser or lime to boost soil available levels of key macro or micro nutrients, addition of organic matter (e.g. the incorporation of inter-row crops into the soil) to enhance the soil structure, improving nutrient availability and soil moisture levels, and direct management of soil water through irrigation (McLaren and Cameron, 1990). Increasing the supply of soil water or key nutrients in a situation where a vine is exhibiting sufficient crop levels and canopy vigour (and has no obvious nutritional deficiencies) may result in excessive canopy vigour, which has been shown to affect juice and wine composition (Crippen & Morrison, 1986; Kliewer & Dokoozlian, 2005; Kliewer & Lider, 1968; Smart *et al.*, 1985). .

The case of potassium is one of particular interest to this study as it is a major nutrient capable of influencing the vegetative growth characteristics of grapevines. It is a nutrient that can be taken up by roots in excess of the plant nutritional requirements (luxury uptake). Increased uptake of this nutrient is believed to increase the vegetative vigour of vines (Figure 2.4) and has been shown to increase the average size of leaves further increasing the impact on canopy density (Li, 2003). Increased potassium supply to vines

has also been implicated in the increased potassium content of harvested fruit (Figure 2.5) (Li, 2003; May, 1994). There is also evidence that, in situations where moderate to high soil available potassium exists, the selection of appropriate grapevine rootstocks may be of use in restricting the uptake of this nutrient (Ruhl, 1989).

2.5.3. Management Factors Affecting Canopy Density.

In addition to the soil related management touched on above there are a number of remedial techniques are available to the viticulturist to help manage crop load and canopy density throughout the growth cycle of the grape vine. Some are in effect pre-season planning and relate to the management and pruning of vines during vine dormancy. At this stage alteration of the trellis design, training system and bud numbers retained can be implemented. These decide the fundamental structure of the canopy and this should be designed to create a balance between crop load, vegetative vine vigour and as a result determine the density of the canopy (Bravado *et al.*, 1985; Clingeleffer *et al.*, 2000; Smart, 1985). The available configurations are almost limitless and the implementation is determined by the grapevine environment and wine quality and style outcomes required. The detail of the theory and implementation into practice will not be discussed in this review. It is sufficient to emphasise that well planned and executed winter pruning must take into account the fundamentals of good canopy design, in doing so this will minimise the incidence of excessively dense canopies. The detrimental impact of excessive canopy density on berry acidity and pH is covered in the following sections (2.6 and 2.7).

There are many situations where the winter pruning design fails to adequately compensate for the vigour of vines. For example, seasonal rainfall, especially in spring and late summer/early autumn, can stimulate excessive growth and the soil variability experienced in many vineyards can create less than ideal canopy densities in sections of rows or blocks in vineyards (Bogoni *et al.*, 1995, Mpelasoka *et al.*, 2003).

There is also a desire by some viticulturists to open up fruiting zones of canopies to enhance compositional development or to improve disease control (spray penetration and aeration). Whatever the reason, the adjustment of canopy during the growing season is

widespread. Shoot and lateral thinning, trimming and leaf plucking are all techniques employed either manually or through the use of machines, and without exception these all add cost to the production of quality wine grapes. However, the ramifications of not employing these techniques may have an impact during wine making. The cost of failing to adequately plan and execute timely canopy management may result in expensive remedial techniques during winemaking (for acidity, pH and disease problems) and the inability to create the wine style of the desired quality.

2.6. Plant Physiology and Acids in the Grape Berry

The two most important grape acids for determining wine pH are tartaric and malic acid, constituting up to 90% of the organic acids found in grapes (Ruffner, 1982a). A number of factors can determine the amount and composition of these two acids in a berry at harvest, including the cultivar, climate and viticultural management (Jackson & Lombard, 1993). The ratio of malic and tartaric acids has been reported to vary according to the grape variety (Kliewer, 1965; Ruffner, 1982a). Relatively high malate producing cultivars have been identified including Carigane, Chardonnay, Grenache, Malbec and Pinot Noir; whereas Chasselas, Merlot, Semillon, Riesling and Thompson Seedless have been identified with a high proportion of tartrate (Kliewer, 1965; Kliewer, Howarth & Omori, 1967).

The growth of grape berries is biphasic; the two phases of rapid growth are separated by a period of slow growth, the lag phase (phase 2). The initial rapid growth phase (phase 1) is characterised by the accumulation of both malic and tartaric acids in green berries (Kliewer, 1964; Skene & Hale, 1971). The concentration of malate and tartrate in grape berries reaches a maximum just prior to veraison during the lag phase (phase 2) of berry growth where growth stops for a period of approximately 10 to 15 days (Kliewer, 1965; Mullins, 1992). Berry concentrations of both acids fall during the ripening process (post veraison) during the second period of accelerated berry growth (phase 3). The net amount of tartaric acid remains relatively constant, the concentration falling due to dilution from berry expansion (Hale, 1977). The concentration of malic acid decreases both by dilution and there is evidence of further significant decrease due to a process of degradation

(Hale, 1977). During phase two the net decrease in organic acid content is dominated by the reduction in malic acid.

2.6.1. The Synthesis of Berry Acids in Grape Berries

To understand the implications of external influences on acid composition we must first consider the internal mechanisms that create and degrade berry acids. In the past the source of tartaric and malic acids found in the fruit of grapes was thought to be grape leaves (Peynaud & Maurie, 1958; Stafford & Loewus, 1958). The importance of the mechanism of external synthesis and subsequent transportation to the grape berry has largely been discounted. The berry has been demonstrated to be an important site of acid synthesis (Hale, 1962), the main translocated substance in the phloem has been found to be sucrose (Swanson & El-Shishiny, 1958) and the synthesis of malic acid from transported sucrose has been proven (Hardy, 1968). Similarly, no evidence of the transport of tartaric acid into the berry has been found (Ruffner, 1982a).

The grape is the most widely cultivated plant that accumulates tartaric acid in the fruit. Tartaric acid in grapes is only found as the optically active L-(+)-sterioisomer (Ruffner, 1982a). Tartaric acid accumulation has been linked to the early growth period in grape berries (phase 1) (Hale, 1962; Ruffner, 1982a). Synthesis and rapid accumulation of tartaric acid occurs in immature berries and leaves during cell division and rapid growth (Hale, 1962; Kliewer, 1965; Kriedemann *et al.*, 1970; Saito & Kasai, 1968). Studies reducing the canopy size (through shoot topping) after veraison have reported no change in the level of tartaric acid found at harvest between the treatments and control (Solari *et al.*, 1988) suggesting the accumulation and stabilisation of this acid occurs earlier in the season.

While a precise categorisation of the mechanism for the synthesis and accumulation of tartaric acid in grape tissues has not been defined, research has indicated a number of potential pathways. Hardy (1968) established that a high proportion of ^{14}C-labelled sucrose was found in organic acids within a few hours of administration to immature grape berries. This indicated a mechanism for the synthesis of organic acids from glucose

and fructose in immature berries. While unable to precisely categorise the mechanism, Ruffner (1982a) indicates the evidence that radioactivity from $^{14}CO_2$ is only found in tartaric acid after an extended period suggests that it is a secondary metabolite. In leaves it appears to be derived from pretaric acid, via the pentose phosphate pathway and in the fruit probably from galacturonic acid (Ruffner, 1982a). The treatment of berries with ^{14}C-malic acid resulted in low transference of radioactivity to the tartaric acid suggesting that the two acids are not closely related biochemically (Hardy, 1968).

Malic acid is known to be an active intermediate compound in grapevine metabolism. Malic acid has a significant role in the assimilation and storage of carbon dioxide (CO_2) during photosynthesis (Ruffner, 1982b) in green tissues throughout the whole plant. The synthesis of malic acid in green berries from imported sucrose is via hexose utilising pathways most notably glycolysis. This is believed to be the mechanism by which the green grape berry stores excess transported assimilates (Ruffner, 1982b).

2.6.2. The Degradation of Berry Acids in Grape Berries

The accumulation of tartrate in the berry is considered to be a result of the acid forming insoluble salts with monovalent metal cations (Saito & Kasai, 1968). The formation of tartaric acid in green berries has been shown to be a dynamic balance of the creation and degradation of the acid in the early stages of growth. The formation of the salt forms of this acid is believed to protect the acid anions from catabolisation by enzymes (Saito & Kasai, 1968). The process of salt formation continues through the process of berry ripening (phase 3) and is considered the primary reason why the levels of this acid remain stable throughout this period (Ruffner, 1982a).

In green berries, prior to veraison (phase 1), malic acid concentrations have been found to be higher in the interior tissues of the berry and lower in the outer mesocarp and the skin (Gutierrez-Granada & Morrison, 1992; Possner & Kliewer, 1985). It has been shown that malic acid in the outer tissues of the berry can be more readily respired (Steffan & Rapp, 1979), offering an explanation for this reported gradient. A change in the distribution of malic acid within the berry occurs from veraison onwards. There is an increase in the

malate content of the skin and a reduction in the interior tissues and in the net berry content during the ripening process (Gutierrez-Granada & Morrison, 1992; Possner & Kliewer, 1985).

Tartaric acid has been identified as having the highest berry concentration just prior to veraison (Kliewer, 1965). Although a small decline in the level of total tartrate has been detected in some studies, it is recognised that the post veraison levels remain relatively constant (Ruffner, 1982a). There is some evidence of the respiration of tartaric acid at high temperatures, typically temperatures above 30^{o} C, however the net effect from this is usually small (Peynaud & Maurie, 1958). Therefore, the decline in concentration of tartaric acid in the ripening berry is primarily attributed to the dilution effect from berry expansion (Possner & Kliewer, 1985).

The onset of ripening alters the physiological behaviour of grape berries, especially the pattern of malate metabolism (Possner *et al*., 1983). During this period malic acid is able to diffuse from the interior of the berry to the periphery where it is more readily respired (Gutierrez-Granada & Morrison, 1992; Steffan & Rapp Vitis, 1979). There is evidence of a switch from carbohydrate breakdown to malic acid degradation as the main source of respiratory substrates (Possner *et al.*, 1983). There is also a sharp reduction in of sugar metabolism to malic acid via glycolysis after veraison (Ruffner *et al.*, 1983). It is thought that these two processes are probably related and it may be the inhibition of glycolysis that triggers the change in the availability and mobility of stored malate in the berry at this time (Ruffner, 1982b).

It has been proposed that a modest proportion of the net reduction of berry malate is as a result of the (net) reversal of the glycolytic flow after veraison. The process of converting malic acid to sugar synthesis is known as gluconeogensis and has been shown to occur in grape berries during phase 3 (Ruffner *et al.*, 1983). This process is believed to compete directly with respiration for the available pool of malate. Berry energy demands and respiratory rates are lower at cooler temperatures and thus may favour gluconeogensis while conversely at elevated temperatures respiration is believed to dominate the

degradation of berry malic acid (Ruffner, 1982b, Ruffner *et al*, 1983). The process of gluconeogensis is thought to account for a significant proportion of malic acid conversion at lower temperatures. This process has not been studied in any detail and estimates of the relative proportion of malate conversion at various temperatures are as yet unavailable. This process must operate at relatively low malate conversion rates relative to berry respiration; the rate of conversion of malic acid is lower at low temperatures than at higher temperatures where respiration is most active (the increase in gluconeogensis does not compensate for the drop in respiration at low berry temperatures) (Ruffner, 1982b).

2.6.3. Factors Influencing the Accumulation of Berry Acids

2.6.3.1. Temperature

A number of studies have identified temperature as a key factor in the rate of malic acid degradation during the ripening period of grape berries. Initially, studies focussed on the differences between regions and seasons within regions to identify this general principle (Jackson & Lombard, 1993). Low acid concentrations were identified with warmer climate regions and relatively higher acid contents found in cooler regions (Coombe, 1987; Kliewer *et al.*, 1967). Subsequent studies focussed on the effect of temperature on the individual clusters and berries throughout grape canopies. Implicit in the study of the effect of temperature on the grape berry is the effect of sunlight exposure on clusters throughout the canopy. Solar radiation is the source of the highest variance in berry temperature, shaded berries accumulate significantly less heat than berries exposed to the sun and total accumulated heat for sun exposed fruits has been measured at 43 to 62% greater than interior shaded fruits (Kliewer & Lider, 1968).

Studies of shaded and unshaded fruit clusters have shown that day temperatures inside the canopy are generally lower and night temperatures higher (Crippen & Morrison, 1986). During the period of one day, shaded clusters are exposed to a narrower range of temperatures than sun exposed clusters. The sun-exposed clusters can exhibit large temperature gradients between exposed and shaded sides (Kliewer & Lider, 1968). The effect of solar heating during the day and radiational cooling at night also intensifies the

temperature differential experienced by the exposed bunches (Crippen & Morrison, 1986). Kliewer and Lider (1968) found that the temperature gradients within sun exposed clusters resulted in significant compositional differences between berries within these bunches at harvest and that the berries in fully shaded interior clusters differed little in composition.

The temperature variation between shaded and exposed clusters has been shown to increase berry average weight in the shaded clusters. This has been attributed to the reduction in transpiration at the lower temperature gradient experienced by the shaded clusters and correspondingly higher water content (Crippen & Morrison, 1986). This phenomenon may help to explain the dilution effect of berry solutes, especially with regard to tartaric acid, and must be considered when comparing acid concentration measurements between berries. It also raises the question whether the smaller average size of sun-exposed berries has any bearing on the concentration of and respiration rate of malic acid.

The rate of malic degradation has been linked to the temperature of a grape berry, with low temperatures resulting in higher concentration of malic acid and moderate to high temperatures resulting in relatively lower berry concentration (Jackson & Lombard, 1993; Kliewer & Lider, 1968). The investigation of sunlight exposure with relationship to the heating of the berry is thought to provide only part of the answer. In a study of two red varieties, in the Pessac region of Graves, Peynaud and Maurie (1958) investigated the seasonal impact upon acidity across five growing seasons. They found variation in the proportion of malic and tartaric acids formed in the fruit depended on the season. The sum of the two acids remained relatively constant and they were unable to explain this phenomenon in relationship to the two general climatic variables collected (temperature summation and total rainfall). Climatically similar seasons produced quite different levels of the two acids and their analysis did not pick up the causal relationships. They noted that the final acid levels at harvest do not necessarily maintain the tartaric/malic ratio (T/M ratio) found at veraison, but that in the years where the grape retains more malic acid the tartaric acid level is lower. They did not observe a relationship between the level

of malic acid and the heat in autumn but did find that the harvest level of total acids (the sum of tartaric and malic) remained relatively constant between seasons.

2.6.3.2. Light Intensity and Radiation Wavelength

It appears that the effect of the climate on the creation and degradation of berry acids is more complex than a broad brush - one season or area is warmer, therefore lower acidity would be expected due to the higher degradation of malic acid. There has been an attempt to define the effect of canopy shading on compositional elements of grapes not just in terms of the seasonal heat accumulated but also the quantity and quality of light reaching grape clusters. The importance of light in the photosynthetically active radiation waveband (PAR) of 400 to 700 nm to grapes has been proven by the fact that this is ca 90% absorbed by grape leaves. Conversely light in the near infrared waveband (750 to 1100 nm) is absorbed at a rate of ca 10% (Smart *et al.*, 1988). The absorption of PAR light by leaves leads to low levels being available within the canopy. The light available to leaves and clusters in the centre of dense canopies is reported to be of a low flux density (reduced to as much as 1% of that reaching exterior leaves) in the PAR band and is relatively high in the near infrared (Smart *et al.*, 1988). Measurement of the flux density of the PAR, the photosynthetic photon fluence rate (PPFR), reveals levels up to or greater than 2000 $\mu Em^{-2}s^{-1}$ above the canopy and values less than 20 $\mu Em^{-2}s^{-1}$ in the canopy interior (Smart *et al*, 1988). The ratio of red (660 nm) to far-red (730 nm) radiation (R: FR) has also been identified as an important measure for phytochrome reactions.

The importance of phytochrome reactions has not been directly established for grapevines. Smart (1987), in a review article, established that key enzymes, thought to regulate grape ripening, were affected by phytochrome in other species. These enzymes included PEP-carboxylase, malic enzyme, malic dehydrogenase, phenylalanine ammonia lyase, nitrate reductase and invertase. At the canopy surface, in sunny or overcast conditions, the R:FR ratio is reported at 1 to1 but this can be reduced to less than 0.1 to 1 in dense canopies (Smart *et al.*, 1988).

The effect of seasonal climatic conditions and canopy shading can therefore have a multiple impact on grapevine physiology. The intensity of PPFR affects vine photosynthetic rates and the availability of the products of these reactions. This is potentially important in the early season for the rapid growth of vines and the production of sugars for transport to the developing berries, both identified as important for the production of berry acids. The level of the R:FR ratio throughout the growing season may alter the composition of grape berries due to its impact on the rate of important enzymatic reactions. This may potentially explain the seasonal variation expressed in the T/M ratio. The role of temperature on the berry, while confused by the latest research into light, may still have an important role in determining the rate of acid accumulation pre-veraison and the rate of acid degradation after veraison. However, it is concluded that (for a given cultivar) the position of a cluster within a canopy, the position on the cluster, the growth rate of the canopy and the level of light exposure throughout the season will largely determine the levels of malic and tartaric acids found in an individual berry at harvest.

2.6.3.3. Yield and Vine vigour

It has been established for some time that high crop loads will reduce the vegetative growth of grapevines. To study the effect of crop load on berry composition, Jackson (1986) set up a glasshouse trial that controlled the climate, light intensity and canopy shading of clusters and leaves. This study highlighted a possible relationship between crop load and the resulting canopy size (separate from shading effects) on the level of organic acids and pH at harvest. Jackson concluded that it appears that shoot vigour can have a direct effect on fruit composition, under the warm conditions maintained in the trial low crop/high shoot vigour vines expressed low acid levels and unacceptably high pH.

This appears to challenge the concept that high vigour canopies with increased cluster and leaf shading cause detrimental alterations to the composition and expression of berry acidity, indicating that this is an effect of the vigour and not the shading. This was a glasshouse experiment conducted in warm conditions where light interception by leaves

and clusters was guaranteed regardless of vigour. These results may not be replicated in the field with commercially grown vines due to variable canopy conditions and temperatures in the canopy interior and around fruit. The result is indicated by a negative linear correlation between total leaf area and acidity. Further investigation explains this in terms of timing of leaf growth.

The acid and pH levels are not significantly affected by total leaf area before the end of stage one and the effect on acidity is most strong when leaf growth occurs later in the season. This study (Jackson, 1986) highlights a relationship between low crop and vine vigour as the lower cropped vines experience stronger vegetative growth later in the season. Therefore, the growing shoot is a stronger sink relative to the fruit (compared to higher cropped vines) and is competing successfully for the supply of photosynthates later in the season. Acid synthesis in the berry from sugar transport may be affected during this extended period of shoot growth (phase1 and 2) and the warm, high, light conditions maintained throughout phase 3 is optimal for the respiration of malic acid. Therefore, it is reasonable to suggest that the low level of acidity found is consistent with the findings presented above, the impact on pH is of interest and this will be discussed in the following sections.

2.7. Factors Affecting the Accumulation of Potassium in Grape Berries

2.7.1. Seasonal Potassium Accumulation in Grape Berries.

The levels of K in grape berries increases throughout the growing season (Hale, 1977). Approximately two thirds of the K accumulation occurs prior to veraison (Li, 2003). K levels in the skin of grapes have been reported to be two to four times that of fleshy tissues throughout the growth of the berry. Skin levels of K increase at a significantly higher rate during phase two of berry development (Gutierrez-Granada & Morrison, 1992).

Gutierrez-Granada & Morrison (1992) investigated the hypothesis that malic acid was the main complimentary anion to K^+ (Storey, 1987). They found no correlation between

malate and K^+ in berry tissues, malic acid levels declined in internal fleshy tissues while K^+ accumulated and by harvest the K^+ levels were nearly three times higher than malate in the skin and outer mesocarp but approximately equal in the interior flesh. This is potentially an expression of the on going respiration of malic acid at the berry periphery, the maintenance of K^+ levels in the skin and mesocarp and the continuing introduction of K^+ into the berry flesh throughout berry expansion during phase 3.

2.7.2. Leaf Shading and Leaf Photosynthetic Activity

The grapevine canopy consists of leaves of different ages situated in positions in the canopy that subject them to different light intensities and lengths of light exposure. The photosynthetic output of a leaf within the canopy is determined by the amount and quality of the light that it receives and this is determined primarily by the position of the leaf in the canopy (Hunter & Visser, 1989). Studies have found a relationship between increased leaf shading and increased K^+ levels in harvested fruit (Smart *et al.*, 1985). The transfer of K^+ from the leaf is may be a result of lower than optimum levels of photosynthetic activity in the shaded leaves. It is thought that the transfer out from shaded leaves results in increased transfer to berries either due to increased K^+ concentration in all plant tissue or as a result of a direct transfer from the leaf to a berry (Iland, 1988; Li, 2003).

Excessive leaf shading is believed to alter the composition of solutes transferred out of leaves via the phloem. Low levels of photosynthetic activity have been implicated in increased K^+ movement out of leaves. This indicates that whenever the leaf is operating at a level below optimum there is potential for the additional mobilisation of K^+. This implies that any influence that reduces the photosynthetic activity of a leaf has the potential to increase berry K^+ levels.

2.7.3. Leaf Area and Leaf Photosynthetic Activity and Potassium in Grape Berries

Leaf shading is the most obvious and well-researched mechanism for reducing the photosynthetic activity of leaves, however, we have mentioned a study by Jackson (1986)

that indicates that vine vigour (not just leaf shading) may have an effect on the composition of grape berries. In his report Jackson reported very high levels of pH (up to 4) along with low berry acidity in relatively warm growing conditions. Despite relatively low levels of reported acidity the levels of pH found in this study are at the extreme high end of the range expected from wine grapes (in this case Cabernet Sauvignon). Potentially, the reported pH levels are a result of increased K^+ accumulation decreasing the dissociation of the remaining organic acids (described in detail in Section 3). The implication of this study is that not just leaf shading but also vine vigour and increased leaf area may have an influence on the accumulation of K^+ in berries and the increase in berry pH.

In this paper (Jackson, 1986) there is a discussion of studies into leaf area to fruit weight ratios that are desirable to ensure adequate ripening of grapes. In this experiment analysis of leaf to fruit ratios established 10 cm^2 per gram of fruit as the point where additional leaf area did not increase the accumulation of berry sugar. This indicates the point at which the photosynthetic capacity of the plant had been reached at the time in the season when the fruit is the dominant sink for photosynthates. There were a number of plants in Jackson's (1986) study with leaf area above this plateau. In this study (Jackson, 1986) in the analysis of pH against total leaf area there is a direct linear correlation between total leaf area and pH, an increase in leaf area corresponding to an increase in pH, also this relationship was shown to apply to vines with high leaf to fruit ratios.

Results from shoot topping experiments have also demonstrated a relationship between pH and leaf area. An experiment conducted in the warm climate Po Valley region of Italy investigated the effect of the leaf to fruit ratio on juice composition of field grown Sangiovese berries. Researchers found that a reduction in the leaf area: fruit weight reduced berry K and lower pH (Solari *et al.*, 1988). The soluble solids level remained constant indicating that this early phase 3 intervention, which reduced shoots to 12 leaves, maintained the leaf to fruit ratio at a level sufficient to maintain the photosynthate flow into the berries to the maximal value.

This study (Solari *et al.*, 1988) also recorded an increase in harvested malate, probably as a result of increased lateral growth in the fruiting zone increasing cluster shading and decreasing malic acid respiration. While the lateral growth in this situation was not excessive, lateral growth was reported and typical lateral growth patterns tend to occur from the basal nodes towards the apical, thus impacting primarily on the fruiting zone of the treated shoots. A reported increase in average berry weight on the topped vines (Solari *et al.*, 1988) strengthens this hypothesis. The increase in lateral growth caused increased cluster shading, resulting in larger average berry size (see Section 2.4.1.3).

The significance of the reduction of berry pH is discussed, the authors (Solari *et al.*, 1988) believing that the effect was a result of a reduction in leaf shading (through reduced lateral growth) and from a reduction in the canopy age since topping proportionally increased younger leaves from the laterals. This relates to the theory that older leaves are more likely to export K^+ and that leaf export of K^+ has been linked to subsequent accumulation in berries. The ideas put forward are contradictory, on one hand the lateral growth is reduced and on the other it is increased due to topping and average leaf age is reduced. As noted, the initial lateral growth that occurs is likely to be from buds lower down on shoots around the fruiting zone and with shoot topping a high proportion of the early lateral growth would be retained. The further growth of laterals after topping will be restrained by the increase in demand placed on the remaining leaves for carbohydrates by the fruit, the primary sink post-veraison. Therefore, it is most likely that the leaf shading throughout the canopy and bunch shading in the fruiting zone was reduced and the activity of leaves throughout the topped canopy maintained by the shoot topping.

In addition, while there has been reported a relationship between leaf age and K^+ export, the net effect must be considered per leaf, the concentration in that leaf, leaf size and the number of leaves of a certain age. The average leaf age may have been reduced overall by the treatment, however just as many basal leaves (which have larger areas than the newer leaves) were retained. These are of the most advanced age and proportionally are likely to contain the highest K^+ concentrations (Li, 2003). Therefore, the relationship between leaf

age, decline in photosynthate output and increase in K^+ export is actually the reverse of that offered as an explanation in this experiment (Solari *et al.*, 1988). In the topped vines proportionally there is a larger percentage of ageing basal leaves, representing a higher percentage of the total leaf area, thus, increasing their representation in the leaf area to fruit ratio, relative to the untreated vines.

If this relationship is investigated relative to the average leaf photosynthetic activity a more robust explanation emerges. The maintenance of the assimilate transport rate to the fruit, after topping, indicates that on average, leaves on the topped vines are more photosynthetically active (Petrie *et al.*, 2000). The smaller leaf area is producing a similar amount of exported solutes as the proportionally larger leaf area from the untopped treatment. The reduction in pH found in this experiment (Solari *et al.*, 1988) is more likely to result from the increased photosynthetic productivity of the leaves, resulting in reduced K^+ export. This is consistent with the evidence that leaf shading increases K^+ export from (senescing) leaves and eventual berry uptake. The fact that the effect is found with all of the oldest, (potentially highest K^+ concentration) leaves retained indicates that the productive life of these leaves can be potentially enhanced, possibly delaying age induced senescence and the export of leaf K^+.

2.7.4. Leaf Area: Yield Ratio and Potassium in Grape Berries

Further evidence of this potential relationship between leaf photosynthetic activity and K^+ export is to be found in a study investigating whole vine photosynthesis (Petrie *et al.*, 2000). In a series of experiments relationships between leaf area, yield, leaf photosynthetic activity and leaf ageing were investigated. Results confirmed that basal leaves on high source to sink vines (i.e. high leaf area: fruit ratio) senesced more rapidly than leaves of low source to sink vines (Petrie *et al.*, 2000). This study concluded that the decline in leaf photosynthesis and early senescence, previously associated with leaf age, was probably caused by the increase in leaf area: yield ratio resulting from extended shoot growth and new leaf development. This study also identified a relationship between inadequate demand for photosynthates and the reduction of the whole vine photosynthetic output to below theoretical capacity, suggesting that the inhibition of photosynthesis is

due to a build up of carbohydrates in the leaves. The results of this study (Petrie *et al.*, 2000) back up the theory that it is potentially the photosynthetic activity of leaves that is the important determinant of K^+ export from leaves back in to the vine.

The relationship of yield to vine canopy area has also been investigated in Australia in warm climate regions, where the fruitfulness of some varieties produces large crops on minimally pruned vines. In an effort to improve harvest fruit composition a series of experiments investigating the effect of mechanical crop thinning were instigated. The results established that a 40% reduction of crop (36 to 25 t/ha) on minimally pruned Shiraz resulted in the grapes 'ripening' 3 weeks earlier, with lower pH (3.6 compared with 4.1) and with a higher titratable acidity (5.2 with 3.5 g/L) (Clingeleffer *et al.*, 2000). The effect in a warm climate of crop reduction is to reduce the time it takes to accumulate sufficient sugars, colour and flavour components to meet the requirements of ripe fruit ready for harvest.

The reduction of time on the vine appears to improve the acid composition and the resulting pH. Whether the effect on the pH is simply a product of higher retained malic acid or a component of both acid retention and lower berry K^+ accumulation is unclear. In relationship to cool climate viticulture the effect of altering the crop load is also to potentially alter the length of time a crop is held on the vine. Cool climates (especially in New Zealand) generally exhibit slower sugar accumulation and malic acid reduction at the end of ripening than that experienced in warmer conditions. An increase in crop load will further extend the period to attain optimal sugar accumulation and berry flavour development. Additional time on the vine offers an extended period of time for accumulation of K^+ in berries. Potentially, high crop levels in cool climates can result in harvested fruit with low sugars, high TA and relatively high pH. This problem can be expanded if the fruit is held on the vine close to the season end when the cool average daily air temperatures and low soil temperatures are prompting the senescence of leaves in preparation for vine dormancy.

2.7.5. Pruning Weights, Yield and Potassium in Grape Berries

In addition to the interpretation of the crop load to leaf area ratio to grape berry composition there has been a number of studies investigating the dry weight partitioning to crop load. While it has been shown that grapevines have the ability to adjust leaf photosynthetic activity, vigour (shoot growth, leaf size, total plant leaf area) and carbohydrate partitioning to meet the demands of increased crop load this is mediated by the competition from other metabolic and vine growth sinks (Edson *et al.*, 1995). Sink demand changes throughout the season and assimilates are allocated according to the priority, locality and demand. The expression of vine balance has been expanded by the development of the ratio of crop load and dormant vine pruning weight. This has been investigated to establish the point where either the crop is too low, aggressively increasing canopy growth or too high restricting water, carbon and nutrient partitioning to the vine, both potentially detrimental to fruit quality and the future productivity of the vine. The generally accepted range is crop load values between 5 to 10 kg of fruit to 1 kg of pruning weight (Bravdo *et al.*, 1985; Kliewer & Dokoozlian, 2005). It has been suggested that this is scion and climate specific, Pinot Noir grown in a cool climate expressing an optimal range of around 3 to 6 kg of fruit per kg of pruning weight (Kliewer & Dokoozlian, 2005). Pruning weight has been shown to correlate with leaf area (Bravdo *et al.*, 1985) indicating that these parameters may be influencing each other with in their relationship to crop load and vine balance.

While the parameters of wine quality to crop load have been investigated, in relationship to the capacity of a vine to ‘successfully’ ripen a crop, there is insufficient data available to establish the relationship between crop load, berry acidity and pH. The parameters used to determine fruit quality are varied and diverse and the assessment of the ‘over cropping’ or ‘under cropping’ of the vines is dependent upon the emphasis of the research. The relationship between vine vigour and crop load is recognised but so far not accounted for in experimental design. Leaf and cluster shading may account for the acidity and pH variances expressed, although no assessment of canopy density has been offered (Bravdo *et al.*, 1985; Kliewer & Dokoozlian, 2005). Whether the size of a crop has any net effect on the concentration of K^+ or acids in harvested berries is unable to be

assessed at this stage. It is worthwhile to note however, the range of values agreed upon for the ratio of crop weight to pruning weight indicates a relatively broad band of potential vine crop loads that can be ripened. This is an indication of the capacity of grape vines to physiologically compensate for additional crop load; especially regarding the allocation of carbohydrate assimilates. It is primarily the effect on vegetative growth (total leaf area) and the subsequent extension of time taken to accumulate sufficient berry sugars for harvest that determined this range and both of these parameters are likely to have a significant impact on the harvest composition of acids, K^+ and pH.

2.7.6. Summary: The Relationships between Yield, Canopy, Photosynthesis and Berry Potassium.

To summarise, it appears that if the plant leaf area exceeds the requirements of that needed to "ripen" the fruit the net effect may be to increase the harvest pH. In phase 3 of berry development the relationship between leaf area and crop load largely decides the average level of photosynthetic activity in vine leaves, as the fruit is the major sink throughout this period. This may have an effect on the amount of K^+ exported from the leaf back into the vine prior to leaf senescence.

The reduction in the photosynthetic capacity of leaves through leaf shading may strengthen this relationship as vine shaded leaf area has been shown to be proportional to harvest berry K (Dokoozlian and Kliewer, 1996; Rojas-Lara and Morrision, 1989; Mpelasoka *et al.*, 2003). There is a direct relationship between harvested berry K^+ levels and extracted juice pH (Li, 2003; Margalit, 1997). It appears theoretically possible to reduce the export of K^+ by maintaining leaf to fruit ratios that maintain leaf photosynthetic capacity as close to optimum as possible, in as many leaves as possible throughout the growing season and in doing so manage grape berry pH (Petrie *et al.*, 2000). Achieving balanced vine growth, reducing the natural vigour of scions to the point where the fruit to canopy balance is sufficient to achieve fruit ripening will reduce leaf and bunch shading and ensure leaves are photosynthetically efficient.

Balanced vine growth has also been defined in terms of its relationship to crop load both by leaf area and pruning wood harvested at dormancy. The optimal range of this measure will vary for different climatic conditions, soils, water regimes and different scions and the eventual crop load may have an impact on the vegetative growth characteristics of the vine and the time taken to accumulate sufficient sugars for harvest. The balanced vine concept must take into account the impact of crop load on acid and K^+ levels. An important element in managing the vegetative vigour of the scion is the management of the vine roots.

The advent of wide spread grafting on to rootstocks offers opportunity to match the root configurations of different rootstocks to the soil and scion combinations to help achieve balanced vine growth. The interaction of crop load and canopy management may also be mediated in part by the nutritional characteristics and vigour of rootstock scion combinations.

3. Chapter 3: Muddy Water Trial Canopy and Yield Results

3.1. Introduction

Any grafted wine grapevine will express the environmental conditions where it has grown in the composition and perceived qualities of the harvested fruit. Any observation of a vineyard will show varying growth and fruit bearing responses of individual grafted vines of similar genetic origin; this reflecting changes in vine aspect, shelter, soil structure, water status nutritional conditions and vine health. Interactions between the rootstock and scion may also alter the degree of response to similar environmental conditions.

Rootstocks express varying responses to environmental conditions and one aspect of this potential response is the degree of vegetative vigour imparted to the scion. This may be a result of the degree of adaptability of the rootstock to a number of environmental conditions and the observed results can have complex determinants. Similarly, the interaction between the genetic character of a rootstock and the environmental conditions at a vineyard site is believed to influence the crop bearing ability of the whole vine and the resulting fruit composition (Pongracz, 1983; Pouget, 1987).

The effect of vine vegetative vigour on fruit quantity and composition is well established. Work investigating canopy area to yield ratios has shown that varying the canopy to fruit ratio can alter sugar accumulation rates in berries (Kliewer & Dokoozlian, 2005). Also, canopy density and subsequent leaf and berry shading has been implicated in the accumulation of berry potassium (K) (Smart *et al.*, 1985) and in altering the acid structure and pH (Crippen & Morrison, 1986; Kliewer & Lider, 1968) of fruit.

What is not well understood is the degree of interaction between the rootstock influence on vine nutrition, and subsequent vine vegetative vigour, on yield and the eventual composition of harvested fruit. Do rootstock influenced changes in vine nutrition (and

water status) directly affect yield and fruit composition or are changes a result of the effect on canopy growth, or is it a synthesis of both?

The trial was designed to expose rootstock derived influences on berry composition, which may be as a result of rootstock derived variation in plant nutrition, water status and physiology or may be from yield and vine vigour differences imparted by the rootstock. Because it is known that choice of rootstock can have an influence on canopy growth (Delas, 1992; Pongracz, 1983; Pouget, 1987), a canopy treatment was incorporated in to the experiment. This allowed assessment of the canopy effects and the rootstock effects (and any interaction between them) on the composition of the berry.

3.2. Materials and Methods

3.2.1. Trial Site

The experiment was established on a rootstock trial site at Muddy Water vineyard, Waipara, North Canterbury in the summer of 2003/2004. The trial block was established in November 1996 (one rootstock, 420A, was planted a year later) and consisted of 8 rootstocks grafted to Pinot Noir clone AM10/5 (Lincoln Selection, sourced from the Wairarapa Vine improvement Group). Plots consist of 5 vines in one bay of the same rootstock-scion combination; the trial design is an 8x8 Latin Square (see Figure 3.1). All rootstocks were sourced from the Te Kauwhata Research Station and grafting was done at the Ormond Nursery, Marlborough. All planting material was ELISA tested for common grapevine viruses and shown to be free of virus.

The Lincoln Selection AM10/5 was suspected to be a selection containing more than one clone. The site was assessed during the growing season for vine ampelography. Two clones were distinguished and defined as “Upright” and “Droopy”. The Upright clone was defined as having upright bunch architecture (bunch tip pointing upwards to the sky) and

Figure 3.1: Trial Map for Muddy Water Rootstock Trial, Waipara, New Zealand.

Plot numbers in parentheses, rootstock code in bold. Rows are oriented North-South and row numbers run East (40) to West (47). Each plot represents a bay of 5 vines.

Rootstock Legend:	
1 = Riparia Gloire de Montpellier	5 = Schwarzmann
2 = 101-14 Millardet et de Grasset	6 = 99 Richter
3 = 420A Millardet et de Grasset	7 = Fercal
4 = 5 C Teleki	8 = 3309 Couderc

Row Number	**North end**							**South end**
40	**1** (1)	**2** (2)	**3** (3)	**4** (4)	**5** (5)	**6** (6)	**7** (7)	**8** (8)
41	**2** (9)	**1** (10)	**7** (11)	**6** (12)	**8** (13)	**4** (14)	**3** (15)	**5** (16)
42	**3** (17)	**7** (18)	**1** (19)	**5** (20)	**4** (21)	**8** (22)	**2** (23)	**6** (24)
43	**4** (25)	**6** (26)	**5** (27)	**1** (28)	**3** (29)	**2** (30)	**8** (31)	**7** (32)
44	**5** (33)	**8** (34)	**4** (35)	**3** (36)	**1** (37)	**7** (38)	**6** (39)	**2** (40)
45	**6** (41)	**4** (42)	**8** (43)	**2** (44)	**7** (45)	**1** (46)	**5** (47)	**3** (48)
46	**7** (49)	**3** (50)	**2** (51)	**8** (52)	**6** (53)	**5** (54)	**1** (55)	**4** (56)
47	**8** (57)	**5** (58)	**6** (59)	**7** (60)	**2** (61)	**3** (62)	**4** (63)	**1** (64)

an upright shoot growth habit (shoots stand straight and upright in the canopy). The Droopy clone had bunches that point downwards towards the ground and the canopy growth tended to spread laterally along the canopy. The assessment allowed the early

identification of plots with both clones and this information was used during collection and analysis of information to assess the impact of clone on results (see Tables 7.2 & 7.3 in appendix).

The soil at this site is sandy clay loam topsoil over non-calcareous clay loam subsoil. The trial block was not situated at the edge of the vineyard to avoid edge effects from lack of competition or wind. A fault in the layout of the 8x8 Latin Square (Figure 3.1) where the rows were not randomised prior to planting resulted in a mirror image of plot layout from a diagonal line running from the north-east to south-west corners of the trial block. Due to the potential of a soil gradient through the block a decision was made to assess the nature of the soil across the trial site. Dr Phil J. Tonkin (soil scientist, Soil Science Department, Lincoln University) evaluated the soil type and profile across the trial block. Dr Tonkin had been involved in an earlier assessment of the Muddy Water site prior to the vineyard planting. His assessment of the soil across the site was: "An even soil type across the block, with no more than 10% variance in the clay content of the clay loam and entirely suitable for a trial of this type." Further to this soil cores were taken across the block to measure top-soil depth, and analysis of these data did not show a soil gradient (see Appendix, Table 7.1 for data).

3.2.2. Experiment Design

The initial set up of this trial occurred during winter pruning (2003), where two canes were laid down horizontally (wrapped on one fruiting wire at a height of 900mm) per plant (i.e. design as standard 2 cane Vertical Shoot Position (VSP) training system), containing where ever possible 24 buds (count nodes). An additional 2 nodes were retained if a spur was required to maintain the structure of the vine. During winter pruning (2004) the vines were pruned to two canes, old wood from the head discarded if removed and the weight of wood (from the cordon and cane from the previous season's growth) removed from each vine was recorded.

Two canopy treatments were applied: an Unthinned treatment and a Thinned treatment. The Thinned treatment was shoot thinned directly after flowering and fruit set. Only a

single primary shoot was retained from each count node along the fruiting canes and the resulting shoot density was maintained through the head region. In the head region count nodes (on spurs and canes) were retained over non-count (water) shoots and water shoots were only retained where necessary to maintain canopy density or to maintain future vine structure. Throughout the growing season all lateral growth was removed from this treatment and shoot orientation was kept as upright as possible to ensure minimal shading of interior leaves.

The Unthinned treatment had an unaltered canopy left to grow naturally, except for normal management interventions such as wire lifting and shoot tucking. The yield was adjusted on this treatment to match the crop removal on the thinned treatment. This intervention was done at the same time as the canopy thinning of the Thinned treatment by removing crop off all non-count shoots. In order to ensure that laterals growing in the Unthinned treatment did not carry crop, distorting fruit to shoot ratios between the two treatments, second set was removed as soon as possible.

The canopy on both treatments was straightened and held in position with clips if shoots began to fall laterally and crowd others shoots (standard practice in commercial Pinot Noir vineyards). This intervention was also designed to mitigate canopy differences caused by the different shoot growth patterns of the Upright and Droopy clones.

It must be noted that irrigation was applied sparingly on 2 occasions, close to harvest to maintain canopy for final ripening, however, the trial was effectively dry-grown for the majority of the season.

3.2.3. Canopy Measurements

To characterise the influence of rootstock on vine vegetative vigour and canopy size and density, a number of measurements were taken throughout the growing season. In all cases information was analysed against the Upright and Droopy assessment to assess the effect of clonal variance on the experiment.

At pruning the number of count nodes and vine pruning weights were recorded. Two pruning periods were required to collect this information. One in the winter of 2003 during trial set up where pruning weights from the previous growing season were recorded and count nodes for the coming season were recorded. The shoot counts and pruning weights for the experimental season were collected in the winter of 2004.

Two measurements were taken of the canopy density once full canopy had been achieved (after the vines were trimmed during the second week of January) and substantial lateral growth had been achieved in the Unthinned treatment. The first measurement was a digital video image, using a hand held magenta background and a motorbike mounted digital video camera. The images were analysed by Kenji Irie of Lincoln Ventures, using posts to delineate bays and removing any missing vines from the images. The computer program establishes raw data as an average percentage canopy cover (canopy fill). This image was converted in to numerical data and assessments made of the whole canopy area, lower half of the canopy and upper half of the canopy. This allowed processing of canopy information relating to the fruiting zone (lower half), as well as the other canopy components.

The second canopy measurement taken 3 days later on the 26 January 2004, was by the Point Quadrat method (Smart & Robinson, 1991). Insertions were made at a height of 1100mm (top of the fruiting zone) at a distance of 180mm apart (10 per vine) for the middle 3 vines of each bay. If a vine was missing then the last vine in the bay was substituted. Leaf Layer Number and Percent Gaps were calculated for all vines.

3.2.4. Yield Measurements and Juice Processing.

In order to assess the effect of the rootstocks prior to harvest, berry samples were taken (initially once a week and then twice weekly for the two weeks prior to harvest) from each bay to monitor the accumulated sugars. The goal was to accurately assess each rootstock to ensure that cluster samples could be taken for crop and compositional measurement with as little variation in the amount of sugar as possible. Five clusters per bay were selected at random and a total of 20 berries randomly taken from them. The

juice was extracted in the field by hand-crushing the berries in a bag and the measure of soluble solids (Brix) taken using a digital refractometer (Atago PR-101, Atago Co. Ltd., Japan).

Additional samples were taken from plots to assess the effect of the Upright and Droopy clones on this experiment. Approximately 30% extra samples were taken at harvest to assess the impact of clone on yield and juice composition. These were taken across the trial site from plots that had both clones.

At sample harvest there was an attempt to mimic the real activities of a winery. A minimum brix is frequently a primary target and this was established as a focus. The attempt made was to ensure the harvested average brix was as close as possible between Rootstock treatments. Coupled with this is the reported potential relationship between brix accumulation and potassium accumulation in the berry (Li, 2003), again indicating that minimising variation in harvested average brix was desirable. There was also an issue with the quantity of samples to be harvested, the amount of fresh juice processing required and the potential for deterioration of the samples while they waited for processing. For these reasons the harvest was split in to two groups (based on the date the target brix was achieved), the eventual harvest consisted of two groups of rootstock, 4 in each group, harvested one week apart. The first harvest on the 12 April (2004) consisted of 4 rootstocks, Riparia Gloire, 101-14, 5C and 3309C. The second harvest a week later on the 20 April consisted of 420A, Schwarzmann, 99R and Fercal rootstocks. Ten clusters were collected per bay from the middle three vines from each bay (1 vine providing a buffer each side). The cluster samples were immediately transported from the field, weighed and refrigerated at 4^{o}C. A random sub-sample of 50 berries was taken from clusters from each bay, weighed to establish the 50 berry weight and average berry weight.

The per-bay cluster samples (including rachis) were transferred into strong plastic bags and the juice extracted by crushing in a ‘Bag-mixer 400’ stomacher (Interscience, St. Nom, France). The time taken to crush all of the berries varied slightly, with larger

clusters taking longer, but the crush time was typically between one and two minutes. The extracted juice was run through a sieve to remove gross solids and combined and mixed in a large beaker prior to sample bottles being filled. Two 30ml sample bottles were filled and stored at 4^{o}C for fresh juice processing. Sodium metabisulphite was added to the fridge samples to provide microbiological protection, at a rate to achieve 10mg/L free molecular sodium dioxide (SO_2). A further 15ml of juice was stored in a plastic screw capped centrifuge tube at -20^{o}C for HPLC processing. A sample of the juice was tested for soluble solids (Brix) using the digital refractometer. A further 10ml of juice was taken and processed immediately through an autotitrator (Metrohm 670 Titroprocessor, Metrohm AG, Switzerland) to determine Titratable Acidity (TA) and pH.

The final trial block harvest for winemaking (by Muddy Water staff) was completed on the 23 April, 2004. Cluster numbers and total cluster weight were recorded for all plants. This process was complicated by the requirements of the host winery that only fruit of acceptable quality was to be collected for processing. A register of acceptable and unacceptable clusters was collated for each plant. Where clusters were rejected because of excessive shrivel or disease damage they were not included when calculating average cluster weight. In some instances large clusters had not fully ripened, and these were rejected for winemaking, but were included in the average cluster weight and yield calculation.

3.2.5. Statistical Analysis of Trial Data

Due to the assessment of the two clones in this trial site the data set was unbalanced. Some plots had information gathered from both clones (approximately 30%) and some from one. As a result the data gathered from this trial was analysed using (Genstat) REML (Restricted Maximum Likelihood) analysis (for analysis of treatment means) and (Genstat) Generalised Linear Regressions (GLM) for regression analysis. Both statistical techniques can analyse unbalanced data sets.

The effect of the two clones on the results from this trial was found to be minimal. The magnitude of influence of the clone on the variables monitored in this experiment was

minor in comparison to the effect of the trial treatments. For this reason the information relating to clonal influence has not been presented in the results (Chapter 3 and 4). The influence of clone may have resulted in an extra variance within the data for some variables but the overall influence for key variables was not significant.

3.3. Canopy Results and Discussion: Point Quadrat and Video Data and the Canopy and Rootstock Treatments.

Table 3.1 Effect of Canopy Treatments on Canopy Variables, REML Analysis

All data collected at full canopy pre-veraison: Point Quadrat (PQ) data taken from the top of the fruiting zone (1100mm from ground), video data assessment done at same growth stage is an assessment of the whole canopy area in the zones indicated. (Means in columns followed by the same letter are not significantly different at P=0.05)

Treatment	Point Quadrat (PQ): Percent Gaps (% Gaps)	PQ: Leaf layer (Leaf layers through canopy)	Video: Lower Half of Canopy Fill (% Fill)	Video: Upper Half of Canopy Fill (% Fill)	Video: Total Canopy Fill (% Fill)
Unthinned	3.9 a	2.3 a	79.5 a	71.4 a	77.6 a
Thinned	17.7 b	1.3 b	68.9 b	52.2 b	62.4 b
l.s.d	5.4	0.1	8.2	4.2	5.3

Every canopy measurement taken confirms that there was a significant difference between the two canopy treatments (Table 3.1). The Point Quadrat data showed 4.5 times the percentage of canopy gaps in the Thinned compared with the Unthinned treatment and the Unthinned treatment had one full leaf layer extra in the measurement zone. The split video data showed that while there was significantly more Canopy Fill overall in the Unthinned treatment the spread of the two treatment means was higher for the upper half

of the canopy than the lower. This indicates that (as we shall see) some rootstocks were unable to fill their allotted trellis volume, indicating poor shoot extension on part or all of the fruiting canes.

Table 3.2 a: Canopy Variables by Rootstock Treatment, REML Analysis.

All data collected at full canopy pre-veraison: Point Quadrat data taken from the top of the fruiting zone, video data assessment done at same growth stage is an assessment of the whole canopy area in the zones indicated. (Means in columns followed by the same letter are not significantly different at P=0.05; ranking letters represent a = heaviest canopy to d = lightest canopy measure)

Treatment	Point Quadrat (PQ): Percent Gaps	PQ: Leaf Layer (Leaf Layers)		Video: Lower Canopy Fill (% Fill)	Video: Upper Canopy Fill (% Fill)	Percent Difference between Lower & Upper fill	Video: Total Canopy Fill (% Fill)
Riparia G.	7.9 a	1.86 ab		71.7 b	52.8 c	-26.4%	64.4 d
Schwarz.	8.8 a	1.94 a		79.5 a	64.6 ab	-18.7	74.3 a
99R	8.8 a	1.93 a		79.7 a	64.0 ab	-19.7	74.0 a
420A	10.1 a	1.69 a		70.0 b	57.8 bc	-17.4	66.0 cd
101-14	11.3 ab	1.80 abc		70.3 b	63.0 ab	-10.4	68.4 bcd
Fercal	11.7 ab	1.70 bc		78.4 a	59.9 abc	-23.6	71.3 abc
5C	12.5 ab	1.69 c		70.5 b	65.8 a	- 6.6	69.8 abcd
3309C	15.9 b	1.68 c		73.5 ab	66.7 a	- 9.3	72.1 ab
l.s.d	5.4	0.16		6.4	6.9		5.5

All canopy measurements showed significant differences between the Rootstock treatments. The data presented in Table 3.2a are an amalgam of the two Canopy treatments and therefore the spread of the Point Quadrat data in both cases is less. It is interesting to note that while the range of Leaf Layers represented is now only 0.25 (compared to a

Table 3.2 b: Interaction of Rootstock and Canopy Treatments for Upper and Total Canopy Fill Video Data.

Least Significant Differences (l.s.d) calculated at a significance level of p=0.05. (UnThin. = Unthinned Canopy treatment, Thin. = Thinned Canopy Treatment). Data collected using digital video with magenta background. The percent canopy fill represents leaf fill in the allotted trellis area for each vine.

Total Canopy Fill (% Canopy Fill)				Upper Canopy Fill (% Canopy Fill)			
Rootstock	UnThin.	Thin.	l.s.d across row	Rootstock	UnThin.	Thin.	l.s.d across row
Riparia G.	64.8	64.0	7.8	Riparia G.	54.1	51.5	9.9
420A	75.9	54.4	7.8	Fercal	66.8	53.0	9.9
Fercal	76.9	65.7	7.8	420A	67.9	47.7	9.9
99R	78.2	69.7	7.8	Schwarz.	68.3	61.0	9.9
Schwarz.	78.7	69.9	7.8	99R	69.8	58.2	9.9
3309C	82.1	62.1	7.8	101-14	80.8	45.2	9.9
5C	82.2	57.4	7.8	3309C	81.7	52.3	9.9
101-14	82.4	54.4	7.8	5C	82.5	49.0	9.9
l.s.d down column	9.1	9.1		l.s.d down column	10.3	10.3	

difference of 1 between the two Canopy treatments) there are still statistically significant differences between the rootstocks. Included in the table is a comparison of the Upper and Lower Canopy Fill video data. This is represented by the percent difference between Lower and Upper Fill. In all cases the Upper Fill is less than the Lower Fill. The percent difference is largest with Riparia Gloire (-26.4%), a reportedly low vigour rootstock (Howell, 1987), indicating poor shoot extension in to the upper reaches of the trellis. The rootstock 5C has the least difference between Lower and Upper Fill (-6.6%); this rootstock ranks second highest in terms of Upper Canopy Fill (65.8%) and second lowest in terms of PQ Leaf Layer (1.69 Leaf Layers) and third lowest in terms of Lower

Table 3.3 Rankings of Rootstocks by Canopy Variables and Canopy Assessments

This table is a manipulation of the rootstock canopy data shown above. The data has been ranked according to its position in the list from highest measurement (1) to lowest measurement (8). The Total Score Lower Canopy is the combination of the rankings for Point Quadrat (PQ) Leaf Layer and the Lower Half Video Data – both data indicating the conditions in the lower half (fruiting zone) of the canopy. The Total Score Canopy Fill similarly is a combination of ranking two canopy measurements, the Upper Half Video Data and the Total Video Data. The data have been ranked and combined in a similar fashion. Data in brackets = rank of total score.

Treatment	**PQ Leaf Layer Rank**	**Video Lower Half Rank**	**Total Score Lower Canopy**		**Video Upper Half Rank**	**Video Total Rank**	**Total Score Canopy Fill**
Riparia G.	3	5	8 (3=)		8	8	16 (8)
Schwarz.	1	2	3 (1=)		3	1	4 (1=)
99R	2	1	3 (1=)		4	2	6 (3)
420A	6	8	14 (8)		7	7	14 (7)
101-14	4	7	11 (5)		5	6	11 (6)
Fercal	5	3	8 (3=)		6	4	10 (5)
5C	7	6	13 (7)		2	5	7 (4)
3309C	8	4	12 (6)		1	3	4 (1=)

Video Canopy Fill (70.5%). This represents a rootstock that is able to fill its allotted trellis area reasonably well without exhibiting excessive vigour in the fruiting zone. Rootstock 5C in combination with this scion exhibits canopy vigour that is well matched to this vineyard site, plant spacing, row width and irrigation management. This is in contrast to Riparia Gloire that under the same growing conditions struggles to fill the allotted trellis area and could be described as devigorated in these conditions.

No interaction was found between the Canopy and Rootstock treatments for the Point Quadrat data or Lower Canopy Fill. An interaction was detected between the treatments for the Total and Upper Canopy Fill data measured using the video (Table 3.2b). For the Total Canopy Fill data, Unthinned Canopy treatment only Riparia Gloire show a significantly different mean from other rootstocks. There are more significant differences between means for the Thinned Canopy treatment (Total Fill) suggesting that the growth of the rootstocks may have been influenced by the thinning of the canopy or indicating rootstocks may have responded to the Thinned treatment differently.

The Upper Canopy Fill data (Table 3.2b) appears to be more sensitive to difference between rootstocks; this and the fact that the Lower Canopy Fill does not show an interaction may point to the Upper Canopy Fill influencing the results of the Total Canopy Fill. It is possible that all rootstock/scion combinations are able to fill the lower half of the canopy (except Riparia Gloire) to a point where this type of data loses the ability to differentiate between the Rootstock treatments. The Unthinned treatment data (Upper Canopy Fill) shows three rootstocks with significantly higher percent canopy fill (101-14, 3309C, 5C) than the other rootstocks. The Thinned treatment data (Upper Fill) shows a more confusing pattern; highest fill rootstocks are Schwarzmann and 99R, lowest fill 101-14, 5C and Riparia Gloire; two out of the three highest Canopy Fill rootstocks in the Unthinned treatment. This may be indicating different responses to the thinning of the canopy; for example it may be that larger leaves are produced as a result of the early thinning of shoots and vine nutrition (or water) differences (caused by the rootstock) influence this. It must be remembered that the Canopy Fill data only measures the extent to which the trellis area allocated to the vine is filled. It does not indicate the density (leaf layer) of that canopy. Also, the canopy was manipulated to maintain upright shoots (to minimise the potential effect of the Upright and Droopy clones on results) so shoot crowding is unlikely to be the cause of canopy fill differences. So it is possible that the rootstock is having some influence on both the Unthinned Canopy treatment (especially relating to filling the upper half of the canopy trellis) and on the vine growth patterns in response to the thinning of shoots and laterals in the Thinned Canopy treatment.

The question remains, what is the best way of assessing grapevine canopies to appropriately categorise relative differences and the potential impact on harvested fruit? Table 3.3 is an attempt to categorise the two canopy zones to allow quick assessment of rootstock performance relating to vegetative growth. This is simply an attempt to break down the data in to more general information that can be used to assess the rootstocks in this trial against observations in the literature. It is not intended to replace the statistically valid results presented relating to grape vine canopy in this experiment.

It is important to define the potential area(s) of the vine canopy that may be of interest in this experiment. Firstly, the area of canopy around the fruit (the lower half of the canopy, represented by the Point Quadrat data and the Lower Canopy Fill video data) is of potential significance due to its importance when considering crop load and fruit composition data (Smart, 1985). The second zone is the total canopy area, considering not just the leaf area or canopy density but also the extent to which the rootstock-scion combination has been able to fill its allotted trellis space, due to the influence this has on the vine response to crop load and insight it gives to the performance of a vine at a site.

For the Lower (fruiting) Canopy Zone, the measures of PQ Leaf Layer and Video Lower Canopy Fill have been chosen to give an estimate of the leaf density in the fruiting zone. The PQ Percent Gaps have been presented (in Table 3.1), but this variable was not considered for this categorisation as it is a sample of the fruiting zone as is PQ Leaf Layer. The PQ Leaf Layer has been chosen because it represents an assessment of the canopy density or thickness (layers of leaves through the canopy from one side to the other); the lower video data is an accurate representation (the whole area is measured not sampled) of canopy fill or light exposed leaf area.

The second area of interest is the total canopy area, as this defines the degree to which the rootstock/scion combination has exploited the potential of its allocated trellis space. The video data measuring Total Fill expresses a representation of total exposed leaf area and the Upper Fill gives an idea of how well the rootstock/scion combination filled the allocated trellis space. The latter is potentially representative of the relative vegetative

vigour of each genetic combination at this site. The Point Quadrat data were gathered in the fruiting zone area and so are less relevant to this measure.

Table 3.4 Rankings of Fruiting Zone (FZ) and Total Canopy (TC) Scores by Rootstock

A manipulation of Table 3.3, where the two combined scores are categorised according to 3 categories: Light, Moderate and Heavy (see legend for scoring).

Rootstock	Parentage	Lower Canopy Score	Fruit Zone Ranking (FZ)	Total Canopy Score	Total Canopy Ranking (TC)
Riparia G.	Rip	8	Mod	16	Light
Schwarz.	Rip x Rup	3	Heavy	4	Heavy
99R	Berl x Rup	3	Heavy	6	Heavy
420A	Berl x Rip	14	Light	14	Light
101-14	Rip x Rup	11	Mod	11	Mod
Fercal	Berl x Vinif	8	Mod	10	Mod
5C	Berl x Rip	13	Light	7	Mod
3309C	Rip x Rup	12	Light	4	Heavy

Legend Table 3.4:
Heavy = 2-6, Moderate (Mod) = 7-11, Light = 12-16
Berl = *Vitis berlandieri*, Rip = *Vitis riparia*, Rup = *Vitis rupestris*, Vinif = *Vitis vinifera*

For ease of assessment the rankings of the two canopy zones (Fruiting Zone, FZ and Total Canopy, TC) have been categorised according to whether they are a light, moderate or heavy density canopy. These assessments are to some degree arbitrary, but represent a generalised relative assessment of the canopies found in this experiment. This manipulation of the numerical data was done to simplify the comparisons that follow, the labelling of the numbers allowing a less confusing comparison of the rootstocks. Table

3.5 provides a summary by parentage that is revealing with respect to the adaptability of rootstock of a certain parentage to this site. It would be reasonable to assume that a rootstock that has vigorous canopy growth and therefore a heavy canopy ranking is one that is well adapted to the conditions at this trial site, in that it is able to exploit the conditions to produce relatively vigorous vegetative growth.

Table 3.5 Summary of Fruit Zone (FZ) and Total Canopy (TC) Rankings

A representation of the Table 3.4, with the rootstocks ranked according to their Total Canopy Ranking, Heavy to Light.

FZ	**TC**	**Rootstock**	**Parentage**
Heavy	Heavy	Schwarz.	Rip x Rup
Heavy	Heavy	99R	Berl x Rup
Light	Heavy	3309C	Rip x Rup
Mod	Mod	101-14	Rip x Rup
Mod	Mod	Fercal	Berl x Vinif
Light	Mod	5C	Berl x Rip
Mod	Light	Riparia G.	Rip
Light	Light	420A	Berl x Rip

Rootstocks that are well adapted to conditions at this site may not be the ones desired for planting in a commercial vineyard at this site. Canopy vigour can create management problems with excessive canopy density shading fruit, increasing disease incident (Gubler *et al.*, 1991; Zoecklein *et al.*, 1992), negatively affecting fruit composition (Clingeleffer *et al.*, 2000; Smart *et al.*, 1988) and potentially affecting yield (Mullins, 1992). To elevate these issues requires increased management intervention and vineyard operational costs to manage the canopy vigour (canopy trimming, leaf plucking and additional spraying). In addition is the potential negative impact on yield in the following season by of excessive shading in the renewal zone (the vine head area in the Vertical Shoot Position (V.S.P) cane pruned system in this experiment) (Smart 1985, Smart, 1987). The rootstock

that is able to fill the allotted trellis without excess vigour and that exhibits moderate density especially in the fruiting and renewal zones is one that is attractive from a commercial management perspective. This is a rootstock that is exhibiting vine balance at the site and will potentially result in the most cost effective and profitable seasonal management. From the summary (Table 3.5) of canopy in the FZ and the TC, 3309C, 101-14, Fercal and 5C may have desirable characteristics, reasonable total canopy fill without excess vigour in the fruiting zone. In general, crosses of *Vitis rupestris* appear to be the most vigorous and conversely, those of *Vitis berlandieri* and *Vitis riparia* the least (Riparia Gloire a 'pure' bred *Vitis riparia* falls into the latter category). It is important to stress that this information comes from heavily manipulated information and is only indicative of what the response of the rootstock/scion combinations may actually be at the this site. To look at the potential of these generalised results it is necessary to reflect them on to information found in literature.

The characteristics of pure *Vitis rupestris* grape vines are plants that thrive in stony, free draining soils, indicating an ability to send roots deep to maintain plant water (Howell, 1987). 99R is classified as a rootstock that favours deep silts or dense loam and is one that has a long growing season and generally vigorous nature (Pongraz, 1983). Schwarzmann also has a reputation for good performance in deep fertile soils. Generally, 3309C is suited to deep soils that are well supplied with moisture, but is sensitive to drought and water logging. 101-14 is extensively used in South African viticulture where it best performs in clay based soils but is not recommended for dry situations (Pongraz, 1983). In general this group of rootstocks prefers deep, heavier soils similar to the type found at this site. The main point of difference appears to be the tolerance of dry conditions; the two lower vigour Rupestris crosses (3309C and 101-14) may be more sensitive to the dry conditions found at this site. However, potentially there appears to be sufficient moisture in the deep clay loam at this site to ensure moderate to heavy vegetative growth.

The *Vitis berlandieri* species is found in hot climates and it has a very long growing season (vegetative cycle up to one month longer than *V. riparia*) (Pongraz, 1983).

Berlandieri x Riparia hybrids generally have been found to have a very shallow growing root system with a reported angle of geotropism of 60-70^{o} (Perold, 1927). This leads to a hypothesis that the dry growing (non-irrigated) conditions at this site and a possible shallow root system are the main cause of the indicated low vegetative vigour of these rootstocks. Fercal, the best performing of the Berlandieri crosses, is a hybrid with the classic European grape species *Vitis vinifera*. This rootstock has a reputation for good performance in dry conditions (Pongraz, 1983).

Riparia Gloire, a pure bred *Vitis riparia* rootstock, was also highly devigourated, which potentially may have been a result of the soil water availability at this site. The trial was largely dry-grown with only two late season irrigations. *Vitis riparia* is typically found in the wild on river and lake banks (or on islands) in deep, moist fertile soils. It would be expected to have poor adaptability to the dense, relatively dry topsoil loam and denser clay subsoil (Pongraz, 1983) found at this site.

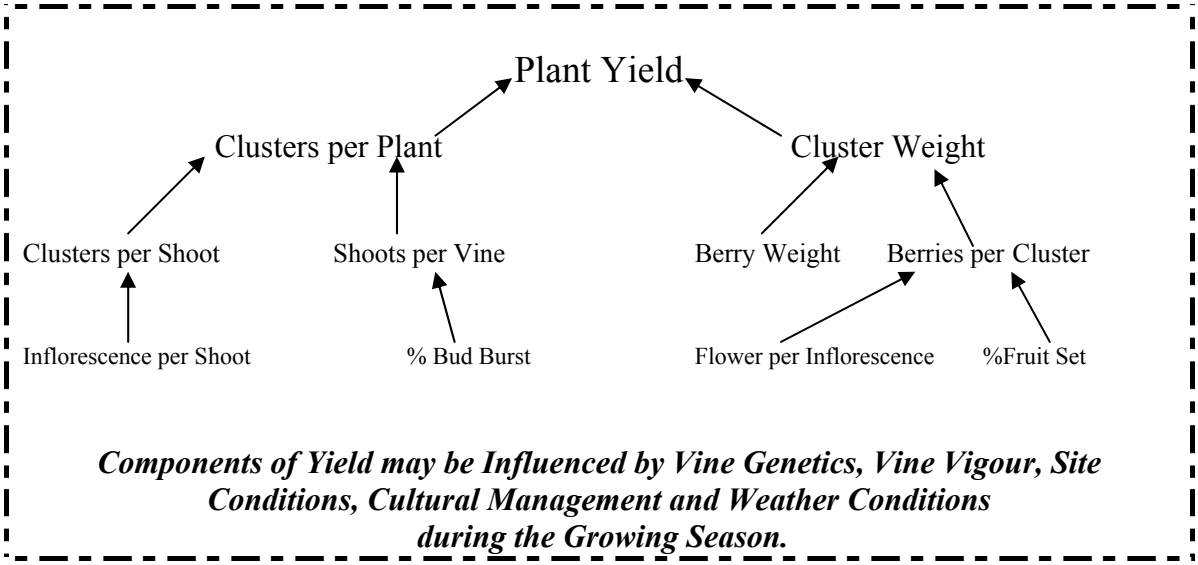
It is interesting to note that 420A, a Berlandieri cross, appears to be the lowest vigour rootstock in the trial. The reason for this may be two-fold. 1) Other Berlandieri crosses appear to express low vigour at this site, so it is a genetic influence; 2) The vines were planted one year later than the other rootstocks. The whole trial site only received regular irrigation for 3 years during establishment and 420A only for 2 years. The trial was established in an irrigation block that had already been planted and once this production block reached maturity it was not regularly irrigated. The net effect of missing a year of irrigation water during establishment is difficult to assess, this potentially could affect the extent of the root zone of the plants, therefore further retarding plant vigour when dry grown. It may be that the vine size and root zone size effect of being one year behind is enough to lower relative vigour. On-going assessment of this rootstock is essential to establish true performance at this site.

3.4. Canopy and Rootstock Treatment Effects on Yield

3.4.1. Introduction: Yield

The effect of Yield on many quality parameters of wine grapes has been documented. Excessive grapevine yields have been implicated in the reduction of harvested fruit sugars, flavour intensity and acidity. The interaction of vine Yield with a number of other variables, for example, vine size (leaf area), vine density, canopy shading and irrigation have all been shown to influence harvested fruit composition (Bravdo *et al*., 1985; Clingeleffer *et al.* 2000, Kliewer & Dokoozlian, 2000; Smart, 1987). When considering Yield it is important to quantify the components that make up Yield and how these Yield

Figure 3.2: A Summary of the Relationship Between Grapevine Yield Components, and Plant and Environmental Factors that Influence Plant Yield (Mullins,1992; Smart, 1985)



components combine to determine the final harvested crop weight (see Figure 3.2). The plant Yield, cluster number per plant, berry number per cluster and berry weight may have an affect on the composition of harvested fruit components (Brix, K, TA, levels of acids, pH) by influencing the accumulation (or degradation) of these compounds during

the growing season. Yield and its component parts must be considered in any evaluation the influence of treatments on compositional variables.

3.4.2. Results: Yield and the Canopy Treatments

The Canopy treatment did not have any effect on the component variables (50 Berry Weight, Berries per Cluster and Clusters per Plant) that determine plant Yield. There was no statistical difference (REML at p=0.05) in Yield per plant between the two Canopy treatments (data not shown).

3.4.3. Results: Yield and the Rootstock Treatments

Rootstock treatment had a significant effect on the Berries per Cluster, average Cluster Weight and average plant Yield (Table 3.6). The interactions between the two treatments were not significant in all cases. Two other variables have been included in Table 3.6, for later comparison purposes that did not show significant differences between rootstock means.

3.4.4. Discussion: Yield and the Canopy and Rootstock Treatments

The fact that there was no statistical difference between the two Canopy treatment means for Yield and the components (50 Berry Weight, Berries per Cluster and Clusters per Plant) was the targeted outcome of the techniques applied during early shoot thinning of the Thinned treatment and crop removal (from non-count shoots) in the Unthinned treatment. This allows comparison of canopy and compositional variables, against the Canopy treatments, without fear of a Yield derived influence in the results.

Analysis of the Rootstock treatments returned significant statistical differences for some of the yield variables; Berries Per Cluster (Berry/Cluster), Average Cluster Weight (Cluster Weight) and Average Plant Yield (Yield) all returned a significant relationship for the rootstock treatment (Table 3.6).

Table 3.6 Average Plant Yield and Components of Yield by Rootstock Treatment

No interactions were found between the Canopy and Rootstock treatments for any variables in this table. Means followed by the same letter are not significantly different at P=0.05. *NSR = Non-significant relationship; variables have been included to show range. The 50 Berry Weight data, used in some calculations, was not statistically linked to the Canopy or Rootstock treatments.

Rootstock	Berries per Cluster	Average Berry Weight (g)	Average Cluster Weight (g)	Number of Clusters per Plant	Average Plant Yield(g)
420A	96.3 a	1.27	122.6 b	31.77	4023 a
Riparia G.	102.1 a b	1.27	103.9 a	38.04	4151 a
101-14	92.3 a	1.37	126.3 b c	35.72	4757 a b
5C	89.7 a	1.55	138.9 c d e	39.17	5279 a b c
3309C	102.8 a b	1.45	149.5 e	41.47	5360 a b c
99R	123.9 c	1.18	146.3 d e	37.85	5538 a b c
Schwarz.	105.8 a b	1.25	132.4 b c d	43.85	5798 b c
Fercal	115.9 b c	1.32	153.3 e	40.97	6200 c
Average	103.6	1.27	134.2	38.61	5138
l.s.d	17.8	*NSR	15.07	*NSR	1406

There were statistically significant differences between Rootstock treatments for Yield (Table 3.6). The range of all rootstocks was 4023 to 6200 grams of fruit per plant. This represents a 54% increase in Yield between the lowest yielding rootstock to the highest. As the trial block is planted at a 3 metre row by 1.8 plant spacing (1850 plants per hectare) this represents a difference in Yield from 7.4 to 11.5 tonnes per hectare (t/ha).

While Yield showed significant differences between some rootstocks, 5 of the 8 means were not significantly statistically different (represented by group b). The range of this

sub-group was from 4757 to 5798 grams on average, representing a 22% variation from the lowest to the highest rootstock. This represents a crop variation of well in excess of 1.5 t/ha, increasing theoretical harvest crop from just under 9 tonnes to 10.7 t/ha, a commercially significant crop variation. The relatively large variation in Yield within this group, without statistical differentiation, indicates that there is a high degree of natural crop variation within the individual plants within the bays that make up these means. The structure of data analysis removed the variation accorded to the spatial position of the component bays at this site. The selection of the central three plants for harvest analysis possibly allowed single plants with significantly higher or lower average Yield to skew the bay average. This leads to the conclusion that Yield variability is an inherent characteristic of the grafted plant material at this site. The fact that a Yield difference was statistically apparent at either end of the range is a testament to the strength of influence some rootstocks hold over crop production at a given site in some seasons. However, these data were only from one season but it will seen (Section 3.5) that there may be a relationship between Yield success and overall plant adaptability to this site.

To illustrate the complexity of the interactions of the components that make up the final Yield it is necessary to revisit the group rootstocks with significantly different means. These means represent the lowest (420A and Riparia Gloire) to the two highest (Schwarzmann and Fercal) crop range amongst the rootstocks in this experiment.

The component variables are at the first level, 50 Berry Weight (50 Berry Weight - the sample estimate of berry size, expressed as Average Berry Weight (Berry Weight), and Berry/Cluster that make up the Cluster Weight. The Cluster Weight multiplied by the Number of Clusters per Plant (Clusters/Plant) gives the final Yield (see Figure 3.2 for pictorial representation).

The analysis was unable to detect any significant difference between the rootstock means for Berry Weight. This variable taken in context of the Berry/Cluster bay averages (which do vary significantly between rootstocks) indicates that independent of the number of berries their average size remained relatively constant. It is possible that environmental

conditions may have dictated the size of berries in this season, while rootstock selection within the environment may have had some influence on berry numbers (Table 3.6).

The two low Yield rootstocks appear to have slightly different sources for their reduced fruit load. 420A has below average Cluster Weight possibly caused by below average Berry/Cluster. Riparia Gloire has a near average Berry/Cluster but has the lowest Cluster Weight of all rootstock. In the light of non significant differences between Berry Weights the Riparia Gloire result is difficult to explain. Trial observations at harvest noted that this rootstock had begun to senesce leaves in the fruiting zone and some bunches had begun to shrivel (i.e. berries in bunches had begun to shrivel). The difference between the sample harvest date (12th April) where the berry weights were established and the final trail harvest (23rd April) where the Clusters/Plant and Cluster/Plant were largely determined (after adding back the number of clusters and their total weight taken by the sample) was 11 days. In hind sight further berry weights should have been taken at the final harvest (23rd) as significant berry shrivel (and other berry weight changes) could have occurred especially in the visually senescing Riparia Gloire rootstock.

The two high Yield rootstocks also exhibit different causal components for their high fruit load. Schwarzmann had a slightly above average number of Berry/Cluster and a slightly lower than average Cluster Weight. The high Yield of this rootstock is difficult to explain, it did record a high Cluster/Plant but this was a non-significant relationship. Fercal was higher than average for Berry/Cluster (2nd highest) and had the highest Cluster Weight of all rootstocks; exhibiting a clearer potential cause for the high Yield recorded.

It is very difficult to draw any hard conclusions from the relationships, but it appears that Berry/Cluster does vary significantly by the rootstock and this does have some influence over Cluster Weight, particularly towards the higher Yields, although this was not true in the case of Schwarzmann. Further analysis of the influence of Cluster Weight of yield is hampered by the non significant relationship between the Berry Weights of the rootstocks.

To conclude this evaluation it is worth while to summarise the findings. Firstly, in this season the Canopy treatment did not have any direct influence on harvested Yield. The Rootstock treatment did influence Yield and the significant variables were Berries/Cluster and average Cluster Weight. Further analysis of the determinants of the Cluster Weights was hampered by the non significant relationship between the rootstocks average Berry Weight. The loss of sensitivity of the Berry Weight data may have been caused by the failure to take berry weights at the final harvest of the trial on the 23rd of April.

3.5. Comparison of Yield and Canopy Data

There is always the possibility of an interaction between plant Yields and the characteristics of the eventual canopy the plant is able to produce. The carbon source and sink relationship at any site may have a bearing on the canopy. Potentially if plant Yield is low this may fail to restrain vegetative growth or where Yield is excessive it may restrict carbon available for structural growth (Coombe, 1992). Similarly, site conditions may have a limiting effect on both crop and canopy due to restrictions in available nutrients and water (Mullins, 1992).

A series of regressions to explore the relationship between the all of the canopy density variables, components of Yield and Yield found no evidence that canopy density had a relationship to Yield. There are three potential explanations for the lack of a relationship: there was no relationship between canopy growth and yield in this experiment, the variability within the data sets was too high to determine the relationship or this situation has highlighted another limitation of canopy measurements, the timeline when they are taken. Because this experiment targeted the acid level and pH of the fruit the canopy measurements were taken at a time where the canopy had matured, better indicating conditions at veraison and throughout final ripening rather than during the green berry development phase. Information was not gathered earlier and conditions around the fruit at flowering, fruit set and cell division was not recorded. Without this information it

cannot be said that differences in canopy between the treatments did not influence Yield (or components of Yield) only that the recorded evidence does not show a relationship.

Future research into the crop bearing potential of rootstocks in New Zealand should certainly include canopy measurements at the onset of flowering to assess the impact of canopy shade and canopy growth on fruit set and cell division in set berries. Categorisation of the canopy throughout the longer phase of bud initiation that extends prior to and beyond flowering for some weeks is needed to better understand the influence of this period on Yield in New Zealand. The timing and the length of this period needs to be defined in different climatic regions around New Zealand and the canopy density and canopy growth (size and vigour) assessed for potential influence. Initiation occurs in the season prior to the eventual flowering of the bunches in these buds, resulting in the potential that canopy conditions over two successive spring and early summer periods can have a great deal of influence on the final plant yield (Mullins, 1992). As a result the seasonal carry over effects from the previous spring weather conditions are certainly of interest to commercial growers, just as the flowering conditions during the current season are of interest. The ability to influence both periods by the manipulation of canopy would be the end goal of any research, and while the climatic conditions are impossible to change, an understanding of the role of canopy conditions on these processes may offer opportunity to alleviate the impact of unfavourable weather conditions.

The relationships between the components of plant Yield (Figure 3.2) indicate the complexity of the relationship between the plant, the environment and Yield. The previous paragraph emphasised the potential of canopy manipulation to alter Yield through the manipulation of the microclimate in the around developing buds and in the fruiting zone during flowering. Other elements of the plant Yield determinant are related to the interaction of the genetics of a rootstock-scion combination to vineyard environmental conditions under certain management regimes. The success of the rootstock at a site has the potential to alter the fruit bearing potential of the scion. Factors such as the percent bud burst, clusters per shoot, flowers per inflorescence, percent fruit

set and berry weight all have the potential to be influenced by plant nutrition and water status (Mullins, 1992, Reynolds, 2000). Therefore the selection of rootstock for a site has potential to influence future Yield.

In addition the categorisation of the rootstock derived influence, the canopy derived influence and the climatic conditions at a site (over two successive spring-summer periods) that influence plant Yield (Mullins, 1992) has the potential to greatly improve crop prediction. The difficulty of providing reliable predictive tools for Yield in New Zealand vineyards has been documented in local publications (Gallopin *et al.*, 2006; Nicholson, 2006; Trought, 2005). The influence of climatic conditions in New Zealand's temperate maritime climate has been implicated in significant annual Yield variations (Jackson, 2001). The complexity of this relationship is that the derivation of Yield in any given season is potentially driven by the macro-climatic conditions in an area, the meso-climate associated with an individual vineyard and the micro-climate in and around the fruiting zone (Gladstones, 1992; Mullins, 1992). Couple this with the influence of cultural management and the genetics within a vineyard the lack of success in building reliable predictive tools become understandable. Any research that attempts to establish the key elements for monitoring, manipulation and assessment of potential Yield would be of great importance to the industry.

As to the viticultural implications, the choice of rootstock for this site would be directed at the desired Yield and canopy mix. The two rootstocks with mid-range Yield (economic without increasing the risk of low fruit quality), open fruiting zones (desirable for disease management and fruit ripening) and the ability to fill their allotted canopy area (an indication of a balanced vine) in this experiment were 5C and 3309C (and possibly 101-14). It is important to bear in mind the growing conditions (clay based soils, dry grown, 1.8 by 3 meter vine spacing) when assessing the results. If closer planting and irrigation are to be used the more devigorating rootstocks may be of use. Certainly, with closer planting and/or regular irrigation 99R and Schwarzmann would be difficult to contain within their allotted canopy area.

3.6. Conclusions Yield and Canopy and the Canopy and Rootstock Treatments.

The incorporation of a Canopy treatment over a Rootstock treatment has allowed the investigation of recorded variables against either treatment. No interaction was found between the Canopy and Rootstock treatments for the Point Quadrat data or Lower Canopy Fill. An interaction was detected between the treatments for the Total and Upper Canopy Fill video data measured using the video (Table 3.2b). The Upper Canopy Fill data is a subset of the Total Canopy Fill data and it appears that it is the formers influence over the latter that defines the relationship. The canopy fill in the lower half of the trellis showed no interaction, possibly because the generally higher average percent fill (Table 3.2a) in this area (relative to the fill in the upper half of the trellis) negates the sensitivity of this method of recording canopy differences. The Canopy treatment influence (Unthinned treatment higher canopy density than Thinned) showed for both Upper and Total Fill for all rootstocks except Riparia Gloire. The Rootstock treatment showed influence on the Unthinned Canopy treatment with regard to the Upper Fill of the canopy trellis (influence was not as strong for the Total Canopy Fill). There was also a measurable response of rootstock to the removal of shoots and laterals associated with the Thinned Canopy treatment (for both the Total and Upper Canopy Fill data), although the pattern amongst the rootstocks was not clear. These interactions confirm that the Thinned Canopy treatment did lower canopy fill and that rootstock had an influence over the canopy fill independent of the Canopy treatment. This was highlighted most strongly by the rootstock influence on Upper Canopy Fill.

The Canopy treatment was also a significant influence on the other canopy variables (Point Quadrat Leaf Layer and Percent Gaps; Lower Canopy Fill, Table 3.1); this was expected as the treatments were managed to have a difference. The Rootstock treatment was also a significant influence on these canopy variables (Table 3.2a). This information and the evidence from the Upper (and to a lesser extent Total) Canopy Fill interaction indicates that, despite the Canopy treatment influence in the data, rootstock did influence canopy density and size. This is a subtle difference but important, as it indicates that despite an intrusive canopy management influence (the Thinned Canopy treatment) the

influence of rootstock on canopy growth was still statistically significant. This suggests that the rootstock can over-ride cultural management and have a significant influence on final canopy growth. The influence of rootstock on canopy growth has been documented (Ponracz, 1983) and in this experiment crosses, of *Vitis rupestris* were found to exhibit the most canopy vigour and those of *Vitis berlandieri* and *Vitis riparia* (including the pure bred Riparia Gloire) the least. This experiment was largely dry-grown (except for two irrigations late season, just prior to harvest), was on soils containing a high percentage of clay and this possibly had impact on rootstock-scion performance (Section 3.3).

The Canopy treatments had no significant influence on Yield or the component variables that make up Yield (Berries per Cluster, Average Berry Weight, Average Cluster Weight and Number of Clusters per Plant, see Section 3.4.2). The influence of the Thinned Canopy treatment may not have been significant at flowering and fruit set (it is very early in the season and canopy growth patterns between Canopy treatments are not that different) but some influence of canopy may have been expected on berry and cluster size between the two treatments (Mullins, 1992). It may be the timing of the measurement of the canopy (just before veraison) clouded the relationship between the canopy variables and the yield components. The Rootstock treatment was shown to influence two of the Yield component variables (Berries per Cluster and Average Cluster Weight) and Yield (Table 3.6). In this experiment rootstock appears to have dominated the determination of these yield related variables; reported canopy influence on Yield (Mullins, 1992) was not evident. If this experiment had studied the effect on these variables without the Canopy treatment overlaid on the Rootstock treatment the conclusion could have been that it was the rootstock effect on canopy that was influencing Yield. However, in this experiment rootstock appeared to have a role in determining both canopy growth and Yield and a direct connection between measured canopy variables and Yield was not found.

4. Chapter 4 - Canopy and Rootstock Treatment Effect on Potassium in Plant Tissue and Berry Composition: Brix, Potassium, Key Organic Acids and pH.

4.1. Introduction to Chapter 4

The composition of harvested grape berries is of great importance to winemakers and is vital for determining the eventual wine-style, perceived quality and longevity of the resulting wine. Traditionally focus has been on the percent soluble solids (measured as brix in this experiment), the composition of the acid structure (measured as Titratable Acidity (TA) and as the concentration of malic and tartaric acids) and the concentration of hydronium ions (H^+) measured as pH. Because of the potential to influence the dissociation of acids in grape juice the concentration of potassium (K^+) was also measured.

4.2. Materials and Methods for Chapter 4

The harvest and juice extraction methods, as well as statistical analyses are covered in Section 3.2.4; where required methods employed to determine juice composition in this experiment will be discussed in a material and methods section for each variable.

4.3. Results and Discussion: Canopy and Rootstock Treatment Effects on Soluble Solids (Brix).

4.3.1. Materials and Methods for Soluble Solids

The juice was extracted from the pre-harvest bunch samples and thoroughly mixed. A measure of soluble solids (which approximates sugar content at 98% of soluble solids) was obtained immediately after extraction using a digital refractometer reading in ^{o}Brix (Atago PR-101). Samples were measured at ambient room temperature and the refractometer cleaned and regularly calibrated using distilled water.

4.3.2. Results: Canopy and Rootstock Treatment Effects on Soluble Solids.

Statistical analysis (using REML) revealed that there were significant effects of both the canopy and the rootstock treatments (Table 4.1).

Table 4.1 Harvested Juice Brix by Canopy and Rootstock Treatment, Muddy Water Rootstock Trial.

There were two sample harvest dates approximately one week apart, harvest 1 and 2 are listed to indicate the harvest group each rootstock belongs to. Harvest 1 was on 12 April 2004, the harvest 2 on 20 April 2004. (Means followed by the same letter are not significantly different at P=0.05)

Harvest	Rootstock	Average Brix
2	**Fercal**	22.76 a
2	**420A**	23.13 a b
2	**99R**	23.20 a b
1	**5C**	23.66 a b c
1	**Riparia G.**	23.75 a b c
1	**3309C**	24.02 b c
1	**101-14**	24.44 c
2	**Schwarz.**	24.56 c
	l.s.d	1.14

Canopy	Average Brix
Unthinned	24.33 a
Thinned	23.04 b
l.s.d	0.89

Because of a reported potential relationship between juice potassium (K) and soluble solids accumulation (sugars measured in degrees Brix) (Mpelasoka *et al.*, 2003; Li, 2003) an attempt was made to harvest the eight rootstocks at similar Brix levels (see Section 3.2.4 for detail). This involved harvesting the rootstocks in two groups approximately one week apart, Table 4.1 shows the two harvest dates related to the Rootstock treatments. This attempt to standardise the harvest Brix across all rootstocks was unsuccessful as three of the eight rootstocks had statistically significant different levels of soluble solids at harvest (the range was 1.8 Brix). Therefore, Brix was used as a co-variate during the analysis of fruit K levels using REML (see results section 4.5.2).

Regression analysis of Brix at harvest versus Yield per Plant by two Canopy treatments revealed that Yield may influence the Brix level (highly significant F Probability (F)< 0.001, $r^2 = 0.26$), with higher plant Yield reducing harvest Brix. The regressions indicate there is still a significant relationship between rootstock (F = 0.01, r^2=0.35), and the interaction between Yield and rootstock was not significant (F = 0.065, $r^2 = 0.41$) in relationship to harvested brix. From the REML analysis the two canopies significantly affect Brix, but there is no relationship between plant Yield and the Canopy treatments. This indicates that while plant crop load may influence the harvest Brix level, there was also a potential effect of both Rootstock and Canopy treatment on the final Brix level obtained.

The analysis summary contained in Table 4.2a shows the results from a series of regressions to investigate the relationship between Brix and canopy variables. The full data set revealed no relationships between Brix, the Rootstock treatments and the canopy variables. To further check this relationship, restrictions were placed on the data set for analysis and the two Canopy Treatments were analysed separately. The Generalised Linear Regression (GLR) analysis of the restricted data sets indicated that there was still no clear relationship between Brix levels found in the harvested fruit of the Unthinned Canopy treatment and the canopy variables. For the Thinned Canopy treatment data, a relationship was found between the Brix level in the fruit by rootstock and the some of the canopy variables. The strongest correlation ($r^2 = 0.50$) was between the Upper Canopy Fill (Upper fill, Table 4.2a) which is a measure of the percent canopy fill, of the upper half of the canopy, when the canopy is viewed from the side against a magenta background.

Further investigation of the Thinned Canopy data incorporated Yield per plant (Yield) in to the GLR. Yield was analysed with the Upper Fill (% canopy fill) data from the video taken of the canopy (at full canopy prior to veraison) by rootstock to assess the relationship of the combination of the two variables to the Brix level of the harvested fruit (see Table 4.2b). The Upper Fill variable was chosen as it had exhibited the best fit (highest r^2) of all the variables in Table 4.2a by rootstock (Thinned Canopy data) to the

harvested Brix level. A possible relationship between Yield and Brix has already been established (Section 4.3.2) and the Rootstock treatment has been implicated in determining the level of all of the variables in this regression.

Table 4.2a Results of Generalised Linear Regressions of Brix vs Canopy Variables by Rootstock Treatment, Muddy Water Rootstock Trial.

Table shows significant F probabilities, NS indicates no significance, * indicates that the interaction was significant and r^2 for significant relationships.

Canopy Variable	Full Data: Rootstock Treatment	Thinned Data by Rootstock Treatment (F Prob)	Thinned Data by Rootstock (r^2)
Point Quadrat (PQ): % Gaps	NS	0.021	0.20
PQ: Leaf Layer	NS	0.017	0.35
Upper Fill	NS	0.004*	0.50
Lower Fill	NS	0.06	0.15
Total Fill	NS	0.026*	0.35

The extended GLR described in Table 4.2b attempts to pull together key variables and treatments to attempt to describe the potential determinants of harvested Brix level. The data analysed has been restricted to the Thinned Canopy treatment Brix levels and the reasons for including Upper Canopy Fill and Yield are described above. The process of adding complexity to the regression has improved the strength of the correlation to harvested Brix. It shows that Upper Fill, Yield and the rootstock all may have some influence on the harvested Brix level in the Thinned Canopy treatment.

Table 4.2b Generalised Linear Regression Analysis of Thinned Canopy Treatment Data and Full Data Set. Incorporating Brix versus Upper Fill, Yield per Plant by Rootstock Treatment, Muddy Water Rootstock Trial.

NS indicates a Non Significant relationship. The Thinned Data is represents the Brix, Yield and Upper Fill data for the Thinned Canopy treatment only. Full Data uses data for these variables from both the Thinned and Unthinned Canopy treatments.

Variable, Treatment & Interaction	**Thinned Data r^2**	**Thinned Canopy Treatment Data by Rootstock Treatment (F Prob)**	**Full Data r^2**	**Full Canopy Treatment Data by Rootstock Treatment (F Prob)**
Upper Fill (UF)	0.2	0.017	0.05	0.002
Yield (per Plant)	0.29	<0.001	0.38	<0.001
Rootstock (Rstock)	0.49	0.002	0.48	0.005
UF. Yield	0.51	0.04	0.48	NS
UF.Rstock	0.60	0.035	0.48	NS
Yield.Rstock	0.74	0.041	0.53	NS
UF.Yield.Rstock	0.74	NS	0.51	NS

4.3.3. Discussion: Soluble Solids (Brix)

The significant difference between the two canopy treatment means (Table 4.1) is of potential interest to viticulturists and winemakers in New Zealand. High brix levels for some varieties (notably Pinot Noir) result in high alcohol levels in table wines. High ethanol levels can result in this chemical dominating the mouth-feel and taste of some wines (cited by Courtney, 2004). The Thinned Canopy treatment has resulted in a significantly lower brix than in the Unthinned treatment in the harvested fruit.

It must be stressed that the Thinned Canopy treatment in this experiment would be considered an extreme cultural intervention on a commercial vineyard; however, the

effect of limiting canopy leaf fill (see Section 3.3) offers potential for Brix reduction. The manner in which the canopy leaf fill was reduced in this experiment (especially the removal of laterals) would be considered an expensive operation in a commercial vineyard. The potential for canopy reduction of any kind (shoot thinning, topping) to reduce Brix in the harvested fruit is of commercial interest. The question remains as to the effect on wine flavour and perceived quality between the two canopy treatments. Unfortunately, small batch vinifications were not made from this experiment and no assessment is available of organoleptic differences between the two canopy treatments. The reduction in alcohol between the two canopy treatments in this experiment was on average modest (0.7^{o} Brix or 0.4% alcohol) but this does indicate that the manipulation of the canopy area has potential to reduce accumulated sugars in harvested fruit (see below for a discussion on the possible reasons for this).

The influence of the Rootstock treatment (Table 4.1) survived attempts to "standardise" the Brix level at harvest. As described in the trial methods (Section 3.2) an attempt was made to minimise variation between average harvested Brix between the Rootstock treatments. Due to the complexity of the 2 x 8 treatment structure and crop load variation between rootstocks this was not achieved. However, the relationship between the two harvest groups and final Brix is not clear, indicating that, while this strategy did not achieve the desired outcome it was not a prime determinant of the Brix of the harvested fruit from the rootstocks. Group 'b' (Table 4.1) incorporates 5 of the 8 rootstocks and the 3 remaining rootstocks include two second harvest rootstocks (rootstocks harvested in the second harvest, see Section 3.2.4 for detail), Fercal and Schwarzmann that represent either end of the Brix range (high and low). This indicates that rootstock rather than harvest time is the dominant influence in determining harvested Brix in this trial. Also the other second harvest rootstocks are 420A and 99R, which lie at the low end of Brix accumulation despite having average Yields of 4023 and 5538 grams per vine (Table 3.6) respectively (a difference of 38%). What was restricted by the two harvest dates was additional sugar accumulation by the four first harvest rootstocks. All four of these are represented in group 'c' (Table 4.1); Schwarzmann is the only second harvest rootstock

in this group, and these rootstocks may have distanced themselves further from the other harvest group 2 rootstocks if they had not been harvested at a earlier date.

As indicated above (Section 4.3.2), at first glance Brix and Yield per plant do not appear to be closely related. The regressions indicate that while this is possibly true, there is a reasonably strong relationship between Brix and the interaction of Yield and Rootstock ($r^2 = 0.41$). This indicates that while Yield may have some influence on harvested Brix, rootstock (a determinant of plant Yield) exerts an additional influence on any potential relationship between the two variables.

The most obvious candidate for an influence on Brix accumulation is leaf area or canopy size. The leaf area was not directly measured in this experiment due to the complexity of doing this in the field, but a number of canopy measurements were taken. Their relationship is summarised in Table 4.2a; the statistical complexity of analysing of the whole data set incorporating both Canopy treatments results in no statistically significant relationships. The GLM does return a statistically significant result when the data set is constrained to just the Thinned canopy treatment.

The Thinned Canopy treatment has (overall) a statistically lower mean Brix than the Unthinned treatment (Table 4.1). This indicates that the thinning undertaken has limited the Brix accumulation across some or all of the rootstocks. That is, in this experiment we have influenced the rate of sugar accumulation in some or all of the rootstocks by removing leaves. The Thinned Canopy treatment has reduced the effective canopy to the point where it has affected the measured Brix at harvest for some of the Rootstock treatments, as a result the canopy variables show a relationship to sugar accumulation in the berry.

To investigate the role of canopy in influencing sugar accumulation in harvested fruit a series GLR was designed (results expressed in Table 4.2a). The full data and restricted data sets were used, incorporating only measurements from the either the Unthinned or Thinned Canopy treatment across all rootstocks. Results from the regressions for the full

data set (both canopy treatments) and the Unthinned data set (no canopy adjustments) showed no significant relationship between Brix and any of the canopy variables (simple relationship or by rootstock). The restricted data set containing the Thinned data for all rootstocks did show a relationship between the canopy variables by rootstock.

The strongest relationship between harvested Brix and the canopy treatments (restricted data set – Thinned Canopy treatment) is the interaction of the Upper Fill canopy measurement (% canopy fill) and the Rootstock treatment ($r^2 = 0.50$). This indicates that the Upper Fill percentage and Rootstock treatment may influence Brix accumulation. This interaction is to a degree logical, as the Rootstock treatment determines to some extent the Upper Fill (Table 3.2) and that the Rootstock treatment is implicated in Brix accumulation. Other canopy variables (Point Quadrat Leaf Layer and Total Canopy Fill) support this relationship as they have expressed reasonable correlations and indicate that canopy density and fill appear to be having some influence on the accumulation of harvested sugars in the thinned canopy treatment.

The Upper Fill measurement is especially interesting because it is a measure not just of the amount of canopy in the upper half of the trellis but also is an indicator of the relative ability of the vine to fill the upper half of the allocated trellis area. It may be that the amount of Upper Fill is related to the Brix level, but it is likely that the rate of accumulation is still influenced by the rootstock driven factor(s) (including, potentially, plant Yield).

Table (4.2b) combines the potential influences of harvested Brix into a GLR to try to define potential relationships more clearly. This regression combines the three identified influences of Brix level: Rootstock, Yield and Canopy. The data set was initially restricted to the Thinned canopy treatment and Upper Canopy Fill was chosen to represent the canopy measurements (due to its high correlation in Table 4.2a). To achieve an r^2 of 0.74 in a complex field experiment of this type indicates a strong potential for a relationship between these variables. It is likely that the influence of Upper Fill, Yield and Rootstock has had some bearing on the Brix accumulation in this growing season for

the Thinned Canopy treatment. Interestingly, although the Upper Fill and Yield are both influenced by rootstock there is still some undescribed element(s) of influence, related to the rootstock, on the rate of sugar accumulation. Implicit in this is that rootstock selection is pivotal in influencing the sugar accumulation in a grape crop either through its influence on Yield, vine canopy or by some undescribed factor(s).

A further GLR was then done incorporating the whole data set (both canopy treatments) in to the analysis. Exactly the same format of variables and the rootstock treatments was used (Table 4.2b). The result was an improved correlation ($r^2 = 0.53$) than the regression using plant Yield and rootstock alone ($r^2 = 0.41$). This indicates that the incorporation of the Upper Fill variable does improve the model and strengthens the arguments presented in the above paragraphs relating to the regression using the thinned data.

The influence of Upper Canopy Fill on the rate of sugar accumulation is potentially important. It defines the level of exploitation of the allotted trellis by a rootstock/scion combination as an important determinant of sugar accumulation. The Upper fill is likely to be influenced by the relative 'success' of a rootstock at a site. The influence of rootstock on Upper Fill is important as correct rootstock selection at a site may reduce the need for canopy intervention to achieve the desired canopy density, vigour or leaf area. Care should be taken in using this information from this experiment, as the vines at this site were dry grown in clay based soils; a different soil type, soil variability, altered plant densities, the use of irrigation or other management interventions could affect the performance of individual rootstocks dramatically.

Other rootstock related factor(s) that affect the rate of sugar accumulation may also be related to the relative success of the plant at a site. For example, altered (rootstock derived) nutrition and water status through the influence on plant metabolic function, may alter the ability of a scion to fix and store carbohydrates or impact the on the ability to carry crop. Any changes to the Yield or carbon fixing ability of a plant may have an impact on the rate of sugar accumulation in the fruit. Further investigation of the

rootstock influence on harvested Brix is required to help define the parameters for sugar accumulation.

4.4. Canopy and Rootstock Treatment Effects on Potassium (K) Percent Dry Weight (% DW) in Plant Tissue (Petioles and Leaf Blades)

4.4.1. Introduction K in Plant Tissue

Plant tissue samples (petioles and leaf blades) were taken during this experiment for analyses of K content. This was firstly to establish the treatment effects on plant tissue concentration of K and secondly to assess if there is any relationship between plant tissue K concentration and K found in juice in this experiment. Previous study has identified different rootstock and scion combinations as potential factors in the final concentration of K in the harvested berry (Mpelasoka *et al.*, 2003). This study of K in the plant tissue and berry may help in the interpretation of the K levels found in the harvested berry from the different treatments and the final impact this has on juice pH in this experiment.

4.4.2. Materials and Methods: K in Plant Tissue

Plant tissue (petioles (Section 4.4.3 and 4.4.4) and leaf blades (Section 4.4.5 and 4.4.6)) was collected on two occasions from this experiment: at flowering (9 December 2003) and at the onset of veraison (6 February 2004). Samples of 20 petioles and leaf blades were collected from the basal area (subtending clusters), separated immediately and stored separately, from the three central vines in each plot. The samples were dried immediately in a kiln at 65^0 C for 48 hours. The samples were ground, mixed and placed in sealed plastic vials for storage. The samples were weighed (approximately 500mg) placed in digester tubes and 10ml Aristar (69%) Nitric Acid was added. The samples were heated using pattern 3 (see Table 4.3) on a digester heating block (Plant Science Department, Lincoln University). In every heating run a blank and tomato standard (known K content) was included, these were processed as the other samples throughout this procedure as a batch check to ensure there was no variation attributable to digestion processing.

The digested sample (free of organic matter) was diluted with deionised water to 50ml. This was mixed and a sub-sample transferred to labelled 30ml plastic screw cap bottles. A further dilution of the sample by 1:100, using the same source deionised water, was completed prior to analysis to reach the required concentration for final analysis. Acidified standards: a blank, 0.2 ppm, 0.5 ppm, 1ppm and 2 ppm standards were prepared using the same deionised water, with 1 ml Aristar nitric acid added per 100ml of standard.

The all acidified standards, digested samples, blanks and digested tomato standards were analysed using atomic absorption spectrophotometry (AAS) in flame emission mode. The samples were run on an Avanta Atomic Absorption Spectrometer using oxygen boost, compressed air and acetylene flame.

Table 4.3 Digester Pattern 3 (Lincoln University Plant Science Department: West Block)

Segments 1,3,5,7 and 9 represent heating and cooling periods.

Segment	1	2	3	4	5	6	7	8	9	10
Temperature ^{0}C	40	40	80	80	125	125	140	140	20	END
Time hour	0.05	0.30	0.05	2.00	0.05	2.00	0.05	2.00	0.05	

4.4.3. Results: K (Percent Dry Weight; % DW) in Petioles and the Canopy and Rootstock Treatments

Significant statistical differences were found (using REML) between the rootstock treatments at flowering (Table 4.4) and veraison (Table 4.5a) and between the canopy treatments at veraison (Table 4.6). There was no detectable difference between the canopy treatment means at flowering. At veraison there was a detectable interaction between the canopy and rootstock treatments (Table 4.5b). While the difference between

Table 4.4 Analysis (REML) of K (% DW) in Petioles at Flowering by Rootstock Treatment, Muddy Water Rootstock Trial.

Samples dried, acid digested and assessed for potassium content using atomic absorption spectrophotometry (AAS) in flame emission mode. Potassium content presented as percent dry weight of sample.

Rootstock	**K % DW**	**Parentage**
420A	1.16 a	Berl x Rip
Fercal	1.65 a b	Berl x Vinif
3309C	1.83 b c	Rip x Rup
99R	2.08 b c d	Berl x Rup
5C	2.19 c d e	Berl x Rip
101-14	2.33 c d e	Rip x Rup
Riparia G.	2.37 d e	Rip
Schwarz.	2.62 e	Rip x Rup
l.s.d	0.53	

the two canopy treatment means was only significant with 3 out of the 8 rootstocks (99R, Riparia Gloire and Schwarzmann) 6 out 8 exhibited the trend of increasing percent K (DW) in petioles from the unthinned to the Thinned Canopy treatment at veraison (Table 4.5b). This reflects the overall trend of the Canopy treatment means (Table 4.6) which shows that the Thinned Canopy treatment had significantly higher percent K (DW) at veraison.

Further exploration of the relationship between the DW percent K at flowering against veraison showed that the majority of the rootstocks (7/8 in both Canopy treatments combined) experienced no change or an increase in K % DW from flowering to veraison (Table 4.7). The interaction between the two treatments was significant for this variable and in 4/8 cases a significant difference between the two Canopy treatment means was

Table 4.5a Analysis (REML) of K in Petioles (% DW) at Veraison by Rootstock Treatment, Muddy Water Rootstock Trial.

Samples dried, acid digested and assessed for potassium content using atomic absorption spectrophotometry (AAS) in flame emission mode. Potassium content presented as percent dry weight of sample.

Rootstock	**K % DW**	**Parentage**
420A	1.09 a	Berl x Rip
Fercal	1.58 b	Berl x Vinif
99R	2.12 c	Berl x Rup
Riparia G	2.14 c	Rip
3309C	2.17 c d	Rip x Rup
5C	2.21 c d	Berl x Rip
101-14	2.34 c d	Rip x Rup
Schwarz	2.45 d	Rip x Rup
l.s.d.	0.32	

found for the same rootstock (Table 4.8). It is also interesting to note that in all of these cases the petioles from the Unthinned Canopy treatment experience a drop in K % DW from flowering to veraison and those in the Thinned Canopy treatment experienced an increase. Generally this trend held for all rootstocks: 5/8 Unthinned treatment rootstocks experiencing a drop in K % DW from flowering to veraison and 7/8 of the Thinned Canopy rootstocks showing an increase.

To help answer some of the questions around these observed findings a series of regressions were undertaken to assess the potential effect of the canopy on K % DW in the petioles. Reasonable correlations were found between canopy video data (Lower and Total Canopy) for the flowering data. The best fit was comparing K% DW in petioles at flowering compared to the Lower Canopy Fill by rootstock, the interaction being highly

Table 4.5b Analysis (REML) of K in Petioles (% DW) at Veraison Showing Interaction Between Canopy and Rootstock Treatments, Muddy Water Rootstock Trial.

Samples dried, acid digested and assessed for potassium content using atomic absorption spectrophotometry (AAS) in flame emission mode. Potassium content presented as percent dry weight of sample.

Rootstock	**Parentage**	**Unthinned Canopy K % DW**	**Thinned Canopy K % DW**	**Significant Difference Between Canopy Treatments?**
420A	Berl x Rip	1.12 a	1.06 a	No
Fercal	Berl x Vinif	1.4 ab	1.76 b	No
99R	Berl x Rup	1.79 bc	2.46 cd	Yes
Riparia Gloire	Rip	1.89 bcd	2.4 c	Yes
Schwarzmann	Rip x Rup	2.01 cd	2.89 d	Yes
3309C	Rip x Rup	2.13 cd	2.21 bc	No
5C	Berl x Rip	2.25 cd	2.18 bc	No
101-14	Rip x Rup	2.29 d	2.39 c	No
l.s.d		0.49	0.49	0.47

significant (F>0.001) with a reasonable explained variance ($r^2 = 0.46$). The explained variance appears to be dominated by the rootstock (Lower $r^2 = 0.03$, Rootstock $r^2 = 0.34$). The Lower Fill plays a minor roll but interacts with the rootstock effect to help explain some of the possible determinant of the DW. The potential strength of this canopy variable over the Total Canopy Fill is easily explained due to the fact that most of the canopy at this stage of growth is in the lower half of the trellis.

Table 4.6 Analysis (REML) of K in Petioles (% DW) at Veraison by Canopy Treatment, Muddy Water Rootstock Trial

Samples dried, acid digested and assessed for potassium content using atomic absorption spectrophotometry (AAS) in flame emission mode. Potassium content presented as percent dry weight of sample.

Canopy Treatment	**K in Petioles (% DW)**
Unthinned	1.86
Thinned	2.17
l.s.d	0.23

Table 4.7 K in Petioles Percent Change: Flowering to Veraison by Rootstock Treatment

(Ratio represents the relative amount at veraison versus that measured at flowering: <100% represents a decrease in potassium percent of dry weight (K % DW) from flowering to veraison; >100% represents an increase; =100% is no change)

Rootstock	**K % Flowering to Veraison**	**Parentage**
Riparia G	97.2 a	Rip
420A	100.0 a	Berl x Rip
Fercal	106.5 a b	Berl x Vinif
99R	106.7 a b	Berl x Rup
Schwarz	107.6 a b	Rip x Rup
5C	111.2 a b	Berl x Rip
101-14	111.3 a b	Rip x Rup
3309C	123.1 b	Rip x Rup
l.s.d	17.8	

Table 4.8 Interaction of K (% DW) in Petioles Percent Change from Flowering to Veraison by Canopy and Rootstock Treatment, Muddy Water Rootstock Trial.

<100% represents a decrease in in potassium percent of dry weight (K % DW) from flowering to veraison, >100% represents an increase, =100% is no change. * Indicates between a significant difference between the two canopy treatments.

Rootstock Treatment	**Unthinned Canopy Treatment**	**Thinned Canopy Treatment**	**Signif. Diff.***	**Average Plant crop Load (gm)**
Riparia G	81.8 a	112.7 abc	Yes	4151
Schwarz	85.4 a	129.8 c	Yes	5798
Fercal	88.2 a	124.9 bc	Yes	6200
99R	91.3 a	122.2 bc	Yes	5538
5C	97.0 ab	125.3 bc	No	5279
420A	106.8 abc	93.2 a	No	4023
101-14	122.2 bc	100.4 ab	No	4757
3309C	130.6 c	115.6 abc	No	5360
l.s.d	26.0	29.8		

Table 4.9 Generalised Linear Regressions: K (percent dry weight) in Petioles at Veraison by Total Canopy Fill and Rootstock Treatment. (NS = Not Significant), Muddy Water Rootstock Trial

Variables/Treatment	**Full Data F, r^2**	**Thinned Canopy Treatment Only F, r^2**	**Unthinned canopy Treatment Only F, r^2**
Total Fill	NS	<0.001, 0.06	<0.001, 0.06
Rootstock	<0.001, 0.56	<0.001, 0.66	<0.001, 0.71
Interaction (Total Fill.Rootstock)	NS, 0.57	NS, 0.69	0.01, 0.80

In addition the regressions that show the Total Canopy Fill by Rootstock treatment at veraison have been included (Table 4.9). The separated Canopy treatments show good correlation between the total Canopy Fill and K in petioles at veraison.

4.4.4. Discussion: K in Petioles and the Canopy and Rootstock Treatments

In this experiment there was a trend of *Berlanderi* crosses exhibiting less K in petioles, both at flowering and veraison (Tables 4.4 and 4.5a). The reported shallow root structure (relative to other commonly used rootstock species) of this species combined with dry growing conditions at this site may have combined to lower overall K uptake and therefore result in lower plant tissue K (% DW). The exception is 5C which has a reputation for suitability for compact, calcareous clay soils (Pongraz, 1983), similar to the description of the soils at this site. The three *Berlanderi* crosses also rank in the lower half of the 8 rootstocks in terms of the percent (increase/decrease) ratio from flowering to veraison (Table 4.7); only the extremely shallow rooting Riparia Gloire (reported reputation, relative to other commonly used rootstock) scores lower. If this is a product of the root purchase in these soils as predicted there would be a struggle to maintain and build plant tissue K in the face of drying top soil and upper sub-soil zones following spring in to the warmer summer months. No information was gathered on soil moisture levels in this experiment or the structure of the roots of the individual rootstocks, so we can only speculate as to the cause of the lower K levels observed.

By considering the breakdown of the data into the two Canopy treatments by the Rootstock treatments (Table 4.6) there is a general trend to the Thinned Canopy treatment having higher average K % DW in the petioles at veraison. The statistical breakdown of this result, in Table 4.6, shows this trend in 6 out of 8 rootstocks (only 3 statistically significant). There was a significant interaction between the Canopy and Rootstock treatments (Table 4.8) for the ratio of percent petiole K (flowering to veraison). In this case, in the comparison of the two canopy means by the rootstocks, 4 out of the 8 rootstocks showed a significant increase in this ratio in the Thinned Canopy Treatment against the Unthinned. These data also show that in 5/8 cases the Unthinned Canopy showed a reduction in K (% DW) from flowering to veraison while 7/8 of the Thinned

showed an increase in K at veraison (Table 4.8). This shows that the relationship between measured K at flowering and veraison may be affected by the Canopy treatments and consequently the different level of canopy density (or leaf area) found in the two treatments.

In summary there is an observed rootstock effect on K % DW at flowering and veraison. There is also possibly an influence of the Canopy Treatment within the Rootstock Treatment. These data indicate some effect on the K % DW in petioles from the adjustments made to the canopy in the Thinned Canopy Treatment. The majority of the vines in the Unthinned Canopy Treatment had lower K % DW at veraison than at flowering (Table 4.8). The majority of the vines in the Thinned Canopy Treatment had increasing K % DW from flowering to veraison. There was also a trend to K gain in the Thinned versus K loss in the Unthinned Canopy treatment from flowering to veraison. The questions to be answered is why is there, in general, higher K % DW in the Thinned Canopy treatment versus the Unthinned, and why is there an observable trend of K gain in the Thinned and K fall in the Unthinned Canopy treatment from flowering to veraison?

The sampling of petioles is in effect a sample of plant sap K % DW at any given point in the growing cycle (Nagarajah, 1999). Therefore nutritional analysis of the petioles is an indicator of the nutritional status of the vegetative parts of the plant. In reflecting on this area of this experiment consideration needs to be given to the factors that may affect the K status of the plant. The soil is the plant store of K; K availability in the soil to some extent will determine K levels in the plant at a given point in the growing season. The other major factor in determining plant K status is the root structure and absorption mechanism(s) for the exploitation of K reserves in the soil. The size of the root ball and effectiveness of the method(s) of extraction in a variety of soil conditions will alter the rate of extraction by the scion-rootstock pairs. As stated the soil in this site is relatively even so that the volume of soil (top and sub-soil) available is a constant and soil water status at any given time can be assumed to be constant over the site. Therefore it is more likely that it is a plant driven factor such as the timing of new root growth, size of the root structure, root position in the soil profile or root effectiveness in absorbing K that is

having an effect on K % DW between the Rootstock treatments. The genetic propensity of the rootstock at a site to gather K for scion use will have some bearing on K concentration measured. But now the effect of the Canopy treatment must be considered: why does the alteration of the vegetative growth of the vine have an effect on the observed plant K concentration?

The reduction in canopy associated with the Thinned Canopy treatment was the early reduction in shoot numbers (meristems) growing from the count nodes and further removal of non-count shoots from the head region of the vines. Later in the season the persistent laterals were removed from the length of the shoot (thus removing more meristems). The effect on the canopy was a statistically significant reduction in Canopy Fill (measured by the video data), higher Percent Gaps and a lower Leaf Layer (Point Quadrat measurements). Thus the thinning of the canopy reduced the density of the canopy and the extent to which the canopy filled its allotted space. While the leaf area per plant was not measured this is a strong indication that the number and/or area of leaves was reduced by this canopy intervention. If the root volume and function is on average the same between the two Canopy treatments within each Rootstock treatment then we can make some assumptions about why the average K concentration in the plant is significantly different.

By reducing the number of leaves and the leaf area in the Thinned Canopy treatment the potential sinks for the plant available K were reduced. While not a structural element of plant tissue, K is used in many plant functions and can be a limiting factor for plant growth if in short supply (Section 2.4.2.2). In this situation we have reduced the growth new of plant tissue that can lock up K and as a result potentially reduced observed concentrations in the plant sap (petioles). If we consider our findings, there is no indication of a Canopy treatment effect at flowering. The first thinning took place three weeks before the on set of flowering probably too soon to have a significant draw down effect on K % DW.

At veraison, the effect of the Canopy treatments had made an impression. The effect may have been amplified by the changes in the soil status from flowering (spring) to veraison (height of summer) due to the soil water availability. All rootstocks potentially will experience a change in the volume of active roots, due to the drying of the upper soil area, from spring to summer at this site. Low summer rainfall is a characteristic of the East Coast of the South Island of New Zealand and soil moisture levels are usually at their peak at the start of a growing season declining as the season continues. This dry grown experiment will serve to amplify any water deficit in the upper soil layers as the season continues and plants at this site will be increasingly dependent on the soil moisture and nutrients available from the lower soil layers. Potentially this could also amplify any effect of the Rootstock treatment, those with shallower root structures faring less well than those with deeper root penetration in to the soil profile. However, there is an additional explanation related to the Canopy treatments that must be considered.

An additional effect of the thinning of grapevine canopies was observed in a series of experiments at Lincoln University (Petrie *et al*, 2000). The reduction of leaf area in these experiments had the effect of increasing the photosynthetic activity of the basal leaves resulting in a reduction in the early senescence of leaves in this area relative to those in canopies with larger leaf surface areas. The hypothesis was that the early senescence of basal leaves was a function of excessive leaf area to the fruit sink and the vine adjusted the active leaf area in response to new leaf growth. The oldest leaves (in the basal area) appeared the first targets of this type of activity. In the section on sugar accumulation (Brix) the thinned canopy appeared to limit sugar accumulation in this experiment. It can be hypothesised that by reducing effective leaf area, in the Thinned Canopy treatment, the early decline of the basal leaves was also reduced. The effect of this decline (initially in photosynthetic activity) would be to reduce amount of K stored in the leaf as it is remobilised back into the plant, reducing the recorded levels in the petioles subtending the senescing leaves. In addition, a lowering of photosynthetic activity brought on by the decline in nutrient status of these leaves may reduce the amount of solute movement in and out of these leaves. The importance of K as a co-transport cation with sucrose has

been hypothesed (Mpelasoka *et al.*, 2003) so we could expect to see a lower concentration of K (and solutes if measured) in the petiole sap.

The petioles of the Unthinned Canopy treatment reflect lower levels of K after veraison than at flowering potentially indicating that these leaves have started the process of decline toward senescence. The Thinned Canopy treatment reflects increased K mobility (in 6/8 rootstocks) from flowering to veraison possibly reflecting the increased plant demand for solutes at this time and the corresponding increase in activity of the basal leaves.

There is a third possible explanation related to the K as co-transport cation: the density of leaves in the Unthinned Canopy treatment may have resulted in partially or wholly shaded leaves being selected. The lessened photosynthetic activity in these leaves could reduce flows and/or concentrations of solutes to and from these leaves. While interior leaves were not selected it is possible that some partial shading may have been involved. However, due to the strength of the effect observed it is not likely that this has had a significant bearing on the results.

The observed findings that K (%DW) in petioles at flowering by rootstock have a correlation with the Lower Canopy Fill ($r^2 = 0.46$, Table 4.9) supports the theory that the situation at flowering is very different from that at veraison. The situation in the Thinned and Unthinned Canopy treatments is closer with regard to the demands placed on the basal leaves. The interesting results occur when comparing K (% DW) at veraison with Total Canopy Fill by rootstock (Table 4.9). For the full data set the interaction is not significant and rootstock alone accounts for the relationship ($F>0.001$, $r^2 = 0.56$). When the data are broken down in to the two component Canopy treatments and two further GLR's are run a different picture emerges. For the Thinned Canopy treatment results are similar to the whole data set but the Unthinned Canopy data reveals a greater influence of Total Canopy Fill on this relationship. The fact that there is an interaction ($F = 0.001$, $r^2 = 0.80$) indicates that there is potentially a mix of canopy and rootstock derived influences on K (% DW).

These results do not lead to a definitive answer but the Unthinned treatment at veraison exhibits the strongest relationship between Total Canopy Fill and K in petioles. It is this treatment that represents the greatest Canopy Fill measures (i.e. the highest average Total Canopy Fill percentage in this experiment) and the influence of this larger average canopy has potentially had time to have an effect on the activity of the basal leaves by veraison. This indicates that while rootstock has an influence on K at both measured phenological stages the canopy derived factors may start, over time, to influence plant K concentrations or K concentrations in the basal leaves or the activity of basal leaves reflected in the measured K in the sap of petioles. The logical extension of this experiment would be to take additional plant K measures over the ripening period. Measuring K levels in plant sap may also help in describing the relationship between K levels in plant tissue (leaves and petioles) and levels in the plant phloem. This may also help to describe how the perceived decline in basal leaves (either senescence or general activity) is affected by the additional draw on plant K in to the fruit in relationship to the two Canopy treatments.

4.4.5. Results: K (% DW) in Leaf Blades and the Canopy and Rootstock Treatments

At both flowering and veraison, K (% DW) in leaf blades was not significantly different between the two Canopy treatment means. In both these cases significant differences were found between the Rootstock treatment means. The results have been listed in the relationship between the treatment means for K (% DW) at veraison (Table 4.10) and there appears to be a relationship between the parentage of rootstocks and the level of K found at veraison. The results for flowering do not show an apparent relationship between rootstock parentage and K (%DW). The ratio of flowering to veraison K (% DW) presented as a percentage in Table 4.11 also does not show a relationship between the Canopy treatments but does express a possible relationship between canopy density levels (especially in the fruiting zone) and this ratio.

Table 4.10 Analysis (REML) of Potassium (K) in Leaf Blades Percent Dry Weight (% DW) at Flowering and Veraison by Rootstock Treatment, Muddy Water Rootstock Trial.

Rootstock	**K Flowering (% DW)**	**K Veraison (% DW)**	**Parentage**
420A	0.91 b	0.77 a	Berl x Rip
Fercal	0.84 a	0.81 a	Berl x Vinif
5C	1.07 c d	0.96 b	Berl x Rip
99R	0.96 b	0.98 b	Berl x Rup
3309C	1.10 c d	1.03 b c	Rip x Rup
Riparia G	1.12 d	1.03 b c	Rip
101-14	1.05 c	1.06 c	Rip x Rup
Schwarz	1.11 c d	1.07 c	Rip x Rup
l.s.d	0.06	0.08	

4.4.6. Discussion K (% DW) in the Leaf Blades and the Canopy and Rootstock Treatments

The percent Dry Weight of K in leaf blades at veraison may be influenced by the parentage of the rootstock. The data in Table 4.10 appears to indicate that crosses of *Vitis berlandieri* have the lowest levels of K at veraison. There is also a trend in the data that indicates that *Vitis rupestris* crosses are able to maintain higher levels of K (% DW) in leaves at veraison. In the discussion of rootstock in Chapter 3 (section 3.4.2) it was found that many crosses of *Vitis berlandieri*, especially those with *Vitis riparia* have been found to have shallow root systems. Conversely, the vines with *Vitis rupestris* as a parent appear to have greater tolerance to dry conditions, indicating a propensity to sending roots to depth and in general this group prefers deep, heavy soils. This trial was effectively dry-grown and this is likely to have affected the relative ability of these vines to extract water and nutrients from the soil throughout the growing season. It would be expected that shallow rooted vines would be restricted by water availability in the upper

areas of the soil profile especially during the hot, dry periods during full summer and this may be characterised by lower K veraison measurements.

Table 4.11 Potassium (K) Percent Dry Weight (% DW) in Leaf Blades Percent Change from Flowering to Veraison by Rootstock Treatment, Muddy Water Rootstock Trial.

Note: <100% represents a decrease in K % DW from flowering to veraison; >100% represents an increase; =100% is no change. Canopy Fruit Zone and Total Canopy Density assessments are from Table 3.5.

Rootstock Treatment	Percent Change Veraison / Flowering	Canopy Fruit Zone Density	Total Canopy Density	Average Plant Yield, Fresh Weight (gm)	Rootstock Parentage
420A	84.8 a	Light	Light	4023 a	Berl x Rip
5C	90.7 b	Light	Mod	5279 a b c	Berl x Rip
Riparia G	92.8 b c	Mod	Light	4151 a	Rip
3309C	94.2 b c	Light	Heavy	5360 a b c	Rip x Rup
Fercal	96.0 c	Mod	Mod	6200 c	Berl x Vinif
Schwarz	96.7 c	Heavy	Heavy	5798 b c	Rip x Rup
101-14	102.7 d	Mod	Mod	4757 a b	Rip x Rup
99R	102.4 d	Heavy	Heavy	5538 a b c	Berl x Rup
l.s.d.	5.2			1406	

In Table 4.11, from the percent change of K concentration in leaf blades from flowering to veraison (expressed as a ratio veraison / flowering % DW) possibly suggests that there is an influence of canopy density and possibly crop load on the ratio. For the generalised evaluation (with a non statistical base) the lightest canopies appear to have the greatest loss of K from the leaves and conversely the heavy canopies the least. Yield may have an influence as in the case of the Fercal/Schwarzmann and 101-14/99R pairings, with both significantly different from one another yet both representing mod/mod and Heavy/Heavy canopy classifications. However the largest shift (downwards) in K from

flowering to veraison is seen in the pair with the highest respective crop loads. This indicates potentially that it is an interaction between crop load draw on plant K resources and the amount of storage in leaves that determines the final shift. The total amount of K storage in leaves is probably determined genetically and may be a result of the net amount of K absorbed by the plant roots. The lower percentage retention of K in the leaf blades from flowering to veraison of *V. Berlandieri* x *V. Riparia* crosses is noticeable and Riparia Gloire (pure bred *V. Riparia*) is also exhibiting poorer K retention between these two periods. These are all rootstocks that would be suspected to have shallow roots systems at this site (Pongracz, 1983) and are exhibiting falling K levels in leaf blades. At veraison this nutrient loss from leaf blades is understandable as hot, dry summer conditions dry out the upper soil layers and the maturing and rapidly expanding fruit causes an increase in plant nutrient demand (especially for K).

Investigation of the potential of the effect of size of Yield or canopy on the % DW recorded in the basal leaf blades was inconclusive. Regressions indicated that there are strong relationships between the level of K in leaf blades and rootstocks but that both Total Canopy Fill and Average Plant Yield had a minor influence on the recorded levels. The dominance of rootstock in determining levels of K in plant material in this experiment is without question. The indications are that the rootstock adaptability to soil and growing conditions at the site has dominated the relationship between K (% DW) in leaf blades. Early season when moisture levels in the upper layers of the soil profile are sufficient for all rootstocks to extract water and nutrients the pattern is less clear (Table 4.10). By veraison when the upper soil layers are drying and the fruit is increasing its draw on plant resources, rootstocks with reported shallow root structures are struggling to maintain K levels in leaf blades.

4.5. Treatment Effects on K in Fruit at Harvest

4.5.1. Materials and Methods K in Fruit

The method of juice extraction is described in section 3.2.4; reserve juice stored at 4^{o} C was used for this measurement as soon as the gross solids had settled. A sample of juice

from each sample was prepared by dilution by 100 with deionised water. Non-acidified standards were prepared using the same deionised water. The range of the standards was a blank, 0.2 ppm, 0.5 ppm, 1ppm and 2 ppm. The samples (and standards) were processed to ascertain K concentration using atomic absorption spectrophotometry (AAS) on a Shimadzu AA-6200 AAS machine in flame emission mode.

4.5.2. Results K Concentration (ppm) and K per Berry in Fruit and the Canopy and Rootstock Treatments

Table 4.12 Analysis (REML) K Concentration (ppm) in Harvested Juice and K per Berry (mg) by Rootstock Treatment, Muddy Water Rootstock Trial.

Information contained in this table is the full data set (i.e. contains data from both canopy treatments and all rootstock treatments). Juice Potassium (K) concentration was determined using atomic absorption spectrophotometry (AAS) in flame emission mode. K per berry calculated from Juice K concentration and average berry weight for the treatment.

Rootstock	Juice K Conc. (ppm)	K per Berry (mg)	Parentage	Avg Bunch Wt (gm)
Fercal	808 a	0.90 a	Berl x Vinif	153 e
Riparia G	832 a b	1.03 a b	Rip	104 a
420A	854 a b c	1.04 a b	Berl x Rip	123 b
Schwarz	862 a b c	1.00 a	Rip x Rup	132 bcd
101-14	890 b c	1.07 a b	Rip x Rup	126 bc
99R	912 c	1.08 b	Berl x Rup	146 de
5C	924 c	1.20 b	Berl x Rip	139 cde
3309C	928 c	1.09 b	Rip x Rup	150 e
l.s.d	75	0.18		8

Two expressions of K in the fruit have been analysed: K Concentration in the juice (ppm) and K per Berry (mg), a calculated value from Berry Weight and K Concentration. The

second expression was of less value due to missing information lowering usable data numbers. For example, it was not possible to break the data down into the Thinned and Unthinned Canopy treatments for analysis.

The initial analysis using REML revealed that for Juice K Concentration (Conc.) the Canopy treatments were not significant. The Rootstock treatments were significant and this relationship was checked using Brix at harvest as a co-variate revealing only minor changes to values for the rootstocks and having no effect on their rankings. The data presented are without Brix as a co-variate (Table 4.12). Similarly, the initial analysis of K per berry revealed that the Canopy treatment was not significant and the Rootstock treatment was.

Table 4.13 Analysis (GLR) of Harvested Juice K Concentration (ppm) and K per Berry (mg) by Average Bunch Weight and Rootstock Treatment, Muddy Water Rootstock Trial.

	Juice K Conc. (ppm)		**Juice K Conc. (ppm)**		**K per Berry (mg)**	
	Full Data Set		**Unthinned Data Only**		**Full Data Set**	
	F	**r^2**	**F**	**r^2**	**F**	**r^2**
Average Bunch Weight	0.006	0.07	<0.001	0.20	<0.001	0.16
Rootstock	0.024	0.19	0.007	0.51	0.002	0.40
Average Bunch Wt.Rootstock	NS	0.13	NS	0.42	NS	0.39

Notes for table 4.13:

1/ The relationships were not significant for the Thinned data set for the Juice K Conc. and division of the K per berry data in to the two canopy treatments was not possible due to low data numbers.

2/ In all cases the trend is for increasing cluster weight to correspond to increasing juice K. The overall amount of K found in the juice on average is to some degree determined by the rootstock.

In addition to the initial analysis using REML the two juice K variables were checked against a list of other variables linked to the Rootstock treatment in order to assess their relationship. The information gathered about K levels in leaves and petioles at flowering and veraison (and the ratios comparing flowering and veraison levels for each one) were analysed using the GLR method. Similarly, Average Plant Crop Load, Average Bunch Weight, the canopy video data (Total, Lower and Upper), Brix and the Point Quadrat data (Leaf Layer and Percent Gaps) were investigated against the two juice K variables. As mentioned earlier the K per Berry data was only analysed against the whole data set but the Juice K Concentration was evaluated as the whole data set and separately as the two Canopy treatments.

No strong evidence was unearthed linking the K in plant tissue to K in the juice for either Juice K Conc. or K per Berry. Apart from a strong correlation between both variables and the Rootstock treatment, Average Bunch Weight was the only variable to exhibit a relationship to the two juice K variables (Table 4.13).

4.5.3. Discussion: K Concentration (ppm) and K per Berry in Fruit and the Rootstock and Canopy Treatments

Two issues have become apparent during the analysis of the Juice K data. Firstly, that rootstock has the strongest determining relationship on juice K of all of our treatments and measured variables. Secondly, the effect of canopy (both treatments and measured variables) appears to be minimal and the main other determinant appears to be the size of the Average Bunch Weight.

There is no clear relationship between the juice K and the measurements made of plant K at flowering or veraison. This evidence was surprising considering the literature relating

to K found in juice frequently indicates that a relationship does exist (Li, 2003; Mpelasoka *et al.*, 2003). The fact that no relationship is evident in this experiment may be due to the conditions found at this site or be due to some experimental error. It must be noted that the methods employed to measure juice K would not have harvested K found in the skins and seeds of the grapes.

The relationship between average bunch weight and juice K is the inverse of that expected. The potential of high Yield rootstock/scion combinations to have diluted K concentration in the juice is borne out by Fercal (Table 4.11) but is not reflected in other high Yield rootstocks in this trial (5C, 99R & 3309C). The general trend in the data was to see higher average cluster weights (a strong determinant of Yield) corresponding to higher levels of juice K. The one possible explanation is that plant success at this site is having an effect on both accumulated juice K and cluster size. The ability of a rootstock-scion combination to extract water and nutrients from the soil at this site is dependent upon the penetration of the root mass in to the soils, specifically the depth and number of roots. The dry grown nature of this experiment means that the plants are entirely dependent on root exploration and exploitation of the soil water and nutrient reserves. As stated before rootstocks with a shallow root structure will have difficulty maintaining supply of water and nutrients and are more dependent on in season rainfall for their supply. The ability to extract water and nutrients from the soil will in part decide the success of a plant at this site.

Maceration of the clusters during the extraction of juice may have resulted in increased solid extraction and with it increased extraction of K. The time difference between the extraction of juice from large versus small clusters was not large (perhaps a minute) (Section 3.2.4). Most of this time was taken turning and repositioning clusters (in sample bags) to ensure all berries had been ruptured. After extraction the juice of all samples were taken when juice had settled off gross solids. There was a further period of settling and juice clarification prior to sampling for juice K analysis. While it remains a possibility that this may have affected these results (higher K from larger clusters due to longer maceration) every attempt was made to minimise this possibility. In the future the

extraction of could juice be improved by crushing the same number of berries (a sample of the berries) minimising maceration time differences. Also, the immediate removal of solids by centrifuging would ensure minimal uptake of K from solids.

The possibility of extraction of additional K from stems, pulp, seeds and skin in this experiment may be a possibility but it can be argued that the chance of this occurring is minimal. The rupturing of berries was to a degree absorbing the energy of the stomacher and cushioning the solid cluster parts from crushing. The maceration was stopped as soon as the berries were all crushed. It was not the berry size that decided the Cluster Weight but the number of berries (Table 3.6, Section 3.4.4) the smaller clusters potentially having their solid parts more exposed to the impact of the extraction process.

The evidence that the rootstock does have some influence on harvested juice K is predicted in the literature (Mpelasoka *et al.*, 2003; Ruhl, 2000). The complexity of the relationship between K and the genetic source of the rootstocks gives no clear indication of genetic propensity for the accumulation of K in the juice. There is no obvious connection between crosses *of Vitis berlandier*i (reportedly shallow rooting compared to other rootstock species) having lower juice K concentration, although two out of the three lowest K rootstocks are crosses of this rootstock species. Similarly, the crosses of *Vitis Rupestris* that have a reputation for deeper soil penetration of their roots are well represented in the top four rootstocks (three out of the four) for juice K concentration.

The difficulty in assessing rootstock performance relative to the accumulation of K in juice reflects the complexity of the relationships surrounding K in the plant. There have been recorded differences in the rate of absorption of potassium ions (K^+) in to the plant from the soil, the transfer of the cation from the root to the aerial parts of the vine (propensity for xylem loading), the efficiency of use of K by the plant (measured as a function of DW accumulation) and the partitioning of K between vegetative and fruiting parts of the vine (Mpelasoka *et al.*, 2003). The situation is further complicated by the reaction of these elements to a range of cultural and environmental factors.

The rootstocks at this site have experienced a low water regime (ample water in spring to a drying soil in summer) in a relatively compact clay-loam soil. The reaction expressed in this experiment is likely to be different from other sites with different soil structures and ample water supply (through rainfall or irrigation). What is consistent with other findings is that the rootstock has played a major role in determining the K content of the harvested juice in this experiment.

4.6. Treatment Effects on Tartaric Acid

4.6.1. Material and Methods Tartaric and Malic Acid

Table 4.14 Tartaric Acid Concentration by Canopy and Rootstock Treatment (REML analysis), Muddy Water Rootstock Trial.

Tartaric concentration in the juice was established using high performance (pressure) liquid chromatography (HPLC), using a C18 column and UV measurement (the optimised RP-HPLC method used is described in Kordis-Krapez *et al*., 2001). Juice samples were collected from fruit that was physiologically ripe.

Rootstock	**Tartaric Conc. (g/L)**	**Parentage**		**Canopy**	**Tartartic Conc. (g/L)**
420A	4.0 a	Berl x Rip		**Unthinned**	4.1
99R	4.2 a b	Berl x Rup		**Thinned**	4.7
3309C	4.3 a b c	Rip x Rup		l.s.d	0.4
101-14	4.4 a b c	Rip x Rup			
5C	4.4 a b c	Berl x Rip			
Schwarz	4.5 b c	Rip x Rup			
Riparia G	4.7 c	Rip			
Fercal	4.7 c	Rip x Vinif			
l.s.d	0.4				

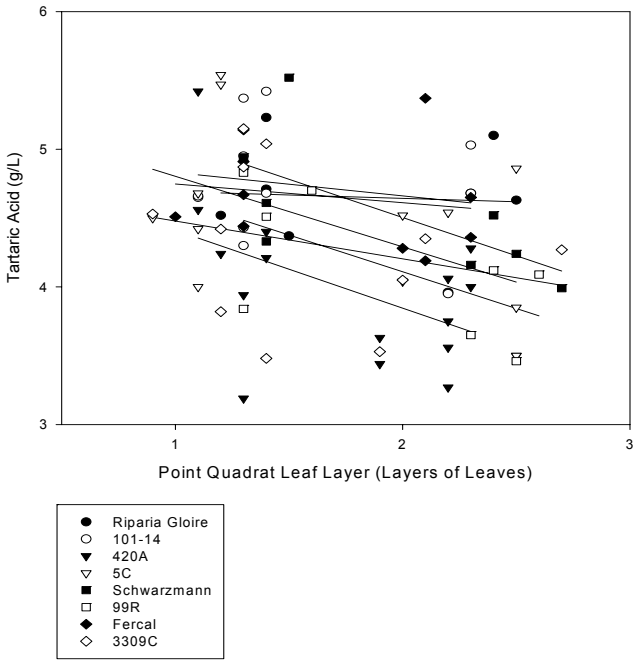
Tartaric and malic acid concentration in the juice was established using high performance (pressure) liquid chromatography (HPLC), using a C18 column and UV measurement (the optimised RP-HPLC method used is described in Kordis-Krapez *et al*., 2001). Juice samples collected just prior to harvest (see section 3.2.4) and stored at –20^{o}C were unfrozen and warmed in an oscillating 50^{o}C water bath for a total of 90 minutes. Once the samples had unfrozen they were inverted to mix any solids and returned to the water bath. Once removed from the water bath the juice was centrifuged and filtered through a 0.45µ syringe-tip filter into sterile, sealable HPLC sample tubes. The HPLC was calibrated using acid standards (Sigma Corporation) and the samples run to establish the concentration (g/L) of malic and tartaric acid.

4.6.2. Results: Tartaric Acid and the Canopy and Rootstock Treatments

Table 4.14 establishes that, for this experiment, there is a relationship between both the Canopy treatments and Rootstock treatments and the Tartaric Acid Concentration (Conc.)in the harvested fruit. The concentration of Tartaric Acid in the Thinned Canopy treatment is on average significantly higher than that in the Unthinned treatment. There is also a significant difference between some rootstock means indicating that altering rootstock has had some influence on the juice Tartaric Acid Conc. found in this experiment.

The regressions that follow are designed to describe the trends in the data (especially for the eight Rootstock treatments). The statistical information presented describes the overall relationship between the variables and the treatments. No attempt has been made to describe the relationship between the individual treatments and the variables.

Figure 4.1: Graph of the Regression of Tartaric Acid Concentration (g/L) by Point Quadrat Leaf Layer (Layers of leaves through the canopy) by Rootstock Treatment (F= 0.006, R^2 = 0.27).



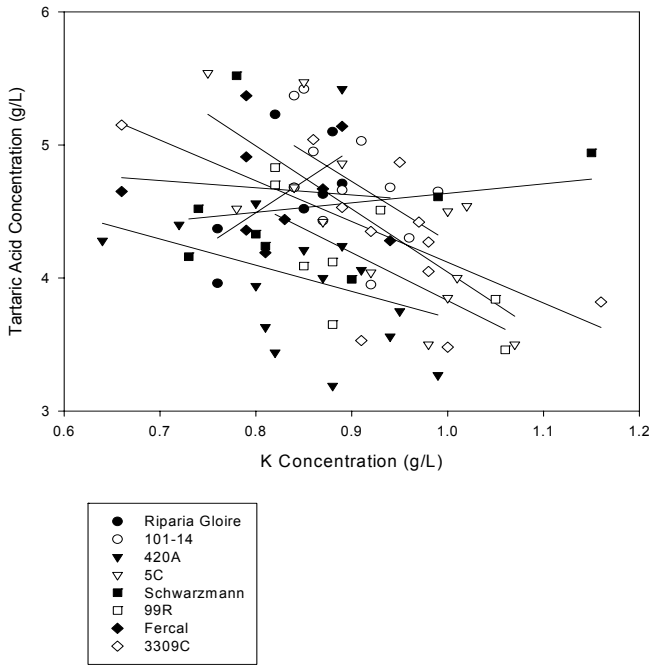
experiment was undertaken over one growing season and therefore the information presented is of general trends rather than specific descriptions of individual rootstocks. Where the statistical information is describes the whole relationship the terms will be capitalised, for example R^2, if it relates to a specific treatment it will be in lower case.

The best visual description of this relationship to the Canopy and Rootstock treatments is the graph of the regression of Tartaric Acid Conc. against the Point Quadrat Leaf Layer data by rootstock (Figure 4.1). This shows that the Rootstock treatments are separated by level and the differences created by the two Canopy treatments are enough to observe the trend of lower Tartaric Acid Conc. to increasing leaf layers. However, it is apparent that there is a fair amount of variation in the data and the R^2 for this graphed relationship is 0.27, a weak correlation. The inter-treatment variation is apparent in most regression relating to Tartaric Acid Conc. (and Tartaric Acid per Berry) making analysis of the

influence of variables on this acid difficult. This graph also has two apparent groupings of the rootstocks by slope of the line. Riparia Gloire, 101-14, Fercal and 3309C all exhibit less of a decline in Tartaric Acid Conc. by increasing Canopy Leaf Layer (as measured by Point Quadrat Leaf Layer, i.e. increased canopy leaf density) than do the other rootstocks. No clear relationship helps to explain this apparent different reaction to increasing canopy density. Once again some other undocumented rootstock driven factor may be altering the response of individual rootstocks to increasing leaf layers or it may be an artefact of the data.

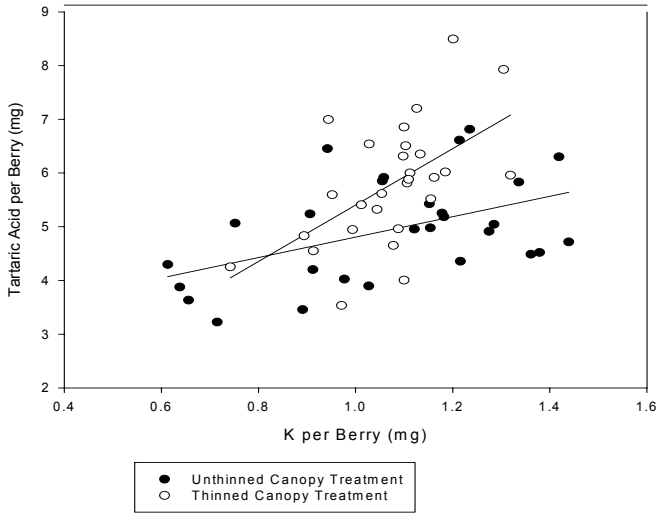
The relationship between potassium ions (K^+) and Tartaric Acid was investigated to check that the integrity of the data set had not been compromised during the sample analysis process. The ability of Tartaric Acid to readily form a salt with K^+ in solution was cause for some concern during the handling of the fruit samples. The initial regression analysis comparing the concentrations of these two variables by the individual Canopy treatments revealed that higher concentrations of K corresponded to lower Tartaric Acid Conc. For the GLR of Tartaric Acid Conc. against K Concentration by Rootstock treatment (Figure 4.2, $R^2 = 0.33$), 5 rootstocks exhibited decreasing Tartaric Acid Conc. with increasing K Conc., 2 had a flat response and Riparia Gloire appeared to increase Tartaric Acid with an increase in K Conc.. The Riparia Gloire case is possibly a result of the short season for this rootstock and the observed berry shrivel (see section 3.4.4 for an explanation) that was apparent at harvest for most of the vines for this rootstock. The Canopy treatment regression confirmed that higher K Conc. corresponded with lower Tartaric Acid Conc. but showed that for any given level of K the Thinned Canopy treatment typically had a higher concentration of Tartaric Acid ($F<0.001$, $R^2 = 0.28$, (interaction not significant), however this is better represented by Figure 4.3 showing the interaction between Tartaric acid per berry and K per berry, see below for further explanation). This situation is mirrored in the main effects analysis using REML, where the Thinned Canopy mean had a significantly higher Tartaric Acid Conc. (Table 4.14).

Figure 4.2: Graph of the Regression of Tartaric Acid concentration (g/L) versus Juice Potassium Concentration (ppm) by Rootstock Treatment. (F= 0.04, R^2 of interaction = 0.33).



This situation was further investigated by the analysis of two other variables, Tartaric Acid per Berry and K per Berry, against the Canopy treatment (Rootstock did not show a significant relationship for these variables). The derivation of these two variables is simply the concentration (of tartaric acid or K) multiplied by the Average Berry Weight (calculated from the 50 Berry Weight) for the plot. The analysis incorporating the Canopy treatments showed increasing Tartaric Acid per Berry to increasing K for both treatments but a steeper increase for the Thinned Canopy (Figure 4.3, $R^2 = 0.35$). This situation was confirmed by a further REML that showed Tartaric Acid per Berry to be significantly higher (5.76 mg versus 4.94 mg, l.s.d = 0.52) in the Unthinned Canopy treatment.

Figure 4.3 Graph of the Regression of Tartaric Acid per Berry versus Potassium per Berry by Canopy Treatment (F=0.04, R^2 of relationship = 0.35).



4.6.3. Discussion: Tartaric Acid and the Canopy and Rootstock Treatments

At the average level of canopy density found in the two Canopy treatments, the lower average canopy density in the Thinned Canopy treatment positively influenced the levels of Tartaric Acid in the juice (Table 4.14). If we assume that the level of degradation of Tartaric Acid within the berry is low, especially post veraison (Kliewer, 1964; Rojas-Lara and Morrison, 1989) and the accumulation of Tartaric Acid is limited to the green berry phase (Ruffner 1982a) then the observed effect must be a result of increased formation of the acid pre-veraison (see Section 2.6.2).

The cause of the canopy influence can only be hypothesised; possibly some enzymatic process has been enhanced in the berry encouraging the formation of Tartaric Acid over the storage of carbohydrates in some other form. It has been hypothesised that Tartaric Acid is a secondary metabolite of sugars (glucose) introduced to the grape berry and is a relatively stable end product (Rojas-Lara and Morrision, 1989; Ruffner, 1982a). It has

been reported that leaf shading and light levels are important determinants of Tartaric Acid production, the pathway is "sluggish at best" and requiring light to be active (Rojas-Lara and Morrision, 1989; Stafford and Lowes, 1958). It may also be a berry temperature related response although this has been found to affect both sugar production and the rate of berry growth (Ruffner *et al.*, 1976). Ruffner (1982a) argues that tartrate formation is not necessarily light dependent, only in situations where precursors are unavailable. He goes on to argue that the observed correlation between plant growth, ascorbic acid and Tartaric Acid (and their derivation from hexose) supports a hypothesis of a precursor-product relationship between the two acids. The derivation pathway of Tartaric Acid is still not clear; however, it appears that there is some relationship to light exposure and berry (cell) growth, and the synthesis of this acid (Ruffner 1982a).

The Rootstock treatment has also been shown to influence the level of Tartaric Acid (Table 4.14). The variability of the Tartaric Acid data within the Rootstock treatments made breaking down of the data using regressions difficult, lower than desirable R^2 values result. The variation within the data could be a result of the observed reticence of the process(s) that create this acid or an interaction of varying influences on this process within the plant. Obvious potential rootstock driven influences on Tartaric Acid are the canopy variation between rootstocks and differences found between Yield derivative variables (and Yield) between rootstocks. The determination of strong relationships between Tartaric Acid and other variables was not possible in this experiment, but it must be stressed that the interaction of elements characterising the growth and fruiting of vines may be the source of the rootstock influence. The variability in the data may be masking the influence of Yield or canopy on Tartaric Acid Conc. within the Rootstock treatments so these variables cannot be discounted as some of the source of the rootstock influence on Tartaric Acid. What can be said is that from our analysis of the treatments using REML is that a canopy effect was observed and there is a rootstock influence on the average level of Tartaric Acid Concentration.

So far this review of the results assumes that there has been no influence of experiment method on the level of Tartaric Acid. When considering measurements of Tartaric Acid it

is important to consider the results in the context of the relationship between Tartaric Acid and K. These two elements can form an unstable salt (Section 2.6.2) and potential precipitation of this salt, due to experiment method, could have an impact on measured levels of this acid. The following paragraphs will explore this relationship and attempt to remove this possibility from the analysis.

A possible influence of experiment method on the recorded results is unlikely for a number of reasons. Firstly, the range of the recorded Tartaric Acid concentration is from 3 to 5.5 g/L (Figure 4.1) an increase of 83%, a very significant increase in the acid. The displacement of over a third of the hydronium ions by K is unlikely, as these have to be absorbed into a molecule in an inactive state for this to have an effect on the measurements. In addition the method of measuring Tartaric Acid (see section 4.6.1), measures the presence of acid anions whether or not they are in the salt form.

The range of K reported here overlapped significantly for both canopy treatments despite significantly lower average tartaric acid in the Unthinned treatment (Table 4.14). When the relationship between Tartaric Acid, K and the Canopy treatments is considered there appears to be a positive relationship between K per Berry and Tartaric Acid per Berry (Figure 4.3). Results presented above (Section 4.5.2) indicated that the average level of K between the two Canopy treatments was similar. There is significant data overlap between levels of K for the two Canopy treatments (and corresponding levels of Tartaric Acid). There is an observable statistical difference between the two treatments for the level of tartaric acid concentration. All of these factors point to the likelihood that levels of K are not affecting Tartaric Acid levels in the Unthinned treatment.

When the amounts of Tartaric Acid and K are calculated as milligrams (mg) per berry the results show that increasing levels of K correspond (in most cases) to increasing levels of Tartaric Acid (Figures 4.3). This is despite the observation that there may be a negative relationship between Tartaric Acid Conc. and K Conc. in the berries of individual rootstocks (Figure 4.2). The positive relationship between the levels of K and Tartaric Acid per Berry (Figure 4.3) indicates that absolute amounts of K are may be having a

positive effect on the amount of Tartaric Acid found in the berry. It is important to note that these measurements are driven by the berry size and we are seeing the effect of berry size on these two variables. The potential for dilution of Tartaric Acid post veraison is known (Ruffner *et al.*, 1983) so the concentration in a large berry versus a small one may be expected to be less. There is also a reported relationship between Tartaric Acid and berry growth (Ruffner, 1982); this possibly indicates that larger berries may exhibit a greater proportion of Tartaric Acid per Berry because they are larger. This assumes that berry size post veraison is in proportion to berry size during the green berry growth phase. It is difficult to ascertain the true relationship due to the variability in the data in this experiment. These issues raise important points regarding the measurement of Tartaric Acid in future experimentation. Berry size measurements are critical at all stages when assessing the level of Tartaric Acid. Also, it may be relevant to assess this acid in the lag phase when berry acid concentrations are at their zenith (Ruffner, 1982) avoiding the distortion of post veraison expansion.

In this experiment the vines were non-irrigated throughout the majority of the season and this may have some bearing on levels of plant nutrients during key periods in berry development. In most cases increased amounts of juice K (mg) correspond to increased amounts of Tartaric Acid (mg) and this may indicate that the level of K (or some other nutrient accumulated in a similar manner to K) was in some way associated with accumulation of Tartaric Acid in the green berry. The work done in this experiment cannot answer these questions; future work in this area needs to concentrate on the key elements of Tartaric Acid accumulation in the green berry phase. Two potential areas of interest are the density of the canopy and level(s) of key plant nutrients that may limit the synthesis of Tartaric Acid in the berry.

In summary the key points to make about the observed level of Tartaric Acid found in this experiment are: K was unlikely to have had an influence on the measured levels of Tartaric Acid in this experiment, the concentration of tartaric acid was influenced positively by lower canopy density and rootstock had a role in determining the level of Tartaric Acid.

4.7. Treatments effects on Juice Titratable Acidity and Malic acid, and the Relationship between Titratable Acidity and Malic and Tartaric acids

4.7.1. Material and Methods Juice Titratable Acidity, Malic and Tartaric Acids

The method to determine the concentration (g/L) of Malic and Tartaric Acid is described in section 4.6.1. As noted in section 3.2.4 as soon as the juice was extracted from the samples juice TA was established using titration with a base (NaOH).

4.7.2. Results Juice TA, Malic and Tartaric Acids and the Canopy and Rootstock Treatments

The difference between malic acid and titratable acidity (TA) was significantly different for the two canopy treatments (Table 4.15). The Unthinned Canopy treatment showed higher levels of both variables. Similar comparison of the two variables with respect two the Rootstock treatments established that there were significant differences between a number of the treatment means (Table 4.16).

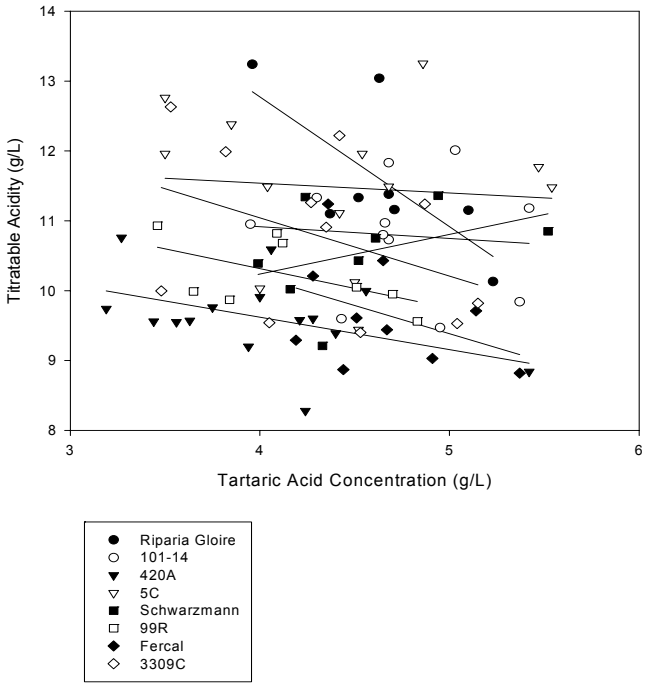
Table 4.15 Juice Malic Acid (by HPLC) and Titratable Acidity by Canopy Treatment (REML Analysis).

	Unthinned Canopy	**Thinned Canopy**	l.s.d
Malic Acid (g/L)	4.7	4.1	0.4
TA (g/L)	11.0	10.2	0.5

A series of regressions (GLR) to investigate the relationship between TA, Tartaric Acid and Malic acid revealed that in this experiment the strongest relationship is between Malic Acid Conc. and TA. There was no observable correlation between Tartaric Acid Conc. and TA. Malic Acid Conc. exhibited a direct correlation with TA ($F<0.001$, $R^2 = 0.58$) exhibiting increasing Malic Acid levels corresponding to increasing levels of measured TA. Including the Canopy treatment in the regression did not improve the

observed correlation but the inclusion of the Rootstock treatment with Malic Acid strengthened the regression relationship (F=0.009, R^2 = 0.64). For Tartaric Acid the relationship improved with the inclusion of the Rootstock treatment (R^2 = 0.40, Figure 4.4) but the impact of Tartaric Acid on TA was flat or negative for all rootstocks. The interaction between the two factors (Malic Acid and the Rootstock treatments) produced a similar result (R^2 = 0.64, see Figure 4.5), with all Rootstock treatments producing a positive increase in TA to increasing Malic Acid Concentration.

Figure 4.4 Graph of the Regression Juice Titratable Acidity versus Tartaric Acid Concentration (by HPLC) by Rootstock Treatment (F<0.001, R^2 of this relationship is 0.40).

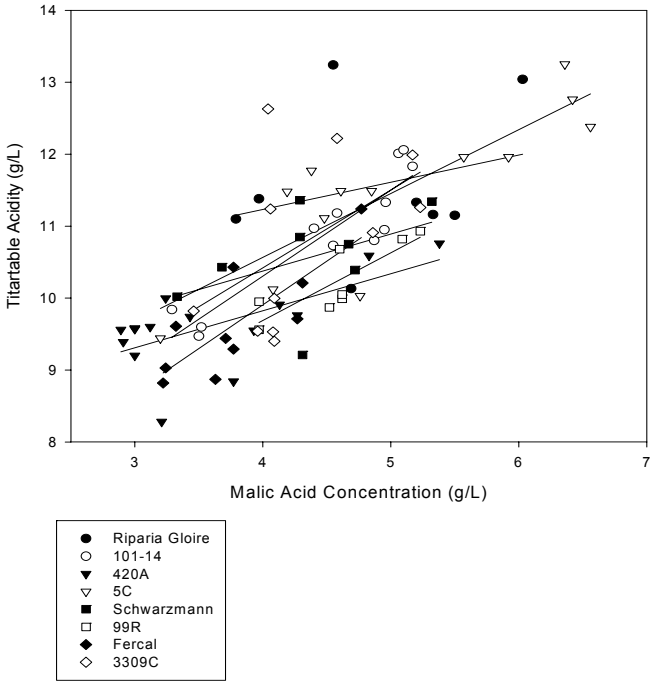


The trend of the incorporation of rootstock treatments into regressions improving correlations continued when a series of regressions were run to explore potential determinants of the level of Malic Acid Conc. in the harvested juice. Regressions were run comparing the level of Malic Acid Conc. to Yield and canopy variables. Either the Canopy treatments or Rootstock treatments were included as another factor. In all cases

the incorporation of the Rootstock treatments strengthened the correlation between the two variables.

Of the crop variables the Malic Acid Conc. versus the interaction of Average Cluster Weight and the Rootstock treatments offered the best correlation, though fairly weak (r^2 = 0.37, data not shown) and six out of the eight rootstocks exhibited a trend of increased Malic Acid Conc. with increasing Cluster Weight.

Figure 4.5 Graph of the Regression of Juice Titratable Acidity verses Malic Acid (by HPLC) by Rootstock Treatment (F=0.009, R^2 of relationship = 0.64)



The canopy variables reinforced the REML analysis of the Canopy treatments showing that increasing canopy density generally increased Malic Acid Concentration. The Point Quadrat data analysed with Rootstock treatments against the Malic Acid Conc. showed a highly significant relationship for Leaf Layer Number (F = 0.001, R^2 = 0.45) and Percent Gaps, but the latter with a lower R^2 (0.36). The strongest relationship for the video data

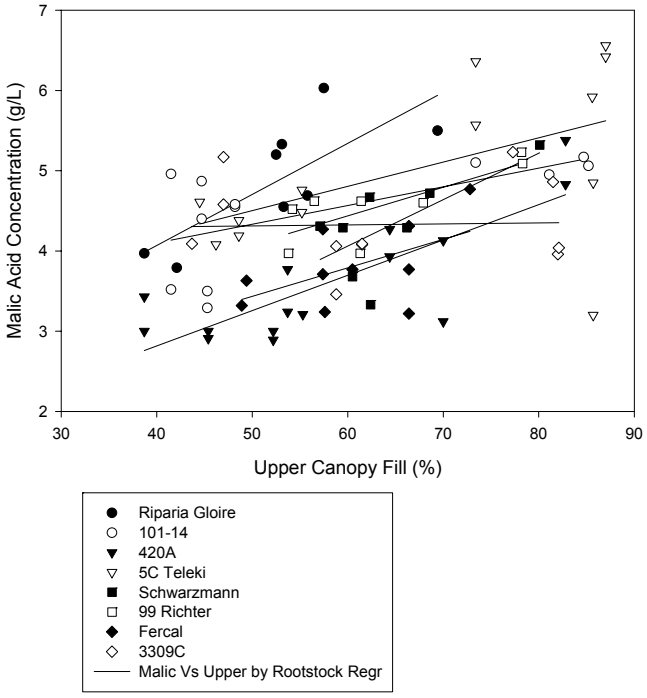
was the Upper Canopy Fill (%) with a R^2 of 0.50 (Figure 4.6), followed by Total Canopy Fill (0.47, data not shown) and Lower Canopy Fill (0.39, data not shown).

Table 4.16 Juice Malic Acid and Titratable Acidity by Rootstock Treatment (REML Analysis).

Rootstock	Titratable Acidity (g/L)	Tartaric Conc. (g/L)	Malic Conc. (g/L)	Parentage	Canopy Fruit Zone	Canopy Total
420A	9.6 a	4.0 a	3.6 a	Berl x Rip	Light	Light
Fercal	9.7 a	4.7 c	3.8 a	Berl x Vinif	Mod	Mod
99R	10.2 a b	4.2 a b	4.6 b c	Berl x Rupest	Heavy	Heavy
Schwarz	10.5 b c	4.5 b c	4.3 b	Rip x Rup	Heavy	Heavy
3309C	10.9 b c d	4.3 a b c	4.4 b	Rip x Rup	Light	Heavy
101-14	11.0 c d e	4.4 a b c	4.6 b c	Rip x Rup	Mod	Mod
5C	11.4 d e	4.4 a b c	5.0 c	Berl x Rip	Light	Mod
Riparia G	11.6 e	4.7 c	4.9 c	Rip	Mod	Light
l.s.d.	0.7	0.4	0.4			

A series of regressions of the Yield and canopy variables with respect to TA (broken down into Rootstock treatments) revealed that plant Yield (and the components of Yield) did not show a strong relationship to TA. There was a positive influence of increasing canopy density on TA, with seven out of eight rootstocks in the regression of TA versus Pinot Quadrat Leaf Layer by Rootstock treatment (Figure 4.7). The strongest canopy variable relationship to TA was the Upper Canopy Fill by Rootstock treatment (Figure 4.6, R^2=0.50).

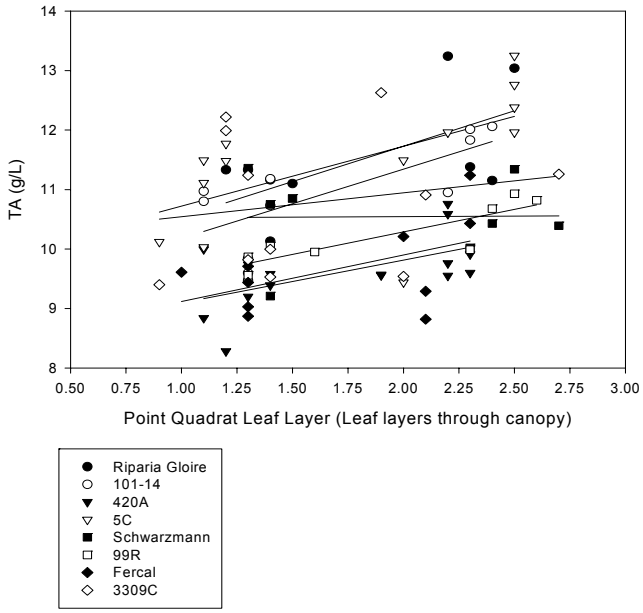
Figure 4.6 Graph of Regression of Malic Acid Concentration (g/L) versus Upper Canopy Fill (%) by Rootstock Treatment. ($F<0.001$, R^2 of relationship = 0.50)



4.7.3. Discussion: Titratable Acidity, Malic and Tartaric Acids and the Canopy and Rootstock Treatments

The relationship between TA and Malic acid and the two Canopy treatments show significant differences between the means for both variables (Table 4.15). The levels of Malic Acid (g/L) and TA are higher in the Unthinned Canopy treatment. The Rootstock treatment means for both variables also show significant differences between means for some rootstocks (Table 4.16). The observation that, in general, as Malic Acid levels rose between Rootstock treatments so did the level of TA led to an investigation of the levels of the two acids (Tartaric and Malic) and their relationship to TA. The most interesting aspect of this investigation was the lack of a strong correlation ($R^2 = 0.40$) and indication

Figure 4.7 Graph of Regression of TA (g/L) versus Pinot Quadrat Leaf Layer by Rootstock Treatment (F<0.001, R^2 of relationship 0.45).



of a relationship (Figure 4.4) between the level of Tartaric Acid (g/L) and TA. This is something that may not have been expected from such an important grape acid. However, Malic Acid Conc. showed a strong positive relationship to TA (R^2 =0.58) and the correlation for this relationship was improved when the data were broken down into individual Rootstock treatments (R^2 = 0.64, Figure 4.5).

Tartaric Acid exhibited less influence on TA across the rootstocks (Figure 4.4) with flat or slightly declining TA to increasing Tartaric Acid Conc. in six rootstocks, sharply negative for one (Riparia Gloire) and a slightly positive relationship for one rootstock (Schwarzmann). The fact that increasing Tartaric Acid did not result in an increase in TA for seven of the eight rootstocks is and indication of a weak relationship between TA and Tartaric Acid. Conversely the correlation for Malic Acid Conc. by rootstock against TA (Figure 4.5) is strong and there is a strong positive relationship between Malic Acid and TA for all of the rootstocks.

By definition the level of Tartaric Acid must have some influence on the level of recorded TA as it provides a component of the total concentration of hydronium ions (H^+) in the solution (Margalit, 1977). It appears however that the Malic Acid Conc. dominates any changes to TA, in this experiment. It is important to note that rootstock has a strong influence on this relationship. An attempt was made to describe the relationship between TA, Malic acid and rootstock by looking at Yield and canopy variables, variables that rootstock has shown some influence over (see sections 3.4.2 and 3.5.4). The regressions showed that canopy density does have some potential influence on TA for the individual rootstocks but Yield did not appear to have a strong influence. The one Yield component that had a positive relationship (with a relatively weak correlation $R^2 = 0.37$) to TA was again Cluster Weight. This can potentially be explained as an effect of berry shading on the degradation of Malic Acid. The increased size of clusters increasing potential shading of other clusters in the canopy and shading of berries within clusters (especially interior berries in the cluster). Malic Acid has been shown to be sensitive to levels of canopy shading (Archer and Strauss, 1989; Lakso & Kliewer, 1975; Rojas-Lara and Morrison, 1989), with increased average berry temperature from increased berry exposure resulting in elevated breakdown of Malic Acid by respiration (Ruffner, 1982b).

The average range of Tartaric Acid Conc. found between rootstocks is from 4.0 to 4.7 g/L (Table 4.14), a difference of 0.7 g/L. The range of Malic Acid Conc. was 3.6 to 5.0 g/L (Table 4.16) giving a difference of 1.4 g/L across the rootstock treatments. The range of Malic Acid is double that of the Tartaric and it is Malic Acid that dominates the relationship between the acids and TA. TA is a measurement of the total amount of hydrogen ions in a solution made available by titrating with a standard solution of sodium hydroxide (Iland, 1987). Both Malic and Tartaric Acids have two H^+ ions that can be cleaved off by this titration (Margalit, 1997). In this experiment, across the range of Malic Acid concentrations (from low to high) there is a increase in the H^+ ions present in solution that is double the increase found across the range of Tartaric Acid. The magnitude of the increase in Malic Acid Conc., relative to Tartaric Acid Conc., results in a greater potential for Malic Acid to affect measured TA. This shows up statistically with Malic Acid having a stronger correlation with TA than Tartaric Acid. As we shall see

(Section 4.8 relating to pH) the higher dissociation constant of Tartaric Acid ensures that it is this acid that dominates the relationship to pH. The measurement of TA does not account for the relative strength of the acids but gives an indication of the combined amounts of the two acids that are present.

The influence of canopy was explored with respect to the concentration of Malic Acid in the fruit. This indicated that the relationship of increased canopy (increased Canopy Fill, Point Quadrat Leaf Layer and fewer PQ Percent Gaps) corresponded to increased Malic Acid concentration in the fruit for the majority of the rootstocks (representative relationship Upper Canopy Fill, Figure 4.8). The effect of increased canopy density measurements in this experiment translated into increased TA, especially when the data are broken down by rootstock. This is most likely a result of the different levels of Tartaric Acid and Malic Acid in the fruit of the individual Rootstock treatments (Table 4.16). The relationship between Tartaric Acid (Table 4.14) and Malic Acid (Table 4.15) is that in the Thinned Canopy treatment Malic Acid is on average lower and Tartaric Acid higher than in the Unthinned Canopy treatment. The inverse relationship of Tartaric Acid and Malic Acid between the Canopy treatments may be an indicator of a similar relationship between the Rootstock treatments; different canopy density affecting the proportions of the two acids within the Rootstock treatments.

The situation is made more complex, as if the (combined) levels of the two acids are compared to TA (Table 4.16) there is an increase in the level of acid in the juice and the corresponding level of TA. There is an effect of the combined amounts of the two acids as well as the relative proportion between the two acids on TA. This is a complicating factor for analysis as is the reported influence of metal cations (particularly K) on the total number of H^+ ions available for titration (see section 2.2.3 for more information). Rootstocks are known to have an influence of the levels of these metal ions in the juice of grapes (see Sections 2.4.2.2 and 2.4.2.3) and there is an observed influence of Rootstock treatment on the level of juice K in this experiment (Table 4.12).

The observations that Tartaric Acid had a flat or negative relationship to TA (for all rootstocks) and that there is a reported increase in TA for increasing canopy density measurements is consistent with reported observations (Ruffner, 1982 a and b) and with other findings in this experiment. Increasing canopy density is likely to negatively influence levels of Tartaric Acid in fruit (Ruffner, 1982a) and positively influence the formation and retention of Malic Acid (Ruffner, 1982b). The potential influence of mineral cations on Tartaric Acid is likely to further lower the influence this acid has on TA and Malic Acid would not be expected to form salts with these ions (Ruffner, 1982b).

In summary, the level of Malic Acid has been found to be a strong influence on the measured TA; Tartaric Acid appears to have little direct bearing on the levels of TA found but by definition (as it is an acid it represents a proportion of the measured TA) is involved in the determination of TA. From the results from this experiment there is a reported (Sections 4.6.3 and 4.7.3) canopy influence on levels of Malic Acid, Tartaric Acid and TA. The conclusion drawn is that Malic Acid Conc. shows a strong positive response to increasing canopy density levels and it is this relationship that is dominating the relationship between the two acids and TA. The Rootstock treatment also has an influence on the levels of TA, Tartaric Acid and Malic Acid in this experiment. It is not clear (and unlikely) that the rootstock influence is simply a result of altered average canopy density between the rootstocks but it is likely that this is one of the rootstock influenced factors that is affecting the level of juice TA in this experiment. The rootstock influence appears to affect not just the proportion of the two acids found but the sum of the Tartaric and Malic Acid levels found and both effects appear to have some influence on TA (for further information refer to section 4.8.2 and 4.8.4).

4.8. Canopy and Rootstock Treatment Effects on pH, and the Relationship between Malic Acid, Tartaric Acid, Potassium and pH.

4.8.1. Materials and Methods for pH

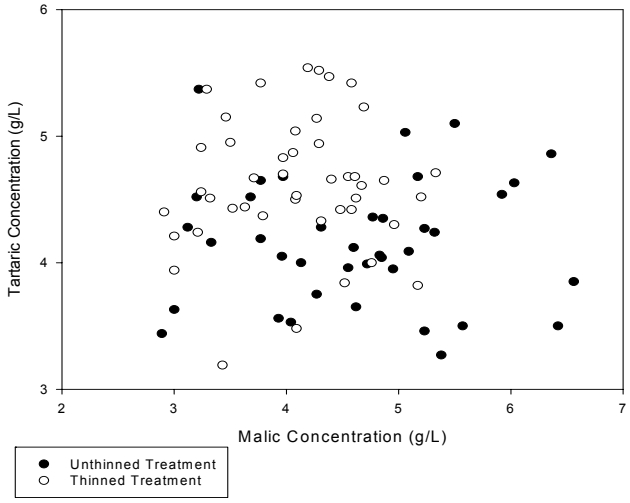
The method of determining the pH of the juice samples is described in section 3.2.4.

4.8.2. Results: The Acids, Potassium, Canopy and Rootstock Treatments and pH.

The broad analysis (REML) of the pH data from this experiment reveals that there is a significant difference between the two canopy treatment means and that the Rootstock treatments did have significant difference between some means (Table 4.17).

The regression of pH versus K Conc. found no relationship between K and pH and the incorporation of either the Canopy or Rootstock treatments into the analysis did not improve this situation. The regression analysis comparing Tartaric Acid Conc. to Malic Acid Conc. by Canopy treatment reveals no relationship between the two acids. The graph (Figure 4.8) does describe pictorially the skew away from Tartaric Acid to Malic Acid Conc. in the Unthinned Canopy treatment and the corresponding increase in average Tartaric Acid Conc. and decrease in Malic Acid Conc. in the Thinned Canopy treatment.

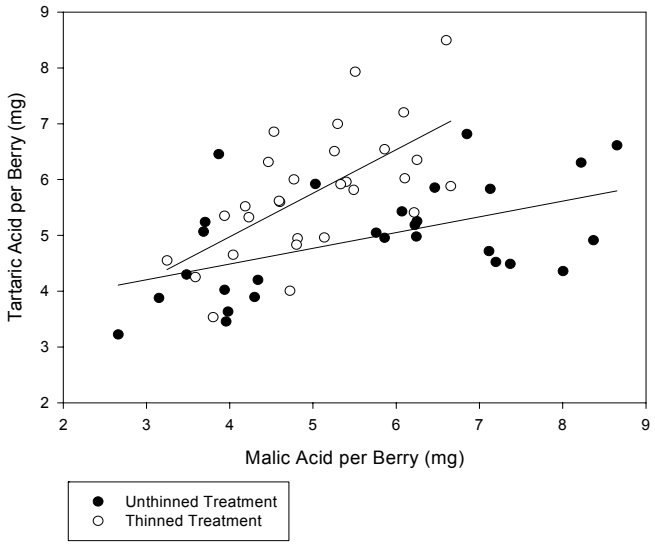
Figure 4.8 Graph of Tartaric Acid Concentration (g/L) versus Malic Acid Concentration (g/L) by Canopy Treatment.



The relationship is more clearly described when the amount of each acid per berry (mg) is considered. This takes into account berry size and reveals a relationship between Tartaric Acid and Malic Acid and the Canopy treatments (see Figure 4.9). This describes the

effect of concentration and berry size on the relationship between the two acids. We find that canopy affects the proportion of the two acids found in the berry: the Thinned Canopy treatment has more Tartaric Acid at the same level of Malic Acid than the Unthinned treatment. At higher levels of Malic Acid per Berry we are likely to find a lower level of Tartaric Acid per Berry for the Unthinned treatment. It must be noted that there is a smaller range of Malic Acid per Berry for the Thinned treatment (approximately 3 to 7 mg/berry) relative to the Unthinned (approximately 3 to 9 mg/berry). The range of Tartaric Acid per Berry is larger for the Thinned Canopy treatment.

Figure 4.9 Graph of Regression Juice Tartaric Acid per Berry (mg) versus Malic Acid per Berry (mg) by Canopy Treatment (F=0.016, R^2 of interaction 0.41).



The regressions of pH by Malic Acid Conc. by Canopy treatment establishes that the Malic Acid Conc. has a lower correlation to eventual pH (Figure 4.10, $R^2 = 0.24$) than that of Tartaric Acid Conc. (Figure 4.11, $R^2 = 0.44$). The basic correlation between pH and Tartaric Acid is 0.43 ($F < 0.001$) and the F probability for the interaction being

significant is F = 0.06. The strength of the relationship appears to lie in a direct connection between pH level and Tartaric Acid Conc.; the inclusion of the Canopy treatment does not improve the description of the relationship between pH and Tartaric Acid. The graph of Malic Acid Conc. by Canopy treatment to pH (Figure 4.10) shows that for both treatments increasing Malic Acid Conc. results in a lower pH. The rate of decline in pH is similar for both treatments indicating that an increase in Malic Acid Conc. has a similar effect on pH regardless of the Canopy treatment (and level of Tartaric Acid). The major difference is that at the same level of Malic Acid the recorded pH is higher in the Unthinned Canopy treatment. This is effectively an expression of the higher levels of Tartaric Acid found in this experiment in the Thinned Canopy treatment (which is expected to result in lower pH).

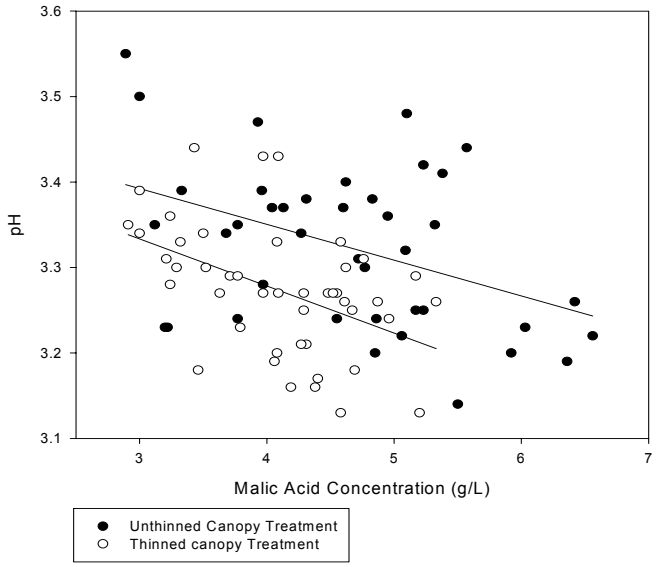
Table 4.17 Analysis (REML) of Juice pH by Canopy and Rootstock Treatment, Muddy Water Rootstock Trial.

Rootstock	**pH**	**Parentage**		**Canopy**	**pH**
Riparia G	3.21 a	Rip		**Unthinned**	3.32 a
5C	3.24 ab	Berl x Rip		**Thinned**	3.27 b
Fercal	3.29 bc	Berl x Vinif		l.s.d	0.04
101-14	3.29 bc	Rip x Rup			
Schwarz	3.30 cd	Rip x Rup			
3309C	3.30 cd	Rip x Rup			
99R	3.35 de	Berl x Rup			
420A	3.39 e	Berl x Rip			
l.s.d	0.05				

A GLR to explore the relationship between the two acids by the Rootstock treatment revealed that the connection between the levels of the two acids is not strong. The

comparison of the two acid concentrations by the Rootstock treatments and then the acids per berry by rootstock both yielded a low R^2 of 0.21.

Figure 4.10 Graph of Regression Juice pH Versus Malic Acid Concentration (g/L) (by HPLC) by Canopy Treatment (F<0.001, R^2 of relationship 0.24).



Two regressions to explore the relationships between the two acids (by the Rootstock treatments) and pH revealed that Malic Acid Conc. had no clear relationship to the measured level of pH (Figure 4.12, $R^2 = 0.28$). The eight rootstock regression curves were dominated by a flat response (4/8 rootstocks) with two rootstocks showing a slight increase in pH to increasing Malic Acid Conc. and two a slightly declining pH to increasing Malic Concentration. The overall effect is no clear relationship between Malic Acid and pH when the data are compared broken down by Rootstock treatments. The relationship between Tartaric Acid and pH is clearer. When the data are broken down by

Figure 4.11 Graph of Regression Juice pH versus Tartaric Acid Concentration (by HPLC) by Canopy Treatment (R^2 of relationship = 0.41)

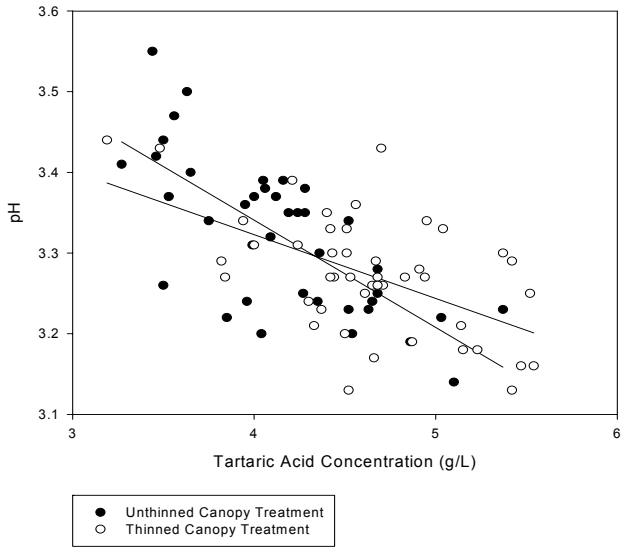
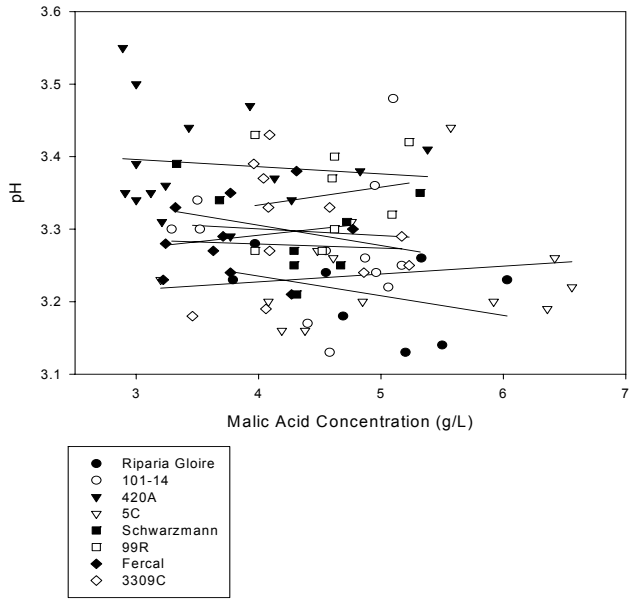


Figure 4.12 Graph of Regression Juice pH versus Malic Acid Concentration (by HPLC) by Rootstock Treatment (R^2 of relationship = 0.28).



rootstock we find the strongest correlation between the two acids and pH (Figure 4.14). The basic relationship between pH and Tartaric Acid Conc. has an R^2 of 0.43 and the inclusion of the Rootstock treatment increases this to 0.61. For all rootstocks increased Tartaric Acid Conc. results in lower pH.

Figure 4.13 Graph of Regression Juice pH versus Tartaric Acid Concentration (g/L) (by HPLC) by Rootstock (F<0.001, R^2 of relationship = 0.61).

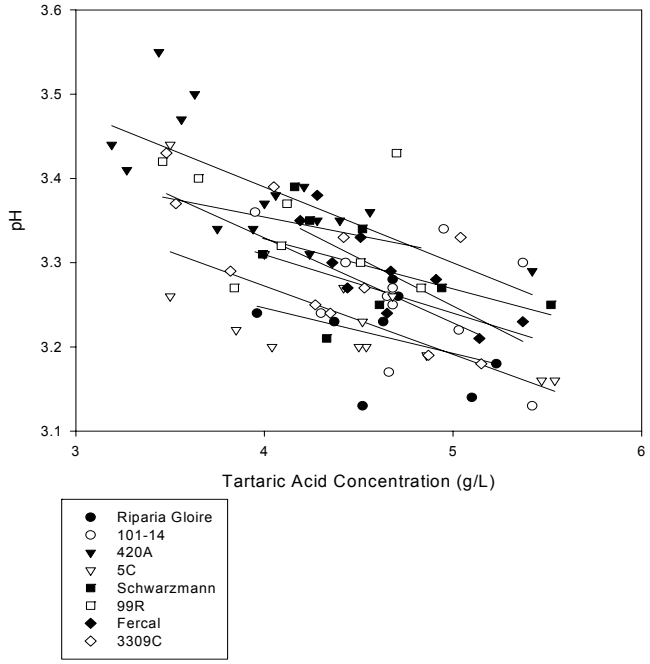


Table 4.17 describes the two acids and their relationship to pH. It is an attempt to highlight the relationship between the two acids in solution and their interaction that results in the final pH. It is a simplification of their relationship designed to highlight the potential (relative) importance of the two acids to pH. It uses a simple idea that recognises that at typical wine pH Tartaric Acid contributes approximately 3 times (actually 2.7 times, excluding the influence of alkaline metals, K and sodium (Margalit, 1997)) the hydronium ions of Malic acid to constitute the eventual pH. The 'Malic Equivalent Total Acid' (META) calculation is the concentration of Tartaric Acid

multiplied by three plus the concentration of Malic Acid. These results were ranked by rootstock and compared with a ranking of pH. The pH match ups correspond exactly to the expected level of acid, low Total Acid rankings to high pH rankings and high Total acid to low pH. This is a simple illustration of the relationship found in this experiment, where K is having minimal influence on pH.

Table 4.17 Calculation of Total Acid levels by Rootstock Treatment and Comparisons of Malic Equivalent Total Acid (META) and pH ranking for all Rootstocks

Note: The source of META calculation: Tartaric Acid is approximately 3 times stronger than Malic Acid at typical wine pH; i.e. 3 times the H^+ ions dissociate at wine pH (Margalit, 1997). Ranking levels for META and pH: 1 = low, 8 = high.

Rootstock	**Malic (1) (g/l)**	**Tartaric (2) (g/l)**	**Tart x 3 (3)**	**META (1 + 3)**	**META Rank**	**pH Rank**
420A	3.6	4.0	12	15.6	1	8
Fercal	3.8	4.7	14.1	17.9	6	3
99R	4.6	4.2	12.6	17.2	2	7
Schwarz	4.3	4.5	13.5	17.8	4	5
3309C	4.4	4.3	12.9	17.3	3	6
101-14	4.6	4.4	13.2	17.8	5	4
5C	5.0	4.4	13.2	18.2	7	2
Riparia G	4.9	4.7	14.1	19.0	8	1

Also note: Low pH matches with high adjusted total acid, high pH with low META:

Acid Rank: 1 2 3 4 5 6 7 8

pH Rank: 8 7 6 5 4 3 2 1

Regressions comparing pH to the META variable showed: pH to META only $F<0.001$, $R^2=0.60$; no improvement to this by including Canopy treatments (not significant); $F=0.035$, $R^2=0.65$ with the inclusion of the Rootstock treatments in to the model (interaction not significant).

4.8.3. Results: Canopy and Crop Variables and pH

The strength of the correlation between pH and Tartaric Acid by Rootstock treatment led to an investigation of the canopy and crop variables against pH. This was an attempt to ascertain if any of these variables was instrumental in describing the relationship between rootstock and pH.

For the crop variables there was a reasonable correlation between Cluster Weight (R^2 = 0.42) and Yield (R^2 = 0.40) with pH. For the canopy variables (by rootstock) the strongest correlations were for the variables that measure density in the fruiting zone. Both Point Quadrat measurements showed a relationship (Leaf Layer, R^2 = 0.36 and Percent Gaps, R^2 = 0.42 see Figure 4.14 and the Lower Video (%) Canopy Fill (see Figure 4.16, R^2 = 0.41)

Figure 4.14 Graph of Regression Juice pH versus Point Quadrat Percent Gaps by Rootstock ($F<0.001$, R^2 of relationship 0.41).

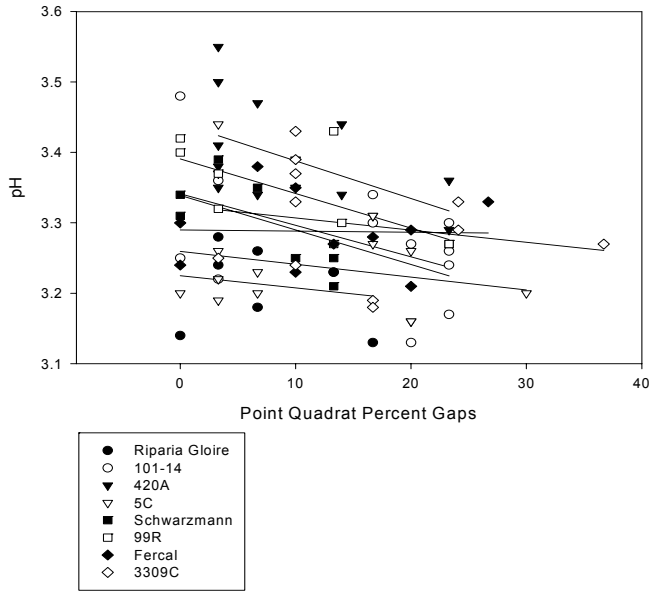
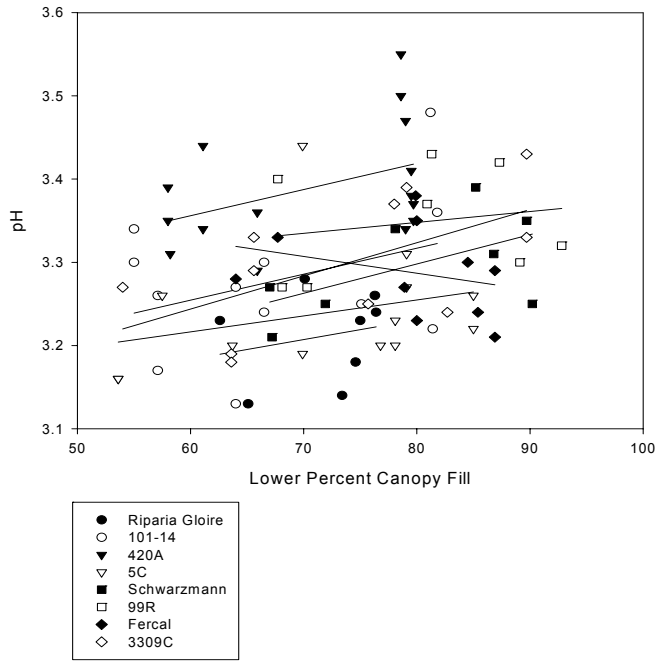


Figure 4.15 Graph of Regression Juice pH versus Lower Video Percent Canopy Fill by Rootstock (F<0.001, R^2 of relationship = 0.41).



There was no relationship between pH and the average size of the berries between the Rootstock treatments. An increase in the Cluster Weight corresponded to a lowering in the pH in seven of the eight rootstocks (3309C the exception, see Figure 4.16). An increase in plant Yield decreased pH in five of the rootstocks (Figure 4.17) and three showing a flat response (Fercal) or pH increase with increasing Yields (3309C and 5C).

The majority of the rootstocks showed a decrease in pH corresponding to a decrease in canopy density. For the Lower Video (% canopy fill) all but one rootstock (Fercal, see Figure 4.15) expressed this relationship and for the Point Quadrat (%) canopy fill Fercal and 3309C showed a flat response to the canopy level the rest declining pH to increased canopy gaps.

Figure 4.16 Graph of Regression Juice pH versus Average Cluster Weight by Rootstock Treatment (F<0.001, R^2 of relationship = 0.41).

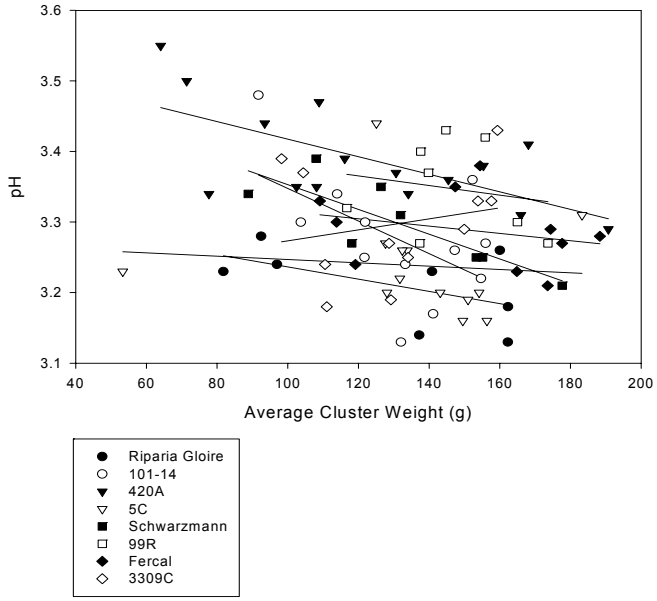
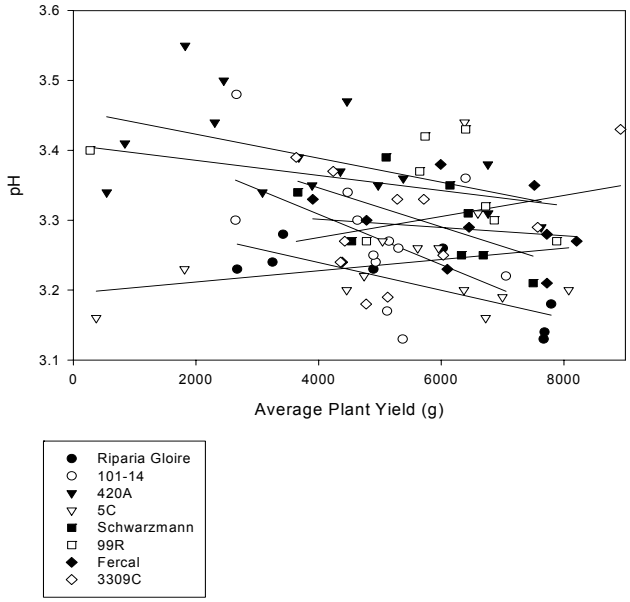


Figure 4.17 Graph of Regression Juice pH versus Average Plant Yield by Rootstock (F<0.001, R^2 of relationship = 0.37).



4.8.4. Discussion: pH, the Canopy and Rootstock Treatments and Related Variables

To help define the relationship between the two acids and pH the influence of K on pH must first be considered. We have found no relationship between the Canopy treatments and K. Similarly no relationship was found between the level of K and pH in this experiment. This is consistent with the findings (section 4.8.2) that the level of Tartaric Acid found appears to be independent of the level of K measured in the juice in this experiment.

It is likely that the low contact time between the skin and the juice in the processing of the grapes to juice in this experiment has minimised the potential impact of K on the results. It is important to stress this point as normal winery practice (especially for red varieties) is for extended skin contact to extract flavour, colour and tannins. A by-product of this extended skin maceration is the extraction of K from the skin, an abundant source of this cation (Mpelasoka *et al.*, 2003). This will have some effect on pH, as the increased level of K in solution is known to affect the dissociation of acids. The likely effect of the break down of skins and the resulting increase in K is a raising of the pH in the ferment; however, in some cases, the precipitation of potassium bi-tartrate may result in a (slight) lowering of the pH (Iland, 1987).

The breakdown of the data on these two acids by the Canopy and Rootstock treatments gives an opportunity to assess two key potential determinants of grape berry acidity. The Canopy and Rootstock treatments have a statistically significant influence on measured pH. The levels within the two treatments altered both Malic and Tartaric acid concentrations. The key to understanding the relationship between the two acids and pH is related to the interaction between the two acids in solution and the resulting pH. This interaction is defined by the proportion of the two acids and by the total amount of the two acids (and metal cations) in the juice (Margalit, 1997). Figure 4.9 shows the level of Tartaric Acid per Berry against the Malic Acid per Berry by Canopy treatment and Figure 4.8 shows the relationship between the two acid concentrations; these two graphs

demonstrate the effect canopy density had on these two acids in this experiment. Clearly, the level of Canopy treatment has had a marked effect on the final concentrations of the two acids at harvest. The level of Malic Acid found in the two treatments overlap but the range is extended by the Unthinned Canopy treatment. The level of Tartaric Acid per Berry is on average higher at the same level of Malic Acid for the Thinned treatment and the level of Malic is higher (on average) at the same level of Tartaric Acid per Berry for the Unthinned treatment.

The increased level of Malic Acid found in the Unthinned treatment is backed by the literature (Archer & Strauss, 1989; Kliewer, 1964; Lakso & Kliewer, 1975; Rojas-Lara & Morrison, 1989, Ruffner *et al.*, 1976) however the increased level of Tartaric Acid in the Thinned treatment is not as well understood. There are references that duplicate the Tartaric Acid and canopy relationships found in this experiment (Archer & Strauss, 1989; Rojas-Lara & Morrision, 1989) but the relationship between Tartaric Acid formation and canopy density has not been extensively explored. What can be said is that the experiment design appears to have highlighted that canopy density has an influence on the ratio of the two major organic acids by altering both the level of Malic and Tartaric Acid retained in the berry. This has obvious implications for pH.

The level of pH, on average, is significantly higher in the Unthinned Canopy treatment data when compared with the Thinned treatment data. This is a reflection of the proportion of Malic and Tartaric Acid Concentrations found (on average) in the two treatments. The graph of pH versus Tartaric Acid Conc. by Canopy treatment (Figure 4.11) shows that the Tartaric Acid Conc. is a major driver of the eventual pH, for both treatments an increase in Tartaric Acid lowers pH. Also on average, the level of Tartaric Acid measured in the Unthinned Canopy treatment is lower than that measured in the Thinned and the resulting pH is higher.

By looking at the same relationships but incorporating the Rootstock treatments in to the analysis (Figure 4.12, pH Vs Malic Acid Concentration and Figure 4.13, pH Vs Tartaric Acid Concentration) it is found that the relationships confirm the trends shown above.

Firstly, Malic Acid is less important in determining pH; the relationship is less convincing with a lower R^2 and relatively flat response of pH to increasing Malic Acid Concentration. Tartaric Acid has a far better defined relationship with a stronger R^2 (0.58) and the response of increased Tartaric Acid Conc. lowering pH for all rootstocks. The table (4.18) ranking Total Acid level to pH may be to an extent an artificial construct but it serves to emphasise the importance of the level of Tartaric Acid to pH. The dissociation constants of the two acids is the key to understanding the two acids, the higher 'strength' of Tartaric Acid relative to Malic in our grape juice (and resulting wine) is significant when determining final pH. A relatively small increase in Tartaric Acid Conc. compared with a much larger increase in Malic Acid Conc. can result in a similar shift in pH.

In this experiment, the Rootstock treatment has been implicated in the determination of the concentration of Malic and Tartaric Acids and the eventual pH. Results indicate that in this experiment the choice of rootstock can influence both canopy growth and Yield (see Chapter 3). The question remains is it a direct rootstock affect or is it a function of the rootstock influence on some other factor(s) (e.g. plant Yield or Fruit Zone density) that is indirectly affecting the level of the two acids and pH.

The Canopy treatments have been shown to broadly influence the levels of the two acids and pH. This is an effect that is evident despite of the Rootstock treatment level inherently influencing the data. The comparison of pH against the canopy variables by rootstock showed the strongest correlation for variables relating to the measurement of canopy density in the fruiting zone. The graphs presented (Figures 4.14 and 4.15, showing pH vs. Point Quadrat Percent Gaps and Lower Percent Canopy Fill by Rootstock treatment) describe the relationship of decreasing pH to decreasing canopy density. This situation is consistent with the findings in this experiment that the canopy density influenced Tartaric Acid Conc. in a similar manner; i.e. increasing Tartaric Acid Conc. to decreasing canopy density levels. The important point to note is that there are still measurable differences between the rootstocks and that the response of pH to changing levels of canopy is influenced by the rootstock.

The component variables of Yield (and Yield itself) in this experiment were influenced by rootstock, though the Canopy treatment level was not shown to have any effect on these variables. The trend shown by the two graphs presented (Figures 4.16 and 4.17, pH vs Cluster Weight and Yield by Rootstock treatment) is that in general the pH declines with both increasing Cluster Weight and Yield per vine despite the rootstock. The exceptions to this rule for Cluster Weight was 3309C, a rootstock with the second highest average Berry Weight (1.45 g) and second highest Cluster Weight (149.5 g). Exceptions for Yield were Fercal, 3309C and 5C. Rootstock 5C has the highest Berry Weight (1.55 g), Fercal has the highest Cluster Weight (153.3 g) and highest average Yield (6200 g). All other crop variables in relationship to these rootstocks are near average. What stands out (even though there was not a strong relationship between pH and berry weight) is that the three rootstocks that have gone against the trend (of declining pH to increasing Yield) represent the top performers in the Berry Weight, Cluster Weight and Yield variables. There is potential that a dilution effect may have skewed the results from these three rootstocks away from the general trend shown by the other rootstocks. This situation cannot be further explored using the data from this experiment

The effect of falling pH to increasing average Cluster Weight and Yield is difficult to explain using current literature. The possibility of a relationship between Tartaric Acid formation and the early growth of the berry has been observed (Kliewer, 1964; Ruffner *et al.*, 1976). The canopy conditions in and around the fruit during the green berry phase may play a role in influencing the amount of Tartaric Acid synthesised. The fact that this was a non-irrigated trial may have had some influence on the measured Tartaric Acid after harvest by influencing the synthesis of Tartaric Acid at this time or by altering canopy conditions in the fruiting zone for each rootstock. Research has identified one of the key elements of harvested Tartaric and Malic Acid Concentrations is the amount of dilution caused by berry expansion after veraison (Ruffner, 1982a). There is potential for some influence on Berry Weight, Cluster Weight or Yield from the alteration of canopy density by the Canopy treatments and/or the Rootstock treatments. The water status of the vines during final ripening may have influenced the potential of the fruit to expand and

the overall influence of fruit expansion on acid concentration may have been determined by the Yield being carried by a vine.

It may be that this is another expression of rootstock (and scion) success at the trial site, with Yield reflecting this success and the level of stored acid another indicator of adaptation to site conditions. The concentration of Tartaric Acid was linked to the concentration of K in the juice. Is the level of Total Acid found in the juice (and resulting pH) a function of plant nutrition (and or water status)? Photosynthate production is inextricably linked to the formation of acid in the berries, as the source of the plant carbon substrate (Ruffner, 1982 a and b). Does the relative success of the plant at the site limit the availability of the carbon for storage as acid in the berries during the green berry phase, therefore limiting acid production (especially in the case of Tartaric Acid)? Do the same limitations enhance or restrict the eventual Cluster Weight and Yield? These are questions this experiment was not designed to answer, so further research into the determinants of berry acid in the green berry phase is required.

What is apparent is that eventual harvested juice pH is linked to both the plant canopy density level (especially in the fruiting zone) and the rootstock selection. This experiment design has offered some insight in to the determinants of berry acidity but further work is needed to describe the rootstock influence on this process. There appears to be a rootstock influence when the level of canopy density in the fruiting zone is correlated with pH (Figures 4.14 and 4.15). When the Yield variables are considered there are still differences in the pH levels and rate of change in response to Yield components by individual rootstocks (Figures 4.16 and 4.17, Table 4.18). The size of this data set and the experiment design restricts the ability to break down the data further with meaningful results. Further work is required to establish the influences of rootstock on pH. The question remains, are the canopy density and Yield components, both influenced by rootstock, sufficient to describe the relationship between rootstock and pH?

What can be said is that there is most likely a rootstock-derived influence that is separate from the canopy-derived influence. This canopy influence appears to affect not just the

creation and retention of Malic Acid, but has an influence on the level of Tartaric Acid in the harvested fruit. The relationship between Malic Acid and pH was strongest when broken down by the Canopy treatment (Figure 4.10), though this relationship was clouded by the Rootstock treatment (exhibiting a relationship visually but with a low correlation coefficient). The dropping the Canopy treatment and including the Rootstock treatment in to the description of this relationship gave a flat response of pH to increasing Malic Acid (Figure 4.12). The inclusion of the Canopy treatments did not improve the description of the relationship between Tartaric Acid and pH (Figure 4.11). The basic relationship was increasing Tartaric Acid Conc. increasing pH (R^2=0.43), at a similar rate, regardless of the canopy treatment. The inclusion of the Rootstock treatment into the relationship between Tartaric Acid and pH did improve the correlation and strength of the relationship (Figure 4.14).

These results indicate that Malic Acid and pH are related through the level of canopy density, but this relationship is dominated by the relationship between Tartaric Acid and pH. The level of Tartaric Acid found in this experiment was the key element in deciding the pH of the harvested juice. The key element in describing the pH and Tartaric Acid relationship appears to be the rootstock. The reasons for the influence of rootstock over Tartaric Acid and final pH are not clear. It appears that canopy density in the fruiting zone and Yield (or Yield components) may have some influence and the levels of these are to some degree influenced by rootstock. It may be the relationship between the rootstock and the environment is another potential determinant of the rootstock effect. The information gathered in this experiment does not adequately describe the relationship between rootstock, Tartaric Acid and pH, but it is sufficient to say that it is this relationship that dominates the determination of pH in this experiment.

4.8.5. Summary of Results for pH for this experiment

Tartaric Acid Concentration in the juice is a key determinant of pH. Malic Acid plays a role though Tartaric Acid dominates the relationship with pH.

Canopy density has some significance in the determination of the ratio of the two acids found in the berry.

Lower canopy density in the fruiting zone tends to reduce average concentration of Malic Acid and increase average concentration of Tartaric Acid in juice at harvest.

Some influence of Yield or Yield components on pH is evident.

Rootstock does have a role in determining the amount of acid found in the berry at harvest as well as the proportion of the two acids found and the eventual pH.

The source of rootstock derived influence on pH can not be described. In addition to the influence rootstock exerts on Yield and Yield components there appears to be additional rootstock derived influence on pH.

K has no observed influence on pH.

5. Chapter 5: Conclusions

5.1. Conclusions: Canopy and Yield

The influence of the two treatment levels (Canopy and Rootstock) on the composition of berry acidity was the primary focus of this experiment. To be able to assess the impact of these treatments a number of key variables were assessed for their influence. Two important areas to evaluate were the canopy characteristics and the plant Yield.

The potential influence of canopy size and density on berry acid composition is documented (See Sections 2.61, 2.6.2, 2.6.3, 2.7.2, 2.7.3, 2.7.4) and Rootstock treatment was found to influence the level of all canopy variables measured in this experiment (Lower, Upper and Total (%) Fill; Point Quadrat Leaf Layer and Percent Gaps). There was a statistically significant difference between Rootstock treatment means despite the recorded influence of the altered Thinned Canopy treatment in the data (Section 3.3 and Table 3.2). Crosses of *Vitis rupestris* were found to exhibit the most canopy vigour and those derived from *Vitis berlandieri* and *Vitis riparia* the least.

The results of the analysis of the canopy variables for this experiment highlighted that rootstock have a significant influence on recorded canopy density. This result is confirmed in the literature (Mpelasoka, *et al.*; Pongracz, 1983) and the root morphology of the rootstocks appeared to influence vine performance in the soil conditions at this site (Section 3.3). This highlights that the interaction of the vine with the environment often has a strong influence over some of the variables measured in an experiment of this type. The potential influence of seasonal variation at the site (rainfall, irrigation and other climate factors) affecting results must be considered when planning an experiment so that any influence can be accounted for in the results.

The Canopy treatment was not found to have influenced Yield or to have had influence on the components of Yield (Berries per Cluster, Average Berry Weight, Average Cluster Weight and Number of Clusters per Plant, Section 3.4.3). The Rootstock treatment was found to influence Yield through influence over numbers of berries set in a cluster and

the final recorded average cluster weight (Table 3.6). There appeared to be a relationship between the rootstock, canopy and Yield (Section 3.5, Table 3.7, Figure 3.4), however the analysis of the individual canopy variables against Yield and the Rootstock treatments was not able to confirm this statistically.

This highlighted the difficulty in measuring canopy in relationship to field experiments. The difficultly is establishing the correct evaluation of canopy (e.g. density, leaf area) and the correct timing(s) and number of assessment(s) (e.g. flowering, pre-verasion, post-veraison). This can be a critical factor in deciding the statistical relevance of canopy variables against other variables in an experiment. In addition recording additional information with regard to Yield could improve the relevance of this information. The measurement of the change in berry and cluster weight from the lag phase (prior to veraison) to harvest would be desirable. The influence of rootstock on the final expansion of the berry (post veraison) was not recorded in this experiment and the degree of berry shrivel just prior to harvest was not recorded. Both of these factors could have bearing on the final concentration of solutes found in the juice and would be useful in improving the assessment of the effect of rootstock on Yield at this site. In addition an assessment of flower numbers and percent berry set may identify additional rootstock differences useful to the interpretation of Yield.

5.2. Conclusions: Potassium in the Plant Tissue and Potassium and Sugar in the Juice.

It was expected that the level of K found in the plant tissue may have an effect on the level of K found in the juice (Section 2.4.2.3 and 2.7). There is also evidence that the accumulation of K and sugars (measured in degree Brix) in juice may be related (Mpelasoka *et al.*, 2003). In the literature there is reported evidence of a canopy density and size influence (Section 2.7) and a rootstock influence on K accumulation in harvested fruit (Section 2.4.2.3).

In this experiment no clear relationships were found between juice K and the K in plant tissue (petioles or leaf blades) at flowering and veraison (Table 4.13). This was despite

recorded relationships between the level of K in plant tissue (for both petioles and leaf blades) and the Rootstock treatment (Sections 4.4.4 and 4.4.6). The Canopy treatment level was not related to juice K in this experiment (Section 4.5.2), but the Rootstock treatment was found to have some influence on the harvested juice K level (Section 4.5.3). The level of Brix was found to have had no effect on the level of K found in the juice. There were no obvious genetic origins of the K level in the juice, but it must be remembered that this was a non-irrigated trial site on clay based soils and this may have had bearing on the recorded levels of K in the fruit for individual rootstocks. The influence of rootstock root morphology may account for the plant and juice K results but the magnitude of the differences in juice K, in this season, was not sufficient to detect a link to pH.

The relationship between average Cluster Weight and juice K was increasing Cluster Weight to increasing levels of juice K. This led to the hypothesis that plant adaptability to the site environment was creating differences in plant nutrition and water status and this was in turn positively influencing both Cluster Weight and juice K Concentration (Section 4.5.3). The results indicate that some rootstocks may be suited (adapted) to environmental conditions at this site, due in part to root morphology and tolerance to the soil water status throughout the growing season, and soil chemical and physical characters at the site. This leads to the conclusion that some assessment of the vine performance at the site is required to evaluate relative vine performance in the trial site conditions. This would incorporate additional canopy measurements designed to assess vine size (an active leaf area measurement, using infrared and video Canopy Fill assessment) throughout the season (at flowering, pre-veraison (phase 2) and post veraison) and assessment total vine carbon fixing throughout the season (pruning weights and estimation of vine capacity).

The fact that no relationship was found between K in the plant tissue measurements (petiole and leaf) and in the juice in this experiment was to some degree unexpected. Rootstock has been implicated in other experiments as an influence on the level of K in the fruit (Section 2.4.2.3) and a similar relationship was expected at this site. A number of

possible explanations were offered as to why this was not so (Sections 4.4.4). The need to clearly identify the status of the leaves that are sampled was established. The potential of rootstocks to influence total leaf area and the length of the growing season (Pongrazc, 1983) could have bearing on the activity of the leaves and the level of K measured. Were the sampled leaves (and petioles) shaded by other canopy, operating below photosynthetic capacity or beginning to senesce? The canopy size (and density) may be influencing the recorded levels of K due to an influence on the photosynthetic activity of the leaf (Petrie *et al.*, 2000). Leaf activity in the basal area may be influenced by root structure differences or seasonal growth differences (e.g. short growing cycle) between the rootstocks, affecting the ability to maintain the full canopy area (with dry summer conditions limiting the plant water and nutrients from the upper soil). The senescence of basal leaves under low water conditions may be influencing recorded K. This may offer an opportunity to assess rootstock differences using a SPAD meter to assess leaf greenness, measurement of gas exchange from the leaves and digital video to assess late season leaf loss in the lower canopy area.

In addition future research may require the drying and digestion of whole berries to assess the true level of fruit K. The time and funding constraints of this experiment did not allow for evaluation of the fruit in this manner. The method of juice extraction in this experiment) did not allow much contact time between the skin, seeds and pulp of the berry. In winemaking (particularly for red varieties) skin contact time is essential for the extraction of tannin and flavour (Jackson, 1994) and this will result in the additional extraction of K.

5.3. Conclusions: TA, Juice Malic and Tartaric Acid

The level of Malic Acid was lower in the Thinned canopy treatment and the Rootstock treatment had an influence on the level of this acid (Section 4.7.2 for results). The Malic Acid level was strongly correlated to the level of TA. TA was influenced by the Canopy treatment in a similar manner to Malic Acid, the Thinned treatment recording lower average TA. The canopy effect on Malic Acid was predicted in the literature (Ruffner, 1982b).

Tartaric Acid had a negative relationship to increasing canopy density, confirmed by the differences in the Canopy treatment (section 4.6.2 for results). There is some evidence in the literature to support that the formation of this acid may be enhanced by increased exposure of bunches (pre-veraison) to increased light levels or higher temperatures (Ruffner, 1982a). The Rootstock treatment also influenced the level of tartaric acid but the variability within the data made establishing whether the rootstock influence on Yield and canopy density had any effect on this result. The influence of K on the level of recorded Tartaric Acid was discounted (Section 4.6.3). The Tartaric Acid Concentration showed a poor relationship to TA.

The relationship of the two acids to TA was explained by the larger range of Malic Acid Concentration recorded in this experiment relative to Tartaric Acid. The influence of Tartaric Acid on TA was further thought to be reduced by the propensity of this acid to form salts (Ruffner, 1982a). The level of TA was influenced by the combined amounts of the two acids (Table 4.16). The combined amount of acid and the proportion of the two acids appear to be influenced by the Rootstock treatments.

The variability within the Tartaric Acid data has caused problems in identifying possible influences on this acid. It is impossible to tell if the Rootstock treatment effect can be attributed to Yield or canopy influences (both influenced by rootstock level) (Section 4.6.2). The confounding factor in this analysis may be the influence of berry and bunch expansion post veraison. Tartaric Acid reaches maximum concentration in the berries at the end of the green berry phase just prior to veraison (Ruffner, 1982a). The measurement of Tartaric Acid at this stage would be beneficial to establishing the relationship between this acid and rootstocks. In conjunction with this the collection additional canopy measurements during the green berry phase, to more closely assess canopy conditions throughout this period, may highlight better the canopy influence on berry acid accumulation. The recording of berry expansion levels post veraison (and shrivel pre-harvest) would help to characterise the influence of rootstock on Tartaric Acid (and Malic Acid and TA).

5.4. Conclusions: K, the acids and pH

In this experiment the level of juice K did not appear to influence pH. This situation was not expected as literature includes many instances where the opposite was found (Mpelasoka *et al.*, 2003). No explanation can be offered for this except perhaps the effect of low skin contact time during processing of the clusters into juice or the recorded variation in juice K at this site between rootstocks was not sufficient to have a measurable influence on pH.

The concentration of Malic Acid appeared to show a weak relationship to pH (Section 4.8.2) and only when analysed in relationship to the Canopy treatment. Tartaric Acid showed a reasonably strong relationship to pH and this was strengthened by the incorporation of the rootstock treatment into the analysis. This situation is supported by the literature with the dissociation of Tartaric Acid versus Malic Acid indicating that it is a significantly stronger acid with respect to pH (Margalit, 1997).

The relationship of the Rootstock to pH was not explained by simply looking at the Yield and canopy density information. In sets of regressions investigating Yield and canopy versus pH the incorporation of the Rootstock treatment improved the relationship. Lower canopy density tended to result in lower pH, which follows the evidence that Tartaric Acid levels tended to increase with lower canopy density (especially in the fruiting zone). What was surprising was an increase in Cluster Weight and Yield (by rootstock) resulted in increased pH. This is difficult to explain but may be related to the reported connection with growth of the berry in the green berry phase (Kliewer, 1964; Ruffner *et al.*, 1976). This could also have a relationship to the level of canopy density in the green berry phase or be related to the capacity or nutrition (and water status) of the vine during this period. The suggested future extensions to the measurement of the canopy and Yield variables may help to resolve this issue. The source of the rootstock derived influence on pH is difficult to describe, it appears the influence rootstock exerts on yield and canopy may

encompass a proportion of the effect. It appears that the rootstock effect on the combined amount of the two acids also influences pH and this was not able to be assessed in this experiment.

5.5. Rootstock and this Experiment

Analysis of the rootstock treatment in this experiment identified a number of instances where the rootstock had influence over measured results either independent of the influence of the canopy treatment or independent of the rootstock influence over other variables that may have some bearing on the relationship (primarily the reported influence of rootstock on canopy variables and yield (and yield determinant) variables). The inclusion of the Canopy treatment overlaying the Rootstock treatment allowed clearer evaluation of the 'pure' rootstock influence independent of the recorded influence of rootstock on canopy growth.

Examples of 'pure' rootstock influence include:

- Canopy variables – the influence of rootstock was apparent for all measured canopy variables despite the inclusion of the data from both Canopy treatments (Table 3.2a). This was highlighted in the analysis of the Percent Canopy Fill data provided by the video evaluation of the whole canopy surface down the rows (Table 3.2b). The influence of rootstock was especially strong in the upper half of the trellis and the ability of the plant to fill this area appeared to be strongly influenced by the rootstock. There was a different response by the rootstocks to the imposing of the Thinned Canopy treatment again clearly highlighted in the Upper Fill Video data (Table 3.2b).

- Yield variables - The Canopy treatment did not influence plant yield (or components of yield) in this experiment. The lack of a Canopy treatment effect was predicted as adjustments were made to standardise the number of bunches in both Canopy treatments (Section 3.2.2); this was done to remove the effect on cluster numbers of removing double burst and excess shoots in the head area of the Thinned Canopy treatment. The Rootstock treatment was found to influence plant yield through an

influence of number of berries set per cluster and the eventual cluster weight; this influence was recorded independent of any influence of the two Canopy treatments.

- Juice Composition:

  - Potassium (K): The Canopy treatment did not influence the level of K found in juice (Section 4.5.2) but the Rootstock treatment influenced the level of K (Section 4.5.3). The yield influence on the level of K was limited to the cluster weight with increasing cluster weight corresponding to increasing recorded K (Section 4.5.3). Cluster weight was also influenced by the Rootstock treatment but not by the Canopy treatment.

  - Tartaric Acid: The information regarding the influence of canopy and rootstock on the level of Tartaric Acid found in the juice was confused by a high degree of variability in the data. There was a Canopy and Rootstock treatment influence on the level of this acid (found independently using REML). There was some evidence of a Rootstock influence on Tartaric Acid independent of the Canopy treatment (Figure 4.1) although this was not able to be conclusively confirmed.

  - pH: The influence of the Canopy treatments on pH is confused by a conflicting influence of canopy level on the two acids. Increasing canopy density increasing recorded Malic Acid (Table 4.15) and decreasing Tartaric Acid (Table 4.14). However, a regression (Figure 4.11) incorporating Canopy treatment into an evaluation of the influence Tartaric Acid on pH reveals that the basic relationship (without the inclusion of the Canopy treatments) offers as good a description of the relationship between the acid and pH. A similar regression incorporating the Rootstock treatments (Table 4.13) shows a marked improvement in the results of the regression by including the Rootstock treatments (R^2 improvement from 0.43 to 0.61). The description of the canopy influence on pH appears to be strongest when the Rootstock treatments are included in the analysis of canopy variables (Figures 4.14 & 4.15). There may be an effect of Average Cluster Weight and Average Plant Yield (Figures 4.16 & 4.17) on pH but only the Rootstock treatment was found to influence these variables. To summarise although the

variability in the data made assessing the influence of the Rootstock treatments on pH difficult, evidence that rootstock has an influence on pH independent of the Canopy treatment exists. This relationship does require further investigation.

In summary there is some evidence in this experiment that the Rootstock treatment influenced the levels of a number of key variables. Rootstock appeared to influence the level of canopy growth and plant yield independent of the Canopy treatment. Rootstock influence was observed in the analysis of a number of key juice composition variables and the Rootstock treatment appears to have an influence on the juice pH independent of the Canopy treatment.

The separation of the rootstock influence on canopy and yield from the influence of canopy density on yield (and other juice composition variables) has not been clearly defined in the literature. This experiment has attempted to clarify the relationship of rootstock selection to harvested juice composition. It has managed to highlight a number of variables that rootstock selection appeared to influence directly (an influence independent of the Canopy treatment) or by way of a rootstock influence on canopy growth or plant yield.

6. Bibliography

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7. Appendix

Table 7.1 Top Soil depths and Sub-Soil Description at Plant Three in Each Treatment Plot

Sub-soil Legend
1: Light clay loam
2: Clay Loam
3: Sandy Clay loam

Bay = Plot number

		Top Soil Depth	
		300-350mm from plant 3	
Row	**Bay**	**in mm**	**Sub-soil Type**
40	1	210	3
40	2	220	2
40	3	200	1
40	4	230	2
40	5	180	2
40	6	160	1
40	7	230	2
40	8	250	2
41	9	180	2
41	10	220	3
41	11	240	2
41	12	220	1
41	13	180	2
41	14	180	1

Row	Bay	in mm	Sub-soil
			Type
41	15	200	1
41	16	240	2
42	17	270	3
42	18	210	3
42	19	260	2
42	20	250	2
42	21	190	2
42	22	200	2
42	23	170	2
42	24	230	2
43	25	260	3
43	26	220	2
43	27	210	2
43	28	160	2
43	29	150	2
43	30	220	2
43	31	200	2
43	32	190	2
44	33	290	2
44	34	260	2
44	35	250	2
44	36	260	2
44	37	180	2
44	38	130	2
44	39	190	2
44	40	260	2
45	41	190	2
45	42	140	3

Row	Bay	in mm	Sub-soil
			Type
45	43	200	2
45	44	290	2
45	45	170	2
45	46	160	2
45	47	160	2
45	48	290	2
46	49	150	2
46	50	280	2
46	51	150	2
46	52	180	2
46	53	180	2
46	54	200	2
46	55	180	2
46	56	150	2
47	57	190	2
47	58	150	3
47	59	200	2
47	60	290	2
47	61	150	2
47	62	160	2
47	63	180	2
47	64	190	2

Table 7.2 Clonal Assessment of Rootstock Trial Conducted 9 January 2004

Table shows assessment of 5 plant bays, north to south. Bay 1 to 8 corresponds to row 1, plant 9-16 = row 2 and a different row for each corresponding 8 bays. Row 1 is the first trial row to the east, row 8 is on the western edge of the trial block. Legend: u = Upright clone, d = Droopy clone, m = Missing Plant, r = Rootstock (no scion).

		PLANT				
BAY	***Rootstock***	**1**	**2**	**3**	**4**	**5**
1	***Riparia G.***	d	d	d	u	u
2	***101-14***	u	u	u	u	d
3	***420A***	d	m	u	u	u
4	***5C***	u	u	u	u	d
5	***Schwarz.***	d	d	d	d	d
6	***99R***	d	d	d	d	d
7	***Fercal***	d	d	d	d	u
8	***3309C***	u	u	u	d	u
9	***101-14***	m	u	u	d	u
10	***Riparia G.***	d	d	d	d	u
11	***Fercal***	d	d	d	d	d
12	***99R***	d	d	d	d	d
13	***3309C***	u	u	u	u	u
14	***5C***	u	u	u	u	d
15	***420A***	u	d	u	u	u
16	***Schwarz.***	d	d	d	d	d
17	***420A***	u	u	u	d	d
18	***Fercal***	d	d	d	d	d
19	***Riparia G.***	d	d	d	d	d
20	***Schwarz.***	d	d	d	d	d
21	***5C***	u	u	u	u	u
22	***3309C***	u	u	u	u	u
23	***101-14***	u	u	u	u	u
24	***99R***	d	d	d	r	m
25	***5C***	u	u	u	u	u
26	***99R***	d	d	d	d	m
27	***Schwarz.***	d	d	d	d	d
28	***Riparia G.***	u	d	u	d	d
29	***420A***	u	u	u	m	u
30	***101-14***	u	d	u	u	u
31	***3309C***	d	u	d	u	u
32	***Fercal***	d	d	d	d	d
33	***Schwarz.***	d	d	d	d	d
34	***3309C***	u	u	u	u	u
35	***5C***	u	u	u	u	d
36	***420A***	d	u	u	d	m
37	***Riparia G.***	u	d	d	d	d
38	***Fercal***	d	r	d	d	d

BAY	*Rootstock*	PLANT 1	2	3	4	5
39	***99R***	d	d	d	r	d
40	***101-14***	u	u	u	u	u
41	***99R***	d	d	d	d	d
42	***5C***	d	u	u	u	u
43	***3309C***	u	u	d	d	d
44	***101-14***	d	d	u	u	u
45	***Fercal***	d	d	d	d	d
46	***Riparia G.***	d	u	d	d	d
47	***Schwarz.***	d	d	d	d	d
48	***420A***	u	u	u	u	d
49	***Fercal***	d	d	d	d	d
50	***420A***	u	d	u	d	u
51	***101-14***	u	u	u	d	u
52	***3309C***	u	u	u	u	u
53	***99R***	d	d	d	d	d
54	***Schwarz.***	d	d	d	d	d
55	***Riparia G.***	d	u	d	u	d
56	***5C***	u	u	u	u	u
57	***3309C***	u	u	u	u	u
58	***Schwarz.***	d	d	d	d	d
59	***99R***	d	d	d	d	d
60	***Fercal***	d	d	d	u	d
61	***101-14***	u	u	u	u	u
62	***420A***	u	d	u	u	d
63	***5C***	d	d	u	u	u
64	***Riparia G.***	u	m	d	d	d

Figure 7.3 Summary of Upright and Droopy Clone by Rootstock

Riparia Gloire

Upright	10	25.6	%
Droopy	29	74.4	%

101-14

Upright	33	84.6	%
Droopy	6	15.4	%

420A

Upright	26	70.3	%
Droopy	11	29.7	%

5C

Upright	34	85.0	%
Droopy	6	15.0	%

Schwarzmann

Upright	0	0.0	%
Droopy	40	100.0	%

R99

Upright	0	0.0	%
Droopy	36	100.0	%

Fercal

Upright	2	5.1	%
Droopy	37	94.9	%

3309C

Upright	34	85.0	%
Droopy	6	15.0	%

Notes on Clone and Rootstock: The analysis of the clonal effect was done using REML, incorporating clone in to the analysis matrix as another treatment. Samples had been taken from rootstocks with a clonal mix and where ever possible and incorporated in to the analysis (resulting in the unbalanced data set and the need to use REML for analysis. The clonal effect was weak for all variables; occasionally it did show a significant relationship or interaction with other treatments but review of the resulting means showed only one rootstock was significant or conflicting results (especially for the interaction means) that required the rejecting of the implied relationship. Overall the effect of clone was weak; there may have been some clouding of the relationships (e.g. for Tartaric Acid) due to the two clones being present but the clone as a treatment failed to establish any relationships with the data being measured. The mitigating effect on the influence of the clone may have been the canopy management employed. Work was done to straighten the Droopy clones' canopy possibly removing any effect additional fruit shading and crowding may have had on results.